J. Vet. Sci. (2002), 3(2), 71-74

JOURNAL OF
Veterinary
Science

Isolation of Subgroup J Avian Leukosis Virus in Korea

Haan-Woo Sung*, Jae-Hong Kim, Sanjay Reddy1 and Aly Fadly1

National Veterinary Research and Quarantine Service, Ministry of Agriculture & Forestry, 480 Anyang 6-Dong, Anyang 430-016, Korea

1U.S. Department of Agriculture, Agricultural Research Service, Avian Disease and Oncology Laboratory, 3606 East Mount Hope Road, East Lansing, MI 48823

Received Feb. 1, 2002 / Accepted Apr. 25, 2002

ABSTRACT

Two subgroup J avian leukosis viurses (ALVs) were isolated from broiler breeder flocks, in which myeloid leukosis had occurred. The isolates could be classified as subgroup J ALV by the positive reaction in polymerase chain reaction (PCR) with primers specific for subgroup J ALV. Two isolates replicated in chicken embryo fibroblast (CEF) cells from the alv6 chicken line in which cells are resistant to subgroup A and E ALVs. In *in vitro* serum neutralization tests with other subgroup ALVs including ADOL-Hc1, the prototype of subgroup J ALVs isolated in the United States of America, two isolates were partially neutralized by antibody to ADOL-Hc1, indicating that Korean isolates and ADOL-Hc1 may be antigenically related, but not identical. When the PCR was done with a primer pair designed to amplify genes of E element and long terminal repeat of proviral DNA, the PCR product size of one isolate (KOAL-PET) was smaller than that of ADOL-Hc1, suggesting that some sequences in these regions are deleted.

Key words : subgroup J avian leukosis virus, myeloid leukosis, virus isolation

Introduction

Avian leukosis is a neoplastic disease caused by avian leukosis viruses (ALVs). ALVs from chickens have been divided into six subgroups (A, B, C, D, E and J) on the basis of their host range, viral envelope interference and cross-neutralization patterns (10). Subgroups A, B, C, and D are exogenous viruses capable of inducing tumors, and subgroup E viruses are endogenous viruses of low pathogenicity. Subgroup J viruses, which have been isolated recently from broiler chickens, are recombinant between exogenous and endogenous viruses (2,3,4,17). Although ALVs can induce various types of tumors, lymphoid leukosis is the most common in chickens. However, subgroup J viruses, first reported in the United Kingdom in 1991, induce primarily myeloid leukosis (11,12). Subgroup J viruses have a broad host range and all lines of chickens tested are susceptible to infection (14). The nucleotide sequence of subgroup J viruses showed multiple changes in *env* gene among the isolates and antigenic variants of the viruses were found in subgroup J viruses (18).

The isolation of subgroup J virus was not reported in Korea. Myeloid leukosis has increased recently in broiler breeder flocks in Korea. This study describes the isolation of subgroup J ALVs from broiler chickens in Korea.

Materials and Methods

Viruses and antibodies.

The prototypes of ALV subgroup A, E, and J were Rous-associated virus (RAV)-1, RAV-0, and ADOL-Hc1, respectively, and were maintained at Avian Disease and Oncology Laboratory (ADOL) in USA. The polyclonal antibodies against ALV were also from ADOL.

Cells for virus isolation.

Cells for virus isolation or virologic assays were C/E (resistant to infection with endogenous ALV) line 0 CEF, C/AE (resistant to infection with subgroup A and E ALV) alv6 CEF and C/O (susceptible to all ALVs) 15B1 CEF (6,7). The presence of ALV in infected cells was confirmed by enzyme-linked immunosorbent assay as reported (15).

Serum neutralization test.

Antigenic relationship of isolates to other subgroups of ALV was determined by cross-neutralization test as described (9). Briefly, one fifth diluted sera were mixed with each viruses which titers were adjusted to 500-1000 infectious units/ml and incubated at 37℃ for 45 minutes. The neutralized samples were then inoculated on line 0 CEF cells and incubated for 7 days. The group-specific antigen was detected from the cell lysates by ELISA.

*Corresponding author:
Tel: +82-31-467-1810, Fax: +82-31-467-1814
E-mail: sunghw@nvrqs.go.kr

Table 1. Korean ALV isolates from meat type chickens.

