Experimentally Introduced Defective Endogenous Proviruses Are Highly Expressed in Chickens

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We have previously described the experimental introduction of recombinant subgroup A avian leukosis viruses (ALV) with Rous-associated virus 0 long terminal repeats into the germ line of line 0 chickens and the generation of 23 transgenic lines. Two of these transgenic lines, *alv6* and *alv11*, do not produce infectious virus. Both of these lines contain defective proviruses but do express the *gag* and/or *env* protein. We have measured viral RNA expression in tissues derived from *alv6*, *alv11*, and the parental line 0. Total RNA was prepared from 9-day embryo, 16-day embryo, 1-day chicken, and 28-day chicken tissues. Viral RNA was detected by Northern RNA transfer analysis. The results indicate that both *alv6* and *alv11* chickens express viral RNA in all tissues tested regardless of the stage of development. No viral transcripts were detected in any line 0 (C/E; *env*-negative) tissue. The levels of biologically active *env* glycoprotein correlates with the *env* RNA levels in both lines. In an in vivo interference assay, *alv6*, *alv11*, and line 0 chickens were infected with Rous-associated virus 1 and monitored for viremia, antibody against Rous-associated virus 1, and ALV-induced pathogenesis from 4 to 21 weeks. None of the 61 *alv6* chickens contained detectable virus or produced antibody against subgroup A ALV. Virus and/or antibody against subgroup A ALV was detected in 34 of the 43 *alv11* chickens, whereas 51 of 52 line 0 birds were viremic and/or produced antibody. ALV-induced pathogenesis was observed predominantly in line 0 chickens (10 of 59), whereas very little ALV-induced pathogenesis was seen in either *alv6* (1 of 62) or *alv11* (1 of 44) chickens. Presumably the mechanism for the increased resistance of *alv6* and *alv11* chickens was subgroup-specific receptor interference. These results clearly demonstrate that experimentally introduced endogenous proviruses can be expressed at high levels in the avian system.

Efficient vectors for gene transfer to eucaryotic cells should be stable, efficient, and capable of inducing expression of the inserted gene. Although several avian and murine retrovirus vectors fulfill these criteria in cell culture, the experimental introduction of murine retroviruses into the germ line early in development often resulted in little or no viral gene expression (4). Jaenisch and co-workers, who generated transgenic mouse lines (*Mov*) by infecting early embryos with Moloney murine leukemia virus, found that most or all of the Moloney murine leukemia virus expression observed in *Mov* mice was due to virus replication from rare new integrations in susceptible tissues (12, 13, 18, 19). Expression from the experimentally introduced endogenous Moloney murine leukemia virus proviral loci was not detected. No Moloney murine leukemia virus RNA transcripts were found in tissues from 16-day embryo, newborn, and adult mice in the nonviremic *Mov* lines studied (3). Subsequent experiments have shown that deletions and mutations in the proviral loci and chromosomal location both play roles in the regulation of proviral expression in the *Mov* lines (2, 20, 21).

We have characterized two transgenic chicken lines that carry experimentally introduced defective avian proviruses. Twenty-three transgenic lines were generated by experimentally introducing recombinant subgroup A avian leukosis virus (ALV), with Rous-associated virus 0 (RAV-0) long terminal repeats (LTRs), into the germ line of line 0 chickens (C/E; *env* negative) (6, 15-17). ALVs have been classified into

five envelope subgroups (A through E) based on host range, reaction with neutralizing antibody, and cross-interference of receptors (23). ALV-infected chicken cells are highly resistant to reinfection by ALVs of the same subgroup. Presumably the envelope glycoproteins produced by the infected cells specifically block the cell surface receptors, preventing the binding and penetration of the second virus. Two of the 23 transgenic chicken lines, *alv6* and *alv11*, did not produce detectable infectious virus (6, 15). *alv6* does not express *gag* p27 protein that can be detected by enzyme-linked immunosorbent assay, and viral particles were not seen with electron microscopy. However, *alv6* does produce biologically active envelope glycoprotein. No genetic lesions were found in the mapping of the *alv6* genome with restriction endonucleases. Small deletions or base substitutions in the *alv6 gag* and/or *pol* genes could account for the absence of *gag* p27 and viral particles. These defects would not necessarily affect the splicing or translation of the *env* RNA, which would not carry the genetic lesions. In contrast, *alv11* expresses both *gag* p27 and envelope proteins and produces viral particles that can be seen with electron microscopy. The *alv11* provirus has lost approximately 0.5 kb from the *pol* gene, which may explain why the *alv11* viral particles are not infectious (6).

