A novel subgroup of exogenous avian leukosis virus in chickens

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An avian leukosis virus with a wide host range belonging to a new subgroup for chickens was isolated from meat-type chicken lines. The virus, of which HPRS-103 strain is the prototype, was of low oncogenicity in chickens but appeared to behave like an exogenous leukemia virus. Neutralizing antibodies to the virus were found in three of five meat-type chicken lines, but not in seven layer lines. The virus and its Rous sarcoma virus pseudotype did not replicate in, or transform, mammalian cells.

Introduction

Avian leukosis viruses (ALVs) isolated from chickens fall into five subgroups, A, B, C, D and E, on the basis of viral envelope properties (Vogt, 1977; Weiss et al., 1982). Viruses of subgroups A and B occur as common pathogenic exogenous viruses in the field (Calnek, 1968; Okazaki et al., 1982), those of subgroups C and D have been reported rarely in the field (Morgan, 1973; Sandelin & Estola, 1974), and subgroup E viruses include the ubiquitous endogenous leukemia viruses of low pathogenicity (Smith, 1987).

Endogenous ALVs of other subgroups have been reported in other avian species. Viruses of the F subgroup have been isolated from ring-necked pheasant and those of the G subgroup from golden pheasant and Lady Amherst pheasant (Hanafusa & Hanafusa, 1973; Fujita et al., 1974; Hanafusa et al., 1976). However, subgroup G viruses apparently belong to a virus group that differs from the viruses which infect chickens (Hanafusa et al., 1976). Endogenous virus of subgroup H has been isolated from Hungarian partridge (Hanafusa et al., 1976) and that of subgroup I from Gambel's quail (Troesch & Vogt, 1985). Other unclassified endogenous pheasant viruses have been isolated also (Chen & Vogt, 1977).

In this report we describe the isolation and properties of a novel ALV of low pathogenicity in chickens. The group properties of the virus are similar to those of viruses of subgroups A to F but in viral envelope properties are different from those of the five chicken subgroups and subgroup F. The relationship, if any, with subgroups H and I remains to be established.

Methods

Chicken and quail lines. The Houghton Laboratory's strains of specific pathogen-free (SPF) Leghorn chickens were used to provide chick embryos or chicks of differing phenotypes: Brown Leghorn (BrL) (C/E), line 131 (C/E), line 6, (C/E), line 72 (C/ABDE), C line (C/ACE) and line 0 (C/E) (Anonymous, 1987). Japanese quail embryos (Q/BC) came from the Laboratory's strain. Five meat-type chicken lines, arbitrarily coded 20, 21, 22, 23 and 24, were used to provide chick embryos, vaginal swabs and sera. Seven commercial layer breeding lines, coded 25, 26, 27, 28, 29, 30 and 31, provided sera.

Mammalian cell lines. Mammalian cell lines used were CV-1 (African green monkey), L929 and L-M (TK-)(mouse), MDBK (NBL-1)(bovine) and PK(15)(pig). Vero cells were obtained from Life Technologies; the other cell lines were from the American Type Culture Collection.

Viruses. The following pseudotypes of Bryan high titre (BH)-Rous sarcoma virus (RSV) of subgroups A, B, C and E, respectively, were used: RSV(RAV-1), RSV(RAV-2), RSV(RAV-49), RSV(RAV-0) and RSV(RPV). ALVs of subgroups A, B, C, E and F were used, respectively, RAV-1, RAV-2, RAV-49, RAV-0 and ring-necked pheasant virus (RPV); HPRS-F42 ALV (subgroup A) was also used (Weiss et al., 1982; Purchase & Payne, 1984). RPV was kindly provided by Dr R. E. Smith, Colorado State University, Denver, U.S.A. Five isolates of a novel ALV, designated HPRS-100, HPRS-101, HPRS-102, HPRS-103 and HPRS-104, were made as described in Results. BH-RSV pseudotypes of these isolates were prepared by activation of R(−)Q RSV-transformed non-producer quail cells (Crittenden et al., 1979; Payne et al., 1979).

Antisera. Antisera against RSV pseudotypes of the A, B, C and E subgroups were collected from BrL chickens in which RSV sarcomas were induced by the appropriate virus. Antiserum against HPRS-100 ALV was collected from BrL chickens inoculated with that virus. Serum samples were collected from normal adult birds of the five meat-type chicken lines and the seven commercial layer lines and tested for antibodies against RSV(HPRS-103).

