Virology **272,** 106–111 (2000)
doi:10.1006/viro.2000.0352, available online at http://www.idealibrary.com on IDEAL®

Hypervariability in the Envelope Genes of Subgroup J Avian Leukosis Viruses Obtained from Different Farms in the United States

R. F. Silva,[1] A. M. Fadly, and H. D. Hunt

Avian Disease and Oncology Laboratory, Agricultural Research Service, U.S.D.A., East Lansing, Michigan 48823

Received January 17, 2000; returned to author for revision February 28, 2000; accepted April 13, 2000

Avian leukosis virus, subgroup J (ALV-J), has a wide host range, preferentially infecting meat-type birds, and produces a high incidence of myelocytomatosis and nephromas. Using the published sequences from HPRS-103 (ALV-J isolated in 1989 in Great Britain), we designed a set of PCR primers that amplified proviral DNA from nine U.S. field samples. The primers were specific for ALV-J, not amplifying DNA from uninfected cells or cells infected with ALV subgroups A–E. These primers expanded a 2.4-kb fragment that encompasses gp85, gp37, the E element, and most of the 3′ LTR. We also developed a set of PCR primers that amplified a 2.1-kb fragment from ALV-J-infected cells and a 1.6-kb fragment from uninfected ev− chicken embryo fibroblasts (Line 0). Upon cloning and DNA sequencing, we determined that the 2.1- and 1.6-kb fragments contained ALV-J gp85- and gp37-like sequences. Comparison of the amino acid sequences demonstrated that the Line 0 sequences were 97.5% identical with the gp85 and gp37 of HPRS-103 and somewhat less identical with the other nine U.S. isolates. This suggests that the envelope genes of ALV-J may have arisen as a result of a recombination event between exogenous ALV and Line 0-like sequences in the chicken. Phylogenetic analysis also showed that the U.S. field isolates were closely related to one another and more distantly related to the European HPRS-103. The pattern of mutations in the U.S. field isolates suggests that the U.S. strains are slowly drifting away from their progenitor Line 0-like sequences. The development of effective vaccines and diagnostic tests is likely to become more problematic as the viruses continue to mutate.

Key Words: retrovirus; poultry; glycoprotein; gp85; DNA sequence; E element; computer analysis.

INTRODUCTION

Avian leukosis viruses (ALVs) primarily cause lymphoid leukosis after a 14- to 25-week incubation period in domestic chickens. ALVs that occur in chickens have traditionally been divided into five subgroups (A, B, C, D, and E) (Payne and Fadly, 1997). Subgroup A ALVs were the most common field isolate. Subgroup B viruses are occasionally encountered in the field, while subgroups C and D are rarely seen. Subgroup E viruses include the ubiquitous endogenous retroviruses of low pathogenicity. Although ALV has not been totally eradicated from commercial flocks, poultry companies have significantly reduced ALV-induced lymphomas by blocking the vertical transmission of virus from dam to progeny by eliminating the dams that test positive for viral antigens (Payne and Fadly, 1997).

In 1989, a new ALV was isolated from meat-type chickens in the United Kingdom. Virus neutralization and interference assays indicated that this virus, designated HPRS-103, is unlike any known ALV and was therefore assigned to its own subgroup: subgroup J (Payne, 1992; Payne *et al.,* 1991a,b). In contrast to other known ALVs, HPRS-103 induced myelocytomatosis and nephromas preferentially in meat-type chickens. Until the isolation of HPRS-103, only the transforming viruses MC29 and CMII were known to cause myelocytomatosis in chickens (Payne, 1992). Upon DNA sequencing, the HPRS-103 provirus was found to be the only known naturally occurring ALV to contain an E element (Bai and Payne, 1995) (Fig. 1). Previously, E elements had been found only in sarcoma viruses (Schwartz *et al.,* 1983; Tsichlis *et al.,* 1982). The gp85 envelope of HPRS-103 is only 40% homologous with subgroups A through E. However, HPRS-103 gp85 is 75% homologous with ancient ALV-like sequences (or ancient endogenous sequences, EAVs) found in the chicken genome. The strong homology of the HPRS-103 env to EAV elements suggests that HPRS-103 arose through a recombination event between an exogenous ALV and these ancient elements (Bai and Payne, 1995; Benson *et al.,* 1998). The extreme variability among different subtypes of ALV-J in the United Kingdom suggests that this virus is mutating much faster than traditional ALVs (Venugopal *et al.,* 1998).