There were several reasons to choose these two lines to study proviral gene expression. Since line 0 chickens have no endogenous ALV, the expression from the *alv6* or *alv11* proviral loci could be measured unambiguously. These two loci are defective; this prevents the rare reintegration events that made the analysis of expression in the *Mov* mice difficult. Subgroup-specific receptor interference can be used

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FIG. 1. Structure of the *alv6* and *alv11* proviruses and of the expected viral transcripts. The proviruses are derived from a recombinant subgroup A ALV with RAV-0 LTRs. The ALV genes *gag*, *pol*, and *env* are indicated. The *alv11 pol* gene has lost approximately 0.5 kb (indicated by a gap within parentheses), including a *Bam*HI site. The black bar indicates the region of *env* used as an ALV probe. Restriction endonuclease sites: B, *Bam*HI; H, *Hind*III; K, *Kpn*I; S, *Sal*I. Abbreviations: SD, splice donor; SA, splice acceptor.

to compare the expression of biologically active *env* glycoprotein with RNA levels. The *alv6* line has been previously shown to be resistant to subgroup A viruses in vitro and in vivo, and it has been proposed that the expressed envelope glycoproteins interfere with the subgroup A receptors to block virus penetration (6, 15).

In an in vitro envelope expression assay between *alv6* and line 0 CEF cells, only *alv6* CEF complemented the envelope defect of the subgroup A Bryan high-titer strain of Rous sarcoma virus (BH-RSV) to produce pseudotype subgroup A virus (6, 15). An in vitro interference assay showed *alv6* CEF to be over 3,000-fold more resistant than line 0 CEF to infection by subgroup A RSV (6, 15). *alv6* chickens were also shown to be highly resistant to infection by subgroup A ALV. *alv6* and line 0 chickens were inoculated at hatch with RPL-42, a subgroup A ALV field strain, and reared together for 40 weeks. No evidence of subgroup A ALV infection or related pathology was observed in *alv6* chickens, whereas line 0 chickens were viremic with ALV and produced antibody against ALV and greater than 50% of line 0 birds contained ALV-induced neoplasms at 40 weeks (6, 15). The increase in resistance was specific for subgroup A ALV, since line 0 and *alv6* CEF and chickens were equally susceptible to a subgroup B ALV infection.

We wished to determine whether the promotor in the LTRs of experimentally introduced endogenous avian proviruses is transcriptionally active and promotes the correct expression of viral gene products. Viral RNA transcripts were assayed in total RNA from tissues of *alv6*, *alv11*, and parental line 0 embryos and chickens by Northern transfer. Four developmental stages were tested: 9-day embryo, 16-day embryo, 1-day chicken, and 28-day chicken. Both

alv6 and *alv11* proviruses correctly expressed viral RNA in all tissues tested regardless of the developmental stage.

MATERIALS AND METHODS

Chicken lines. Line 0 White Leghorn chickens are genetically susceptible to all ALV subgroups except E (C/E) and are free of all endogenous proviruses with close homology to ALV cDNA (*ev* negative) (1, 10). Line 0 breeding stock has been maintained free of exogenous ALV infection for several generations. The generation of *alv6* and *alv11* transgenic chicken lines from line 0 parents has been described (16, 17). Heterozygous offspring from the sixth generation were used in these experiments.

Virus assay. Assays for cellular resistance to virus infection were conducted on secondary CEFs from individual 10-day embryos (6). Tenfold serial dilutions of BH-RSV with RAV-1 as the helper virus were used to quantitate resistance by focus formation.

DNA probes. The ALV *env* probe was a 1.0-kb *Kpn*I-*Sal*I fragment isolated from the *env* region of the RCAS vector (11) (Fig. 1). The glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe was a 0.7-kb *Pst*I fragment that contains most of the chicken GAPDH cDNA from pGAD-28 (9). The DNA fragments were gel purified and 32 P labeled by nick translation (14).