Isolate	Age (weeks)	Type of flocks	Organs virus isolated	Year
KOAL-PET	50	grandparent stocks	Liver	1997
KOAL-HD	25	parent stocks	Liver	1998

Table 2. ALV group-specific antigen positive rate of flocks which ALVs were isolated.

Flocks	Weeks	Samples	Positive rates (%)
PET1	50	Serum	4/24 (16.7)
		Egg albumen	5/30 (16.7)
HD2	25	Serum	25/74 (33.8)

1 KOAL-PET was isolated from this flock
2 KOAL-HD was isolated from this flock

Polymerase chain reaction (PCR).

PCR was done for the detection of proviral DNA from cells infected with isolates. DNA was prepared with the Qiagen DNA isolation kit (Qiagen Inc., USA) and about 10 0 150ng of genomic DNA was used for PCR of 25ul volume. Primer pair S1/S2 (S1; 5'-AATTCTGCTTGAAATATG-3', S2; 5'-AGTTGTCAGGGAATCGAC-3') was derived from the unusual E element and the long terminal repeat of proviral ALV, and PCR was specific for subgroup J ALV or some Rous sarcoma viruses as described (16). The cycling conditions of PCR were denaturation at 94℃ for 4 min, followed by 30 cycles at 92℃ for 1min, 48℃ for 1min, and 72℃ for 1min. A final extension was conducted at 72℃ for 5min. PCR products were resolved in 1.5% agarose gel with electrophoresis in tris-acetate-EDTA buffer for 30 min at 95 volts.

ALV antigen test from flocks.

Samples of plasma or egg albumen from flocks in which ALVs were isolated, were tested for ALV group-specific antigen by ELISA with ELISA kit (KPL, USA).

Results

Virus isolation.

Two ALVs, KOAL-PET and KOAL-HD, were isolated from broiler breeder flocks. One of the viruses was isolated from grandparent, and the other was from parent stocks (Table 1). These two viruses were isolated from liver tumors of myeloid leukosis, previously confirmed by microscopic examinations. The group-specific antigen for ALVs was tested by ELISA from the samples of sera or egg albumen of flocks in which ALVs were isolated. Two flocks tested were positive for ALV antigen and positive rates in the sera were high, ranging from 16.7 to 33.8% (Table 2).

Virus replication in CEF of different genetic types.

ALV isolates were inoculated on CEF cells of different genetic type to examine the interactions between virus-specific cell receptor and virus envelope glycoproteins. Two isolates replicated in line 0 CEF cells, indicating that Korean isolates were not subgroup E ALVs. Two isolates also grew on alv6 CEF cells, which are resistant to subgroup A and E ALVs, suggesting that those viruses were neither endogenous nor subgroup A ALVs (Table 3).

Serum neutralization test.

Table 4 is the results of the *in vitro* serum neutralization tests with two isolates, ADOL-Hc1 and RAV-1. Two isolates were not neutralized by antibodies to subgroup A and E,

Table 3. Replication of ALVs on different chicken embryo fibroblast (CEF) cells.

Virus	CEF1 of		
	15B13	Line 04	alv65
KOAL-PET	1.428 (+)2	1.574 (+)	1.543 (+)
KOAL-HD	1.504 (+)	1.508 (+)	1.490 (+)
ADOL-Hc1	1.248 (+)	1.694 (+)	1.594 (+)
RAV-1	1.464 (+)	1.565 (+)	0.054 (-)
RAV-0	0.397 (+)	0.028 (-)	0.045 (-)
Uninfected cell	0.028 (-)	0.048 (-)	0.014 (-)

1Viruses were simultaneously inoculated on different cells, and 7 days later, cell lysates were tested for the presence of ALV group-specific antigen by ELISA.
2Absorbances (+; virus replication positive, -; virus replication negative).
315B1; C/O CEF (susceptible to all ALVs).
4Line 0 CEF; C/E CEF (resistant to infection with endogenous ALV).
5alv6; C/AE CEF (resistant to infection with subgroup A and E ALV).

Table 4. Neutralization test with subgroup-specific antiserum.

Virus	Antiserum against1			Negative serum	Virus control	Cell control
	A	E	ADOL-Hc1			
KOAL-PET	1.0942	0.742	0.156	0.558	0.687	0.008
KOAL-HD	1.194	0.886	0.368	0.859	0.955	0.007
ADOL-Hc1	0.721	0.776	0.023	0.837	0.813	0.013
RAV-1	0.010	0.779	0.422	0.446	0.673	0.036

1Virus neutralization was done as described (9).
2Absorbance.

however, these isolates were partially neutralized by antibody to ADOL-Hc1, indicating that Korean isolates and ADOL-Hc1 may be antigenically related, but not identical.