A similar pattern of late-onset myeloid tumors began occurring in meat-type chickens in 1993 in the United States. Based upon virus neutralization and host range, the isolates were identified as being subgroup J. The virus continued to spread, such that ALV-J-induced

[1] To whom correspondence and reprint requests should be addressed at Avian Disease and Oncology Laboratory, Agricultural Research Service, U.S.D.A., 3606 E. Mount Hope Road, E. Lansing, MI 48823. Fax: (517) 337-6776. E-mail: silvar@msu.edu.

0042-6822/00 $35.00

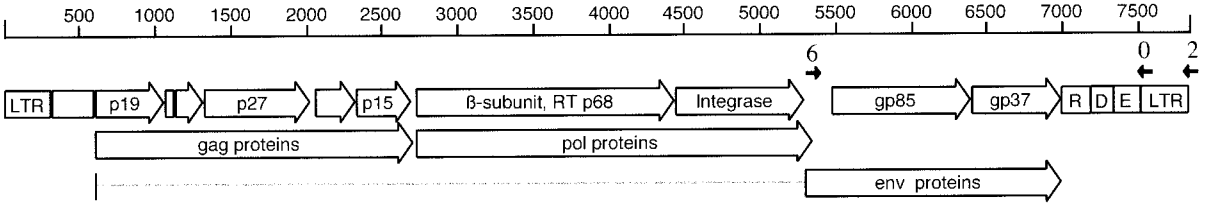


FIG. 1. Genomic organization of the HPRS-103 proviral genome (Bai and Payne, 1995). Boxes with single letters are as follows: R, redundant region; D, DR1; E, E element. The PCR primer binding sites are shown as arrows. Right arrow 6 denotes forward primer 6; 2 left arrow denotes reverse primer 2; 0 left arrow denotes reverse primer 0.

myeloid tumors have now appeared in all the major broiler breeder farms in the United States. Furthermore, recent ALV-J isolates from broiler breeders appear to be increasing in virulence. With latent periods as short as 4 weeks, ALV-J-induced myeloid leukemias are now causing problems in poultry companies in Africa, Europe, and South America. In the United States, at least seven broiler breeder companies are experiencing mortality and serious production problems due to ALV-J infections. The total loss in commercial broiler breeders is currently estimated by industry veterinarians to be 1.5% per week in excess of normal mortality and represents a major economic loss for the poultry industry.

Attempts to develop vaccines and diagnostic kits will be severely hampered if ALV-J field strains prove to be highly variable and demonstrate an unusually high mutation rate. Evidence that ALV-J can be highly variable has come from laboratory studies in Great Britain (Venugopal *et al.,* 1998). Unfortunately, it is not known how different ALV-J field isolates vary in their genetic makeup. Consequently, we initiated a study to evaluate genetic variations in the envelopes of U.S. ALV-J field isolates obtained from different farms at various times.