Extraction and analysis of RNA. All glassware and solutions were RNase free. RNA was extracted from tissues by using RNazol (Cinna/Biotech Laboratories International, Inc., Friendswood, Tex.). Approximately 100 mg of tissue was homogenized in 2 ml of RNazol with a Kinematica GmbH Polytron homogenizer. Chloroform (0.3 ml) was

added to the homogenate and mixed vigorously for 15 s, and the tissue was incubated 4°C for 15 min. The suspension was then separated by sedimentation in a centrifuge ($12,000 \times g$ at 4°C for 15 min), and the upper aqueous phase was collected. An equal volume of isopropanol was added to the aqueous phase, and the mixture was chilled to -20°C for 45 min. The precipitates were collected by sedimentation ($12,000 \times g$ at 4°C for 30 min). The pellets were suspended in 2 ml of water, extracted with 2 ml of phenol-chloroform (1:1), and separated by centrifugation ($12,000 \times g$ at 4°C for 5 min). The aqueous phase was collected, and NaCl was added to 100 mM. Two volumes of ethanol were added, the RNA was allowed to precipitate at -70°C for 1 h, and the precipitate was collected by sedimentation ($12,000 \times g$ at 4°C for 30 min). The RNA pellets were dried and suspended in water.

RNAs were analyzed by modifications of the protocols of Maniatis et al. (14). Total RNA (20 µg) was fractionated on 1.5% agarose-formaldehyde gels in 20 mM MOPS (morpholinepropanesulfonic acid) (pH 7)–1 mM EDTA–5 mM sodium acetate–2.2 M formaldehyde. RNA samples were dissolved in 20 mM MOPS (pH 7)–1 mM EDTA–5 mM sodium acetate–50% formamide–2.2 M formaldehyde and heated at 65°C for 5 min before the gels were loaded. The fractionated RNA was transferred to nitrocellulose with $10 \times$ SSC ($1 \times$ SSC is 0.15 M sodium chloride plus 0.015 M sodium citrate [pH 7]) for 16 to 24 h. The filter was rinsed in $6 \times$ SSC for 5 min, air dried, and baked at 80°C for 2 h under vacuum. The filter was then prehybridized in 50% formamide–50 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (pH 7)–0.1% Ficoll–0.1% polyvinylpyrrolidone–0.1% bovine serum albumin– $3 \times$ SSC–150 µg of salmon sperm DNA per ml at 42°C for 12 to 24 h. For hybridizations, 3.5×10^7 to 7×10^7 cpm of 32 P-labeled, nick-translated ALV *env* DNA or GAPDH DNA (specific activity, 1.5×10^8 cpm/µg) was added to the prehybridization solution, and the DNA was incubated for 24 to 48 h at 42°C. Hybridized filters were washed in $2 \times$ SSC–0.1% sodium dodecyl sulfate at 25°C and then in $0.2 \times$ SSC–0.1% sodium dodecyl sulfate at 55°C and exposed to Kodak X-Omat film. Filters were then stripped in $0.1 \times$ SSC–0.1% sodium dodecyl sulfate preheated to 100°C before the second probe was added to the filter.

In vitro virus challenge. Hemizygous *alv6*, hemizygous *alv11*, and line 0 chickens were inoculated intra-abdominally with 10^3 to 10^4 infectious units of RAV-1 (a subgroup A ALV) on the day of hatch and then reared intermingled. Serum was collected at 4, 12, and 21 weeks postinfection and assayed for subgroup A ALV and antibody against subgroup A ALV by standard procedures (6, 15). All birds that died after 4 weeks of age or were killed at 21 weeks were necropsied for three types of ALV-induced pathogenesis: lymphoid leukemia, non-neoplastic syndrome (5, 8), and bursal-thymus atrophy.

RESULTS

***alv6* and *alv11* proviral loci.** The structures of the *alv6* and *alv11* proviruses are diagrammed in Fig. 1. Two RNA species were expected to be transcribed from these proviruses. The larger genomic RNA is initiated at the U3/R boundary in the upstream LTR and extends to the polyadenylation site in the U3 region of the downstream LTR. The *env* RNA results from splicing of the genomic RNA at the splice donor, six codons inside the *gag* gene to the splice acceptor just 5' of the *env* gene (22) (Fig. 1). In replication-competent ALV, the *gag* and *pol* proteins are translated

FIG. 2. Northern analysis of RNA from 9-day embryos. Total RNA (20 µg) from *alv6*, *alv11*, and line 0 whole 9-day embryos was fractionated on 1.5% agarose-formaldehyde gels and analyzed for ALV transcripts by hybridizing Northern transfers with 32 P-labeled *env* fragment (ENV) (Fig. 1). The filter was stripped and probed with 32 P-labeled cDNA for GAPDH. The positions of the expected *alv6* 7.1- and 2.4-kb viral transcripts and *alv11* 6.6- and 2.4-kb viral transcripts are indicated. The film shown in the panel labeled ENV was exposed for 3 days. The film shown in the panel labeled GAPDH was exposed for 10 h.

from genomic RNA, whereas the *env* glycoproteins are translated from spliced RNA (22). A 7.1-kb genomic RNA and a 2.4-kb *env* RNA were expected from the *alv6* locus. A 6.6-kb genomic RNA and a 2.4-kb *env* RNA were expected from the *alv11* locus, since a 0.5-kb deletion in *pol* (which encompasses a *Bam*HI site) affects only the genomic RNA.