Polymerase chain reaction.

Fig. 1 shows the PCR result of two isolates with primers specific for subgroup J ALVs. The isolates were positive in PCR, however, the PCR product size of one isolate(KOAL-PET) was was smaller than that of ADOL-Hc1.

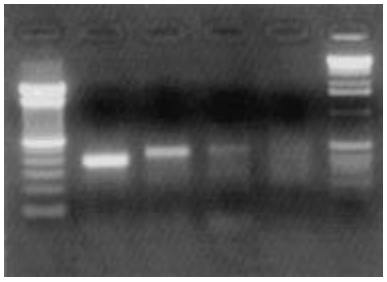

Fig. 1. PCR reaction of Korean isolates with subgroup J specific primer pair S1/S2.
Lane 1, MW marker of 100bp multiples(Gibco BRL); lane 2, KOAL-PET; lane 3, KOAL-HD; lane 4, ADOL-Hc1; lane 5, uninfected line 0 CEF; lane 6, MW marker of 1kb(Gibco BRL)

Discussion

This paper is the first report for the isolation of subgroup J ALVs in Korea. The isolates were classified as subgroup J by the positive reaction in PCR with primers specific for subgroup J ALV.

Two Korean ALVs, KOAL-PET and KOAL-HD, were isolated from flocks in which mortality due to myeloid leukosis was observed. In addition to liver, kidney and spleen, the lesions of myeloid leukosis were sometimes found in the ribs of affected chickens and were clinically similar to those described in chickens experimentally inoculated with subgroup J ALVs (1,12). HPRS-103, the prototype of subgroup J ALV, has tropism for the cells of the myeloid rather than the lymphoid lineage and induces primarily myeloid leukosis (1,12).

The group-specific antigens for ALVs were positive from samples of two flocks where ALVs were isolated. Some sera of chickens which have endogenous ALVs could be false positive when group-specific antigen was tested by ELISA (5,13). The positive reaction against group-specific antigen from sera in this study, however, was unlikely due to endogenous ALVs, because positive rates in sera was usually high and some egg samples tested were also positive. Tolerant infected chickens, a state in which chickens develop no neutralizing antibodies, are capable of shedding ALV in the eggs and vertical transmission is possible. Egg samples from a grandparent flock tested in this study were positive, indicating that the positive flocks might be an role on the spreading of subgroup J ALV to the next generation, parent flocks of meat type chickens in Korea.

Two Korean isolates were not neutralized by antibodies to subgroup A and E, indicating that these isolates may be antigenically distinct from subgroup A and E. Two isolates, however, were partially neutralized by antibody to ADOL-Hc1, suggesting that Korean isolates and ADOL-Hc1 may be antigenically related, but not identical. Subgroup J ALV has many antigenic variants (18). Venugopal et al (18) reported that ten of twelve isolates were not neutralized by antibodies to any of ALV subgroups including J, and only two isolates were neutralized with a specific serum of HPRS-103, the prototype of subgroup J ALV. ADOL-Hc1, the prototype of American isolates, neutralized HPRS-103 virus, whereas antibody to HPRS-103 did not neutralize ADOL-Hc1 (8).

Two isolates were positive by PCR with primer pair S1/S2, specific for subgroup J ALVs, suggesting that the Korean isolates are subgroup J ALVs. It was interesting that the PCR product size of a Korean isolate was somewhat different from ADOL-Hc1. The PCR product size of one isolate, KOAL-PET, was smaller than that of ADOL-Hc1. The primer sequences were derived from the beginning of the E element and the end of the long terminal repeat

(LTR). The smaller size of the PCR product, compared with ADOL-Hc1, suggests that some sequences of this isolate in E element or LTR regions may be deleted. In order to characterize which part of gene sequences of Korean isolates is deleted, further study will be needed.

Acknowledgments

This work was partially supported by the Korea Science and Engineering Foundation.