RESULTS

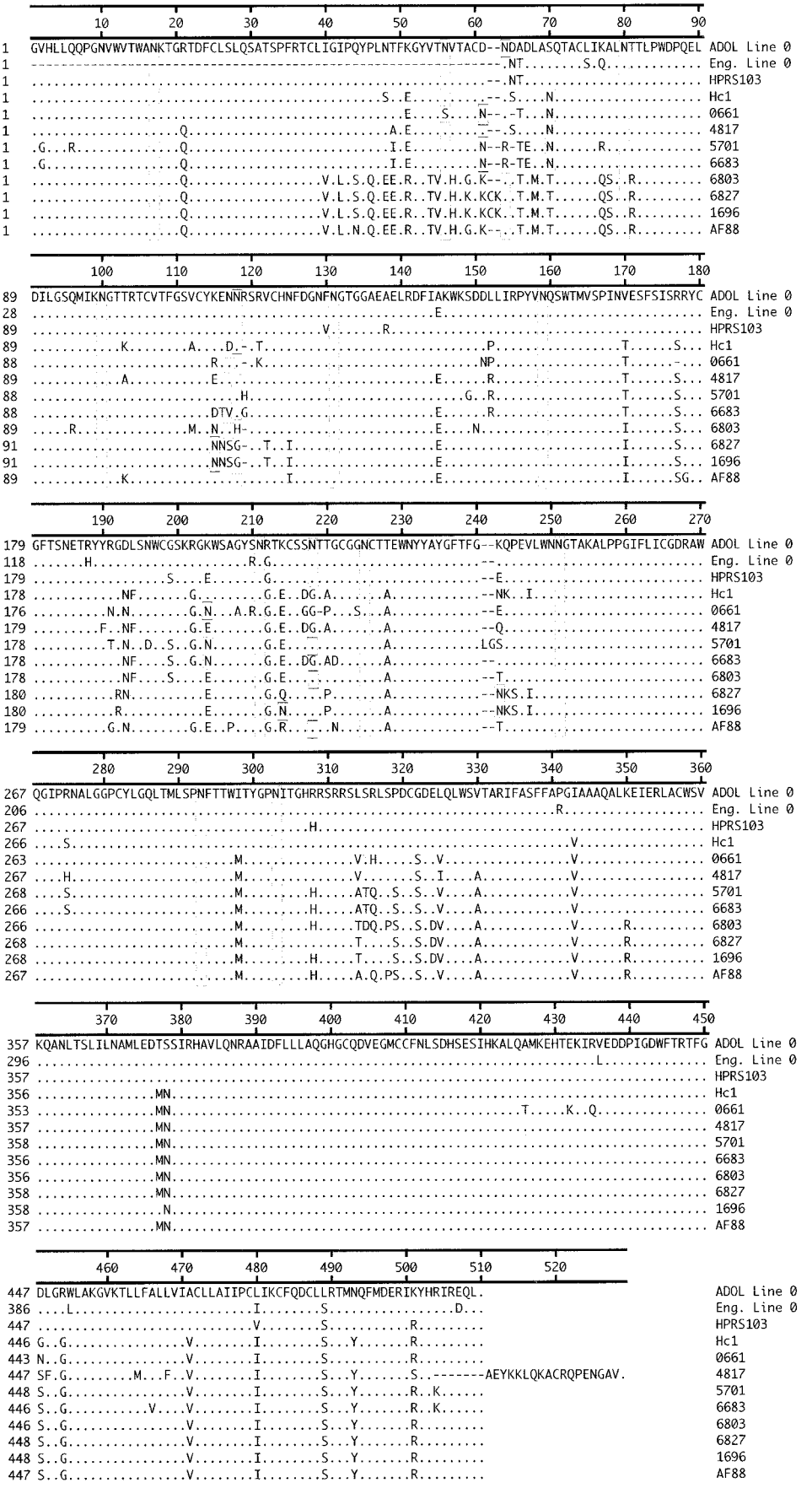
PCR amplification with the 6-2 primers amplified an approximately 2.4-kb DNA fragment from all the DNAs extracted from the ALV-J-infected chicken embryo fibroblasts (CEF). Nothing was amplified from DNA extracted from uninfected Line 0 CEF or CEF infected with Rous-associated virus (RAV)-1, RAV-2, RAV-49, RAV-50, or RAV-0 (data not shown). With the 6-0 primers, we PCR amplified a 2.1-kb DNA fragment from HPRS-103-infected CEF and a slightly smaller fragment from the other ALV-J-infected CEF. The 6-0 primers also amplified a 1.6-kb fragment from uninfected Line 0 DNA. The translated amino acid sequence from the Line 0 DNA was aligned with the translated amino acid sequences from the ALV-J field isolates (Fig. 2).

While the sequences displayed in Fig. 2 contain stretches of conserved amino acids, there are several hypervariable regions. All but one of the hypervariable regions are in gp85. The extreme amino end of gp37 (amino acids 313 to 325 in Fig. 2) contains the only hypervariable region of gp37. In general, potential N-glycosylation sites are conserved. This ability to conserve potential N-glycosylation sites is especially evident in the hypervariable region around amino acids 116–118. Despite numerous amino acid substitutions, each virus has retained a single potential N-glycosylation site. In other areas, strict conservation of potential N-glycosylation sites is not maintained. The number of potential N-glycosylation sites in gp85 varies from a low of 15 in HPRS-103, Hcl, 0661, 4817, and 1696 to a maximum of 18 sites in 5701. This variability is especially evident around amino acids 61–63, 204, 218, and 243. Whether these sites are actually glycosylated or not is not known.

The percentage of amino acid identity is shown in Fig. 3. The ADOL Line 0 env-like sequences are not identical to the Line 0 sequences isolated by the British group (98.8% identical). The noninbred nature of Line 0 chickens and the fact that the two flocks were established 15–20 years ago probably account for the 1.2% difference in sequences. Among the ALV-J strains, identities range from a maximum of 99% identity between 6827 and its progeny, 1696, to a low of 87.5% identity between 0661 and 1696. The relationship between the viruses is more clearly seen when the viruses are arranged in a phylogenetic tree (Fig. 4). Figure 4 also shows the U.S. isolates forming a separate group from the older HPRS-103 isolate from Great Britain.