Analysis of viral transcription. Total RNA (20 µg) was fractionated on agarose-formaldehyde gels and analyzed for ALV RNA transcripts by hybridizing Northern transfers with nick-translated 32 P-labeled *env* fragment (Fig. 1). Whole 9-day embryos were extracted for RNA (Fig. 2). A variety of tissues were dissected from 16-day embryos (Fig. 3), 1-day chicken (Fig. 4), and 28-day chickens (Fig. 5) and were extracted and analyzed separately. Northern transfers were reprobed with GAPDH to demonstrate the quantity and quality of the RNA.

All *alv6* and *alv11* tissues tested contained one or both of the expected viral transcripts. No viral transcripts were detected in any line 0 tissues. Intact GAPDH RNA was detected in all *alv6*, *alv11*, and line 0 tissues. The levels of the GAPDH message varied somewhat among tissues (Fig. 2 to 5) but were fairly consistent in a particular tissue type (e.g., muscle) among birds of a similar age (Fig. 4 and 5). *alv6* tissues contained predominantly *env* RNA with very low levels of genomic RNA in 9- and 16-day embryos (Fig. 3 and 4). In 1-day and 28-day chickens, the level of genomic RNA increased (Fig. 4 and 5). *alv11* tissues contained significantly higher levels of genomic RNA relative to *env* RNA at all four developmental stages (Fig. 2 to 5). *alv11* *env* RNA was barely detectable in some tissues (e.g., heart and liver; Fig. 3). Total viral RNA expression varied among the *alv6* and *alv11* tissues. Several tissues expressed comparatively high ALV RNA levels (e.g., thymus and spleen; Fig. 5).

Viral interference assay of *env* glycoproteins. Biologically

FIG. 3. Northern analysis of RNA from 16-day embryos. Total RNA (20 μ g) from *alv6*, *alv11*, and line 0 tissues was analyzed by Northern transfer. The filters were hybridized first with an *env* probe, stripped, and then probed with a GAPDH probe as described in the legend to Fig. 2. Tissues: H, heart; L, liver; Br, brain; M, muscle; G, gizzard. The positions of the expected 7.1-, 6.6-, and 2.4-kb viral transcripts are indicated. The film shown in the panel labeled ENV was exposed for 3 days. The film shown in the panel labeled GAPDH was exposed for 10 h.

active *env* glycoproteins were quantitated by a viral interference assay that measured resistance of *alv6*, *alv11*, and line 0 CEFs to RSV infection (6) (Table 1). *alv6* CEFs showed an average increase in resistance to BH-RSV (RAV-1) infection of 4,430-fold compared with that of line 0 controls. *alv11* CEFs showed an average resistance increase of 11-fold. The relative resistance varied somewhat among embryos in both *alv6* and *alv11* lines. Previously, *alv6*, *alv11*, and line 0 CEF were shown to be equally susceptible to subgroup B BH-RSV (RAV-2) infection (6).

In vivo virus challenge. To test whether the subgroup A

FIG. 4. Northern analysis of RNA from 1-day chicken. Total RNA (20 μ g) from *alv6*, *alv11*, and line 0 tissues was analyzed by Northern transfer. The filters were hybridized first with an *env* probe, stripped, and then probed with a GAPDH probe as described in the legend to Fig. 2. Tissues: H, heart; Bu, bursa; L, liver; Br, brain; M, muscle; G, gizzard. The film shown in the panel labeled ENV was exposed for 19 h. The film shown in the panel labeled GAPDH was exposed for 10 h.

env glycoproteins produced in *alv6* and *alv11* transgenic chickens interferes with a subsequent subgroup A ALV infection and pathogenesis, *alv6*, *alv11*, and line 0 chickens were inoculated with RAV-1 at hatch and raised intermingled. The presence of subgroup A ALV, antibody against subgroup A ALV, and ALV-induced pathogenesis were monitored at 4, 12, and 21 weeks postinjection (Table 2).