Virological methods. RSV pseudotypes of the five ALV isolates were prepared by growing the isolates in C/E BrL chick embryo fibroblasts (CEF's) and co-cultivation with R(−)Q cells. Interference and host range assays were performed using standard methods (Solomon et al., 1971; Spencer, 1987). Virus neutralization tests were carried out in C/E CEFs or quail embryo fibroblasts (QEFs) using the RSV focus
microneutralization test (Solomon et al., 1971; Spencer, 1987) or by a microneutralization test (Fadly et al., 1989). Polybrene (2 μg/ml) was used routinely in virus titrations. ALV gs antigen was detected by an ELISA test (Payne et al., 1985); the novel ALV was detected using an R(-)Q test (Crittenden et al., 1979; Payne et al., 1979) and attempts to detect the novel virus in an endogenous state were made following polyethylene glycol-induced fusion between R(-)Q cells and CE

The ability of HPRS-103 ALV and RSV(HPRS-103) to replicate and/or transform six mammalian cell lines was examined by inoculating cultured cells grown at 37 °C in the appropriate medium with virus at a multiplicity of 1 and testing extracts of inoculated and control cells for ALV gs antigen by the ELISA test 7 to 14 days later. Cultures of line 0 (CEF) CEFs and QCEFs (QB) were included as virus-susceptible and virus-resistant controls.

Protein detection. Virus pellets were obtained by centrifugation of media harvested from line 0 CEFs 1 week post-infection (p.i.) with HPRS-103 ALV or RAV-1. Pellets were resuspended in sample buffer and separated by SDS-PAGE on 10% acrylamide gels (Laemmli, 1970). Proteins were transferred to nitrocellulose using a Bio-Rad Trans-Blot apparatus; transfer was overnight at 30 V, 150 mA using the recommended Tris-glycine buffer (25 mM Tris, 192 mM-glycine, 20% methanol). Blots were blocked using 3% bovine serum albumin in phosphate-buffered saline.

Proteins were identified using either a polyclonal rabbit antiseraum raised against bromelain-treated HPRS-F42 ALV followed by a commercial peroxidase-conjugated anti-rabbit IgG (Sigma), or by using biotin-labelled concanavalin A (Sigma) followed by streptavidin-biotinylated horseradish peroxidase complex (Amersham), and developed using chloronaphthol.

DNA isolation and hybridization. DNA was prepared from line 0 CEFs 1 week post-infection (p.i.) using standard procedures (Maniatis et al., 1982). Digestion of the DNA samples, gel separation and hybridization were carried out as described in Bumstead et al. (1987) overnight at 42 °C in the presence of 50% formamide. After hybridization, blots were washed twice at 55 °C for 30 min and then twice at 65 °C for 30 min.

Probes used for hybridization were pRAV-1, containing a complete RAV-1 ALV genome (kindly provided by P. Enrietto, Imperial Cancer Research Fund, London, U.K.) and pPB101, containing a complete spleen necrosis virus (reticuloendotheliosis virus; REV) virus genome (Bandyopadhyay & Temin, 1984).

Pathogenicity tests. Pathogenicity tests on ALV isolates HPRS-100 and HPRS-104 were carried out by inoculating 1-day-old line 151 chicks intra-abdominally with 3 × 10^4 tissue culture infectious units (TCIU) of virus; diluent-inoculated chicks were kept as controls. The tests were terminated at 502 days p.i. A pathogenicity test on ALV isolate HPRS-103 was conducted by inoculating 3 × 10^4 TCIU of virus intravenously in 11-day-old line 151 chick embryos; diluent-inoculated embryos were kept as controls. Chicks were hatched and kept for 352 days p.i.; treatment groups were kept in separate isolators. Birds which died during these tests were autopsied and gross tumours were recorded and examined histologically.

Electron microscopy. HPRS-103 ALV-infected CEF cultures were prepared for electron microscopy by suspending the cells in culture medium, fixing them by mixing with an equal volume of 4% glutaraldehyde in 0.2 M 2,4,6-collidine buffer pH 7.2 and centrifuging to give a pellet. The pellet was post-fixed in 1% osmium tetroxide, dehydrated with ethanol and embedded in Araldite. Thin sections were stained sequentially with uranyl acetate and lead citrate and examined using a Philips 301 transmission electron microscope.