DISCUSSION

Probably the most interesting observation derived from Figs. 3 and 4 is that the endogenous sequences in the ev- Line 0 cells are very similar to the gp85 and gp37 sequences in ALV-J. This close relationship, especially the 97.5% identity between HPRS-103 and Line 0, suggests that the envelope genes of ALV-J arose through a recombination with these endogenous sequences and an exogenous virus. The other known endogenous sequences in all chickens, designated EAV, are more distantly related to the ALV-J envelopes and are probably not the progenitors of our current ALV-Js (Boyce-Jacino *et al.,* 1992; Venugopal *et al.,* 1998). The U.S. field isolates appear to be related to one another and somewhat distantly related to the oldest, HPRS-103 isolate (Fig. 4).


10 20 30 40 50 60 70 80 90
1 GVHLLQQPGNVWVTWANKTGRTDFCLSLQSATSPFRTCLIGIPQYPLNTFKGYVTNVTACD--NDADLASQTACLIKALNTTLPWDPQEL ADOL Line 0
1 ----------NT.....S.Q..... Eng. Line 0
1 ..--.NT..... HPRS103
1 .....S..E.....--.S....N..... Hc1
1 .....E....S....N--.-T...N..... 0661
1 .....Q.....A.E.....--.S....N..... 4817
1 .G...R.....Q.....I.E.....N--R-TE..N.....R..... 5701
1 .G.....Q.....I.E.....N--R-TE..N..... 6683
1 .....Q.....V.L.S.Q.EE.R..TV.H.G.K--..T.M.T.....QS..R..... 6803
1 .....Q.....V.L.S.Q.EE.R..TV.H.K.KCK..T.M.T.....QS..R..... 6827
1 .....Q.....V.L.S.Q.EE.R..TV.H.K.KCK..T.M.T.....QS..R..... 1696
1 .....Q.....V.L.N.Q.EE.R..TV.H.G.K--..T.M.T.....QS..R..... AF88
100 110 120 130 140 150 160 170 180
89 DILGSQMIKNGTTRTCVTFGSVCYKENNRSRVCHNFDGNFNGTGGAEAELRDFIAKWKSDDLLIRPYVNQSWTMVSPINVESFSISRRYC ADOL Line 0
28 .....E..... Eng. Line 0
89 .....V.....R..... HPRS103
89 .....K.....A....D.-.T.....P.....T.....S... Hc1
88 .....R...-.K.....NP.....T.....-... 0661
89 .....A.....E.....E.....R.....T.....S... 4817
88 .....H.....G..R.....T.....S... 5701
88 .....DTV.G.....E.....R.....T.....S... 6683
89 .....R.....M..N..H-.....E....N.....I.....S... 6803
91 .....NNSG-..T..I.....E.....I.....S... 6827
91 .....NNSG-..T..I.....E.....I.....S... 1696
89 .....K.....I.....E.....I.....SG.. AF88
190 200 210 220 230 240 250 260 270
179 GFTSNETRYYRGDLSNWCGSKRGKWSAGYSNRTKCSSNTTGCGGNCTTEWNYYAYGFTFG--KQPEVLWNNGTAKALPPGIFLICGDRAW ADOL Line 0
118 .....H.....R.G.....-- Eng. Line 0
179 .....S....E.....G.....--E..... HPRS103
178 .....NF.....G..-.....G.E..DG.A.....A.....--NK..I..... Hc1
176 .....N.N.....G.N...A.R.G.E..GG-P...S...A.....--E..... 0661
179 .....F..NF.....G.E.....G.E..DG.A.....A.....--Q..... 4817
178 .....T.N..D..S..G.N.....G.E.....A.....LGS..... 5701
178 .....NF....S..G.N.....G.E..DG.AD.....A.....-- 6683
178 .....NF....S....E.....G.E.....A.....--T..... 6803
180 .....RN.....E.....G.Q.....P.....A.....--NKS.I..... 6827
180 .....R.....E.....G.N.....P.....A.....--NKS.I..... 1696
179 .....G.N.....G.E..P....G.R.....N.....A.....--T..... AF88
280 290 300 310 320 330 340 350 360
267 QGIPRNALGGPCYLGQLTMLSPNFTTWITYGPNITGHRRSRRSLSRLSPDCGDELQLWSVTARIFASFFAPGIAAAQALKEIERLACWSV ADOL Line 0
206 .....R..... Eng. Line 0
267 .....H..... HPRS103
266 ....S.....V..... Hc1
263 .....M.....V.H.....S..V.....V..... 0661
267 ....H.....M.....V.....S..I....A.....V..... 4817
268 ....S.....M.....H.....ATQ..S..S..V....A.....V..... 5701
266 ....S.....M.....H.....ATQ..S..S..V....A.....V..... 6683
266 .....M.....H.....TDQ.PS..S.DV....A.....V.....R..... 6803
268 .....M.....T....S..S.DV....A.....V.....R..... 6827
268 .....M.....H.....T....S..S.DV....A.....V.....R..... 1696
267 .....M.....H.....A.Q.PS..S..V....A.....V.....R..... AF88
370 380 390 400 410 420 430 440 450
357 KQANLTSLILNAMLEDTSSIRHAVLQNRAAIDFLLLAQQHGCQDVEGMCCFNLSDHSESIHKALQAMKEHTEKIRVEDDPIGDWFTRTFG ADOL Line 0
296 .....L..... Eng. Line 0
357 ..... HPRS103
356 .....MN..... Hc1
353 .....MN.....T.....K..Q..... 0661
357 .....MN..... 4817
358 .....MN..... 5701
356 .....MN..... 6683
356 .....MN..... 6803
358 .....MN..... 6827
358 .....N..... 1696
357 .....MN..... AF88
460 470 480 490 500 510 520
447 DLGRWLAKGVKTLLFALLVIACLLAIIPCLIKCFQDCLLRTMNQFMDERIKYHRIREQL. ADOL Line 0
386 ....L.....I.....S.....D... Eng. Line 0
447 .....V.....S.....R..... HPRS103
446 G..G.....V.....I.....S...Y.....R..... Hc1
443 N..G.....V.....I.....S.....R..... 0661
447 SF.G.....M...F..V.....I.....S...Y.....S..-------AEYKKLQKACRQPENGAV. 4817
448 S..G.....V.....I.....S...Y.....R..K..... 5701
446 S..G.....V....V.....I.....S...Y.....R..K..... 6683
446 S..G.....V.....I.....S...Y.....R..... 6803
448 S..G.....V.....I.....S...Y.....R..... 6827
448 S..G.....V.....I.....S...Y.....R..... 1696
447 S..G.....V.....I.....S...Y.....R..... AF88