In susceptible chickens infected with RAV-1 at hatch, an initial viremia is followed by clearing of the virus as the level of antibody against RAV-1 increases. In the infected line 0 control chickens, only 2 of 59 chickens were viremic at 4 weeks, but 8 of 59 chickens already had high antibody levels. At 12 to 21 weeks, 51 of 52 infected line 0 chickens had high

FIG. 5. Northern analysis of RNA from 28-day chicken. Total RNA (20 μ g) from *alv6*, *alv11*, and line 0 tissues was analyzed by Northern transfer. The filter was hybridized first with an *env* probe, stripped, and then probed with a GAPDH probe as described in the legend to Fig. 2. Tissues: H, heart; T, thymus; L, liver; S, spleen; Br, brain; M, muscle; G, gizzard; Go, gonad; M*, muscle. The film shown in the panel labeled ENV was exposed for 19 h, except lane M*, which was exposed for 7 days. The film shown in the panel labeled GAPDH was exposed for 10 h.

antibody levels against RAV-1 with no viremia. No RAV-1 or antibody against RAV-1 was detected from 4 to 21 weeks in the infected *alv6* chickens. We believe that this high degree of resistance is due to the *alv6* subgroup A envelope glycoproteins interfering with RAV-1 infection. The effect of RAV-1 infection on *alv11* chickens was less clear. Virus was detected in 17 of 40 of the infected *alv11* chickens at 4 weeks and was cleared by 12 weeks. However, only 34 of 43 *alv11* chickens had antibody against RAV-1 at 12 weeks and only 27 of 43 had antibody at 21 weeks. Nine *alv11* chickens never produced antibody to RAV-1, and seven *alv11* chickens went from antibody positive at 12 weeks to antibody negative at 21

TABLE 1. Relative resistance of *alv6* and *alv11* CEF to subgroup A RSV infection

Embryo CEF sample	Titer ^a	Relative resistance ^b
<i>alv6</i>		
1	1.7×10^2	2,290
2	2.1×10^1	18,570
3	3.0×10^1	13,000
4	1.3×10^2	3,000
<i>alv11</i>		
1	4.3×10^4	9
2	4.1×10^4	10
3	5.0×10^4	8
4	8.9×10^3	44

^a Titters (focus-forming units per milliliter of virus stock) were estimated by averaging duplicate titrations. Average titers were as follows: 8.8×10^4 for *alv6* CEF, 3.6×10^4 for *alv11* CEF, and 3.9×10^5 for line 0 CEF (the average line 0 titer was determined from six embryos).

^b Ratio of titer of test embryo to line 0 titer average. The average relative resistances, determined by using the ratio of line 0 average titer to *alv6* and *alv11* average titers, were 4,430 and 11, respectively.

weeks. ALV-induced pathogenesis (lymphoid leukosis, non-neoplastic syndrome [5, 8], and bursal-thymus atrophy) was observed predominantly in infected line 0 chickens (10 of 59) through 21 weeks (Table 2). Little ALV-induced pathogenesis was observed in either infected *alv6* (1 of 62) and *alv11* (1 of 44) chickens.

DISCUSSION

The results reported here demonstrate that it is possible to achieve high-level expression from experimentally introduced endogenous proviruses in the avian system. The LTRs of two experimentally introduced defective proviruses, present in the transgenic chicken lines *alv6* and *alv11*, actively promote the transcription of viral RNA that accumulates in all tissues tested over four developmental stages (Fig. 2 through 5). Both *alv6* and *alv11* birds contain biologically active *env* glycoproteins (Table 1). No viral RNA transcripts or protein products were detected in any line 0 tissue.

Why are these results different from the results obtained in the murine system? The chicken *alv* and mouse *Mov* transgenic lines were generated by different methods of infection at a different stage of development. To create transgenic chickens, fertilized eggs were infected by injecting virus into the egg on the first day of incubation near the embryo (16, 17). At the time of injection, the eggs contained embryos of approximately 50,000 cells. To generate the *Mov* transgenic mouse lines, preimplantation mouse embryos (4 to 64 cells) were incubated in vitro in the presence of virus-producing cells and then reinserted into pseudopregnant foster mothers (12, 13). These differences may, in part, account for the differences in the proviral transcriptional activity seen in the two systems. Alternatively, it is possible that differences in either the hosts or the viruses were responsible for the observed differences. In any case, the ALV-chicken system can be used to obtain expression of transgenes experimentally introduced with retroviral vectors.