References

1. **Arshad, S. S., K. Howes, G. S. Barron, L. M. Smith, P. H. Russel, and L. N. Payne.** Tissue tropism of the HPRS-103 strain of J subgroup avian leukosis virus and of a derivative acutely transforming virus. Vet. Pathol. 1997, 34, 127-137.
2. **Bai, J., K. Howes, L. N. Payne, and M. A. Skinner.** Sequence of host range determinants in the env gene of a full-length, infectious virus proviral clone of exogenous avian leukosis virus HPRS-103 confirms that it represents a new subgroup (designated J). J. Gen. Virol. 1995, 76, 181-187.
3. **Bai, J., L. N. Payne, and M. A. Skinner.** HPRS-103 (exogenous avian leukosi 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. 1995, 69, 779-784.
4. **Benson, S. J., B. L. Ruis, A. M. Fadly, and K. F. Conklin.** 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. 1998, 72, 10157-10164.
5. **Crittenden, L. B., and E. J. Smith.** A comparison of test materials for differentiating avian leukosis virus group-specific antigens of exogenous and endogenous origin. Avian Dis. 1984, 28, 1057-1070.
6. **Crittenden, L. B., and D. W. Salter.** A transgene, alv6 that expresses the envelope of subgroup A avain leukosis virus reduces the rate of congenital transmission of a field strain of avian leukosis virus. Poult. Sci. 1992, 71, 799-806.
7. **Crittenden, L. B., S. McMahon, M. S. Halpern, and A. M. Fadly.** Embryonic infection with the endogenous avian leukosis virus Rous-associated virus-0 alters response to exogenous avian leukosis virus infection. J. Virol. 1987, 61, 722-725.
8. **Fadly, A. M., and E. J. Smith.** Isolation and some characteristics of a subgroup J-like avian leukosis virus associated with myeloid leukosis in meat-type chickens in the United States. Avian Dis. 1999, 43, 391-400.
9. **Fadly, A. M., and R. L. Witter.** Oncornaviruses: leukosis/sarcoma and reticuloendotheliosis. In: A laboratory manual for the isolation and identification of avain pathogens, 4th ed. J. R. Glisson, D. J. Jackwood, J. E. Pearson, W. M. Reed, and D. E. Swayne, eds. American Association of Avian Pathologists, Kennett Square, PA. pp. 185-196. 1998.
10. **Payne, L. N., and A. M. Fadly.** Leukosis/sarcoma group. In : Calnek, B. W., Barnes, H. J., Beard, C. W., McDougald L. R. and Saif Y. M. (ed.) Disease of Poultry, 10th ed. Iowa State University Press, Ames, IA. pp. 414-466. 1997.
11. **Payne, L. N., A. M. Gillespie, and K. Howes.** Induction of myeloid leukosis and other tumors with the HPRS-103 strain of ALV. Vet. Rec. 1991, 129, 447-448.
12. **Payne, L. N., A. M. Gillespie, and K. Howes.** Myeloid leukemia and transmission of the HPRS-103 strain of avian leukosis virus. Leukemia. 1992, 6, 1167-1176.
13. **Payne, L. N., A. M. Gillespie, and K. Howes.** Unsuitability of chicken sera for detection of exogenous ALV by the group-specific antigen ELISA. Vet. Rec. 1993, 132, 555-557.
14. **Payne, L. N., K. Howes, A. M. Gillespie.** Host range of Rous sarcoma virus pseudotype RSV (HPRS-103) in 12 avian species: support for a new retriovirus envelope subgroup, designated J. J. Gen. Virol. 1992, 73, 2995-2997.
15. **Smith, E. J., A. M. Fadly, and W. O. Okazaki.** An enzyme-labeled immunosorbent assay for detecting avian leukosis-sarcoma viruses. Avian Dis. 1979, 23, 698-707.
16. **Smith, E. J., S. M. Williams, and A. M. Fadly.** Detection of avian leukosis virus subgroup J using the polymerase chain reaction. Avian Dis. 1998, 42, 375-380.
17. **Smith, L. M., A. A. Toye, K. Howes, N. Bumstead, L. N. Payne, and K. Venugopal.** Novel endogenous retroviral sequences in the chicken genome closely related to HPRS-103 (subgroup J) avian leukosis virus. J. Gen. Virol. 1999, 80, 261-268.
18. **Venugopal, K., L. M. Smith, K. Howes and L. N. Payne.** Antigenic variants of J subgroup avian leukosis virus: sequence analysis reveals multiple changes in the env gene. J. Gen. Virol. 1998, 79, 757-766.