Percent Identity

1	2	3	4	5	6	7	8	9	10	11	12		
	98.8	97.5	92.9	91.0	90.4	90.0	89.6	88.4	88.0	88.2	89.0	1	ADOL Line 0
		96.2	91.0	89.9	84.7	89.4	88.8	90.1	89.2	89.4	90.1	2	Eng. Line 0
			92.4	91.2	90.0	90.8	90.2	89.2	88.0	88.6	89.4	3	HPRS103
				92.9	93.3	91.6	92.7	89.2	89.4	88.8	89.4	4	Hc1
					91.2	91.8	92.0	88.4	88.0	87.5	88.8	5	0661
						91.4	92.9	89.0	88.2	87.6	89.4	6	4817
							96.1	90.6	88.6	88.4	91.0	7	5701
								90.8	89.0	88.8	90.6	8	6683
									95.1	94.9	95.9	9	6803
										99.0	94.3	10	6827
											94.1	11	1696
												12	AF88

FIG. 3. Amino acid percentage identity in gp85 and gp37.

Either the U.S. strains arose independently of HPRS-103 or HC1 has several generations of ancestors that we have not detected.

The U.S. isolates tend to be plotted on the phylogenetic tree in same order that they were first isolated. Hc1 is most closely related to the Line 0 sequences, and the 1997 isolates are grouped the furthest away from the Line 0 sequences. As expected the U.S. strains are also grouped together and are more closely related to one another if they were isolated from the same farm. Thus, 5701 and 6683 are closely related viruses, both isolated at different times from the same farm. Similarly, AF88LT, 6803, 6827, and 1696 all were isolated from the same farm at different times and are grouped together.

The phylogenetic pattern of the U.S. isolates suggests that the U.S. strains may be continuing to mutate. This trend of ongoing mutation is particularly evident when the percentage of divergence from the ADOL Line 0 sequences are plotted versus the date of isolation (Fig. 5). There are several possible implications of continuing ALV-J mutations. Additional recombinations with EAVs or other sequences related to the ADOL Line 0 sequences could result in the emergence of new strains with new host ranges and pathogenicity. Slower, more gradual mutations could also have profound effects. Antigenic epitopes can be altered, confounding ELISA-based diagnostics, and may also result in reducing the likelihood of developing effective vaccines. It is not known whether ALV-Js emerging in 1998 and 1999 continued this trend. Unfortunately, no 1998 or 1999 samples are available.