We do not know why the levels of genomic and *env* RNA vary within lines. For example, *alv6* embryonic stages (Fig. 2 and 3) have very little genomic RNA, whereas later in development the genomic RNA levels are more prominent (Fig. 4 and 5). Although no detectable defects were found in

TABLE 2. RAV-1 infection interference and oncogenicity in *alv6*, *alv11*, and line 0 chickens

Progeny	Viremia ^a at the following age (wk):			Antibody ^a at the following age (wk):			ALV-induced pathogenesis ^b
	4	12	21	4	12	21	
<i>alv6</i> × line 0	0/62	0/62	0/61	0/62	0/62	0/61	1/62
<i>alv11</i> × line 0	17/44	0/43	0/43	1/44	34/43	27/43 ^c	1/44
Line 0 × line 0	2/59	1/52	0/39	8/59	51/52	38/40 ^d	10/59

^a Ratio of positive to total observed.^b Ratio of chickens with lymphoid leukosis, non-neoplastic syndrome, and bursal-thymus atrophy to total chickens observed.^c Seven chickens were antibody positive at 12 weeks and then became antibody negative at 21 weeks.^d Two chickens were antibody positive at 12 weeks and then became antibody negative at 21 weeks. Twelve chickens died from nonspecific causes. Three chickens with ALV-induced neoplasms were alive at 21 weeks, accounting for the difference in the total number of birds.

the *alv6* provirus by restriction analysis, *alv6* birds do not produce either *gag* p27 or viral particles. We have not yet found a structural defect that can explain the low levels of *env* RNA in *alv11* tissues seen throughout development. It is possible that the *alv11* provirus has a small defect that affects either the splice junctions or the splicing efficiency.

Anomalies were seen in some of the Northern transfers. What appear to be additional RNA transcripts were seen in several tissues (Fig. 3 and 5). We believe that the additional bands were the result of hybridization artifacts due to the presence of 18S and 28S rRNAs in the samples, since these bands are located just above or below the rRNA positions. It remains possible, however, that additional transcripts can arise from these defective proviruses.

The relative levels of *env* RNA from *alv6* and *alv11* correlate with the relative levels of active *env* glycoprotein as measured by the increases in cellular resistance to RSV infection (Fig. 2, Table 1). The interference experiments employed CEFs from 10-day embryos and can be compared with the Northern analysis done on 9-day embryos shown in Fig. 2. *alv6* gives rise to high levels of *env* RNA and shows a correspondingly large (4,430-fold) increase in cellular resistance to RSV. The *alv6* resistance increase is consistent with the level reported by Crittenden et al. (6), suggesting that there is no major variation in the level of susceptibility from different generations of *alv6* transgenic birds. Since the preliminary in vitro interference assays on *alv11* CEF measured increases in resistance ranging from 20- to 2,000-fold relative to those in line 0 CEF (6), we repeated the experiment to verify the *alv11* *env* glycoprotein levels in this generation of transgenic birds. We found that *alv11* embryos contained barely detectable levels of *env* RNA (Fig. 2), and we found an approximately 11-fold increase in resistance to RSV infection (Table 1).

The viral proteins expressed by the *alv6* and *alv11* loci clearly increase the resistance of these chickens to subgroup A ALV-induced diseases (Table 2). The *alv6* resistance increase had been documented previously (6) and correlates well with the results reported in Table 2. However, *alv11* chickens had not been previously investigated. The majority of the *alv11* chickens were susceptible to RAV-1 infection, as shown by the production of antibody against ALV in 79% of *alv11* birds. This was in contrast to *alv6* chickens, which showed no evidence of RAV-1 infection. Yet neither *alv6* and *alv11* chickens showed significant ALV-induced pathogenesis through 21 weeks. A background level of ALV-like pathogenesis has been previously noted in both line 0 and *alv6* chickens and may account for the single neoplasm in an *alv6* chicken, since no evidence of ALV was detected.

We did not expect to see a high level of resistance to ALV-induced pathogenesis in the *alv11* line of chickens,

since the level of envelope RNA (Fig. 2 through 5) and glycoprotein (Table 1) was low and the majority of the birds were infected with the virus. The low level of ALV-induced pathogenesis in *alv11* chickens may reflect the relatively high levels of viral RNA (and presumably of viral protein) expressed in bursa (Fig. 3), the major target tissue for ALV pathogenesis. The level of envelope glycoprotein expressed specifically in the bursa may determine the magnitude of ALV interference in that organ and thereby prevent ALV-related neoplasia even in viremic birds.

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