Although the gp37 sequences are fairly well conserved, there is one anomaly in gp37 of 4817. A deletion has occurred in the 3′ end of the 4817 coding region such that the normal termination codon has been lost. Consequently, the ORF for the 4817 gp37 continues until the next stop codon is encountered. Interestingly, the deletion in 4817 is actually 229 bp long, completely deleting the redundant region (region R in Fig. 1). The stop codon for the 4817 gp37 is not encountered until just before the DR1 sequences (D box in Fig. 1). Despite major deletions and additions in the carboxy end of gp37, the virus is still infectious and presumably gp37 is still functional. The conformation of the nearby transmembrane region of gp37 (approximately amino acids 460–485) is therefore not adversely affected.

PCR amplification with the 6-2 primers is highly specific, amplifying DNA from every ALV-J sample we tested, yet not amplifying DNA from other avian retrovirus subgroups. These primers may be useful as part of an ALV-J screening program. However, one caution with using these primers is that the amplified product is approximately 2.4 kb. We have found that with long PCR amplicons the concentration of the template DNA becomes critical. Thus the 6-2 primers may not be optimal for detecting low levels of ALV-J in blood samples. In addition, the highly variable nature of ALV-Js does not preclude the possibility of encountering a variant subtype that the 6-2 primers will not detect.

MATERIALS AND METHODS

Virus strains and cell culture

ALV-J strain HPRS-103 was obtained from Dr. L. N. Payne, Institute of Animal Health, Compton, United Kingdom. Blood samples obtained from various U.S. broiler breeder flocks experiencing myeloid leukosis mortality were initially passed on CEF cells from alv6 embryos (very resistant to infection with ALV subgroups A and E) (Crittenden and Salter, 1992). ALVs growing on the alv6 cells were subjected to further analysis. Table 1 lists the

FIG. 2. Amino acid alignment of the gp85 and gp37 polyprotein from ALV-J field isolates. ADOL Line 0 represents the sequence amplified from Line 0 DNA with the 6-0 primers. Eng. Line 0 is the sequence obtained from Line 0 by researchers in Great Britain (Smith *et al.,* 1999). Dots indicate amino acids that are identical with the Line 0 amino acids. Dashes indicate gaps that were introduced to improve the alignment. Potential N-glycosylation sites in gp85 are boxed. The serine at position 312 is the beginning of gp37.

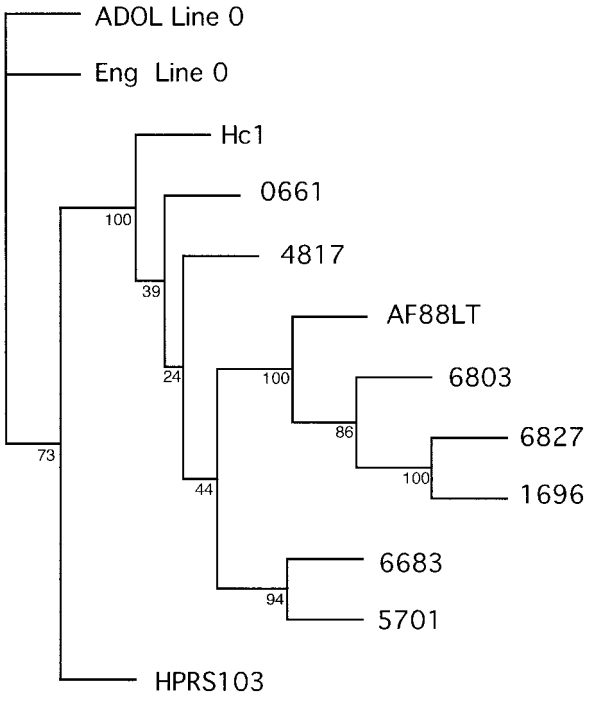


FIG. 4. Phylogeny of endogenous Line 0 sequences and ALV-J viruses. This is a rooted tree using the ADOL Line 0 as the outgroup. The numbers at the branch points represent the result of bootstrap analysis of 100 samples and indicate how many times the group that consists of the viruses to the right of (descended from) the fork occurred.

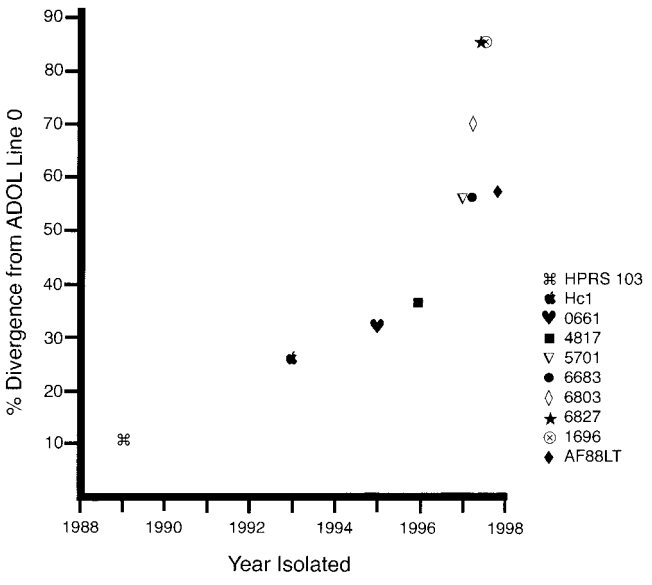


FIG. 5. Plot of percentage divergence from ADOL Line 0 vs year of isolation. Percentage divergence was calculated from the bootstrapping numbers in Fig. 4 as follows: Total bootstrapping numbers = 660 and

$$\% \text{ Divergence} = \frac{100 \times \text{Total bootstrapping numbers for a particular virus}}{\text{Total bootstrapping numbers}}.$$

different ALV-J isolates used in this study. ALV subgroups A, B, C, D, and E were obtained from CEFs infected with RAV-1, RAV-2, RAV-49, RAV-50, and RAV-0, respectively.

Line 0 chickens are a noninbred line of endogenous virus-negative White Leghorn chickens developed by back-crossing F1 chickens (Line 7_2 × SPAFAS Line 11 cross) with SPAFAS Line 11 (Astrin *et al.,* 1979). Around 1980, Line 0 chickens were introduced and maintained at the Avian Disease and Oncology Laboratory (ADOL Line 0). Subsequently, a few of the ADOL Line 0 birds were shipped to England, where they were used to establish a second Line 0 flock (Eng. Line 0).

PCR amplification

The oligonucleotide primers were synthesized by Operon Technologies (Alameda, CA). The ALV-J-specific primers include the forward primer "6" (5′-CTT GCT GCC ATC GAG AGG TTA CT-3′), annealing just upstream from the protein coding region of gp85, and the reverse primer "2" (5′-AGT TGT CAG GGA ATC GAC-3′), annealing to sequences in the 3′ LTR (Fig. 1). ALV-J-like sequences were PCR amplified from Line 0 CEF by using forward primer 6 and reverse primer "0" (5′-ACA CTA CAT TTC CCC CTC CCT AT-3′) (Fig. 1).

All the PCRs were in 50-μl volumes and contained 20 mM Tris–HCl (pH 8.8), 2 mM $MgSO_4$, 10 mM KCl, 10 mM $(NH_4)_2SO_4$, 0.1% Triton X-100, and 0.1 mg/ml nuclease-free BSA, 2.5 units *Pfu* polymerase (Stratagene, La Jolla, CA), a 200 μM concentration of each deoxynucleoside triphosphate, 25 pmol of each primer, and 50–200 ng proviral DNA. Following an initial template melting step at 95°C for 3 min, the DNA was amplified during 30 cycles of 95°C for 1 min, 57°C for 1 min, and 72°C for 2 min. A final elongation step at 72°C for 10 min completed the PCR.

TABLE 1

ALV-J Field Isolates

Strain	Year isolated	Farm	Comments
HPRS-103[a]	1989	Great Britain	Earliest known ALV-J isolate
Hc1	1993	A[b]	Earliest known U.S. isolate
0661	1995	B	
4817	1996	C	
5701	1997	D	
6683	1997	D	
6803	1997	E	
6827	1997	E	
1696	1997	E	Derived from 6827
AF88LT	1997	E	

[a] ALV-J strains are listed in the order in which they were isolated.
[b] Viruses isolated from the same farms are shown with the same letter.

Three independent PCR amplifications were performed on each ALV-J isolate. DNA from each amplification was independently cloned into a pCR-Blunt vector (Invitrogen, Carlsbad, CA).

DNA sequencing and analysis

Both strands from two of the three clones from each field isolate were DNA sequenced on an ABI Model 373A automatic DNA sequencer (Applied Biosystems, Foster City, CA) and contigs were constructed using Sequencer (Gene Codes Corp., Ann Arbor, MI). If the two sequences were not identical, then the region of discrepancy in the third clone was sequenced and compared to the other two clones. The "correct sequence" was the sequence that matched two of the three sequences. Gene Construction Kit 2 (Texaco, Inc., West Lebanon, NH) was used for restriction endonuclease analysis, ORF identification, and protein translation. We identified the gp85 and gp37 sequences by comparing our sequences with the published HPRS-103 sequences (Bai and Payne, 1995). Protein sequences were aligned using the Clustal V method (Higgins and Sharp, 1989), as implemented in MegAlign (DNASTAR Inc., Madison, WI). Phylogenetic relatedness of the isolates was calculated using bootstrap analysis with the maximum-parsimony method, as utilized in PHYLIP (Phylogeny Inference Package, version 3.57c, 1995, distributed by J. Felsenstein, Department of Genetics, University of Washington, Seattle).

GenBank

The nucleotide sequence data reported in this paper were submitted to GenBank and the following accession numbers were assigned: ADOL Line 0, AF247392; Hcl, AF247391; 0661, AF247566; 4817, AF247385; 5701, AF247386; 6683, AF247387; 6803, AF247388; 6827, AF247389; 1696, AF247384; and AF88, AF247390.

ACKNOWLEDGMENTS

We thank Lonnie Milam and Melanie Flesberg for their excellent technical assistance.

REFERENCES

Astrin, S. M., Buss, E. G., and Haywards, W. S. (1979). Endogenous viral genes are non-essential in the chicken. *Nature* **282**(5736), 339–341.

Bai, J., and Payne, L. N. (1995). HPRS-103 (exogenous avian leukosis virus, subgroup J) has an env gene related to those of endogenous elements EAV-0 and E51 and an E element found previously only in sarcoma viruses. *J. Virol.* **69,** 779–784.

Benson, S. J., Ruis, B. L., Fadly, A. M., and Conklin, K. F. (1998). The unique envelope gene of the subgroup J avian leukosis virus derives from ev/J proviruses, a novel family of avian endogenous viruses. *J. Virol.* **72**(12), 10157–10164.

Boyce-Jacino, M. T., Odonoghue, K., and Faras, A. J. (1992). Multiple complex families of endogenous retroviruses are highly conserved in the genus *Gallus*. *J. Virol.* **66,** 4919–4929.

Crittenden, L. B., and Salter, D. W. (1992). A transgene, alv6, that expresses the envelope of subgroup A avian leukosis virus reduces the rate of congenital transmission of a field strain of avian leukosis virus. *Poultltry Sci.* **71**(5), 799–806.

Higgins, D. G., and Sharp, P. M. (1989). Fast and sensitive multiple sequence alignments on a microcomputer. *CABIOS* **5,** 151–153.

Payne, L. N. (1992). Biology of avian retroviruses. *In* "The *Retroviridae*" (J. A. Levy, Ed.), pp. 299–376. Plenum, New York.

Payne, L. N., Brown, S. R., Bumstead, N., Howes, K., Frazier, J. A., and Thouless, M. E. (1991a). A novel subgroup of exogenous avian leukosis virus in chickens. *J. Gen. Virol.* **72,** 801–807.

Payne, L. N., and Fadly, A. M. (1997). Leukosis/sarcoma group. *In* "Diseases of Poultry" (B. W. Calnek, H. J. Barnes, C. W. Beard, L. R. McDougald, and Y. M. Saif, Eds.), pp. 414–466. Iowa State Univ. Press, Ames.

Payne, L. N., Gillespie, A. M., and Howes, K. (1991b). Induction of myeloid leukosis and other tumours with the HPRS-103 strain of ALV. *Vet. Rec.* **129,** 447–448.

Schwartz, D. E., Tizard, R., and Gilbert, W. (1983). Nucleotide sequence of Rous sarcoma virus. *Cell* **32,** 853–869.

Smith, L. M., Toye, A. A., Howes, K., Bumstead, N., Payne, L. N., and Venugopal, K. (1999). Novel endogenous retroviral sequences in the chicken genome closely related to HPRS-103 (subgroup J) avian leukosis virus. *J. Gen. Virol.* **80**(Pt 1), 261–268.

Tsichlis, P. N., Donehower, L., Hager, G., Zeller, N., Malavarca, R., Astrin, S., and Skalka, A. M. (1982). Sequence comparison in the crossover region of an oncogenic avian retrovirus recombinant and its nononcogenic parent: genetic regions that control growth rate and oncogenic potential. *Mol. Cell. Biol.* **2,** 1331–1338.

Venugopal, K., Smith, L. M., Howes, K., and Payne, L. N. (1998). Antigenic variants of J subgroup avian leukosis virus: Sequence analysis reveals multiple changes in the env gene. *J. Gen. Virol.* **79**(Pt 4), 757–766.