Statistical analysis. The Mann-Whitney U test (two-tailed) was used to compare median pock counts on chorioallantoic membranes (Siegel, 1956).

Results

Origin of the novel ALV in commercial chickens

Five isolates of the novel ALV were made from meat-type chicken lines. The isolates were derived from heart (and other tissues) from a line 20 bird with ascites syndrome (Julian, 1988) (designated isolate HPRS-100), from vaginal swabs from two normal hens in line 20 (isolates HPRS-101 and HPRS-102), from a vaginal swab from a normal hen from line 23 (isolate HPRS-103), and from tumour tissue from a case of myeloid leukaosis in line 20 (isolate HPRS-104). BrL C/E CEF cultures of the three vaginal swabs from which ALV was isolated were positive in an ALV ELISA test for gs antigen. Preliminary studies indicated that the five ALV isolates had identical properties; isolate HPRS-103 was adopted as the representative prototype.

Group properties of HPRS-103 ALV

HPRS-103 ALV is morphologically typical of an avian C-type retrovirus, replicating by budding from the cytoplasmic membrane and having a mean (± s.e.m.) diameter of 107 (± 3.2) nm (range 80 to 130 nm, n = 20). Like other avian retroviruses, HPRS-103 ALV and the other isolates complemented a defective BH-RSV genome in R(-)Q cells, forming infectious RSV pseudotypes.

Titres of four stocks of HPRS-103 ALV grown in line 0 CEFs ranged from 3 × 10^5 to 10^7 TCIU/ml, and those of two stocks of RSV(HPRS-103) grown in line 0 CEFs were 3 × 10^3 and 4.2 × 10^4 f.f.u./ml.

Western blots of HPRS-103 ALV, and of RAV-1 ALV for comparison, are shown in Fig. 1. Antibody staining revealed a protein band of approximately 27K, whereas staining with concanavalin A identified a glycoprotein band of apparent Mr, 89K. The ELISA test for p27 ALV gs antigen detected gs antigen in HPRS-103 ALV-infected CEFs.

DNA prepared from HPRS-103 ALV-infected CEFs hybridized strongly to probe pRAV-1 even under the stringent washing conditions used, but parallel blots probed with pPB101 showed no evidence of hybridization. Fig. 2 shows a typical blot in which DNA from line 0 cells infected with HPRS-103 or HPRS-F42 ALV were digested with BamHI and probed with pRAV-1. Digestions show similarities between HPRS-103 and HPRS-F42 ALV. The lanes containing undigested DNA indicated the presence of a substantial proportion of unintegrated linear DNA in both viruses. The mobility of the uncut viral bands of HPRS-103 ALV suggested a size of approximately 8 kb, closely similar to that for HPRS-F42 ALV.
A novel avian leukosis virus in chickens

Fig. 1. (a) Western blot of pelleted supernatants from (lane 1) line 0 CEFs infected with HPRS-103 ALV, (lane 2) uninfected line 0 CEFs, or (lane 3) line 0 CEFs infected with RAV-1. The blot was probed using antiserum prepared in rabbits against bromelain-treated HPRS-F42 ALV followed by a commercial peroxidase-conjugated anti-rabbit antiserum. (b) Western blot of pelleted supernatants from (lane 1) line 0 CEFs infected with HPRS-103 ALV or (lane 2) line 0 CEFs infected with RAV-1, probed using biotin-labelled concanavalin A followed by peroxidase-conjugated avidin.

Interference between ALV isolates and RSV pseudotypes of different subgroups

Interference assays were conducted between the five ALV isolates and RSV of subgroups A, B and C. No interference with RSV subgroups A, B and C was observed but the isolates did interfere with their own RSV pseudotypes. ALV of subgroups A, B and C did not interfere with these new pseudotypes (Table 1), nor was there interference between HPRS-103 ALV or RPV (subgroup F) and the reciprocal RSV pseudotypes (Table 2). These results exclude the isolates from subgroups A, B, C and F. Subgroup D RSV was not included in the tests, but would be expected to behave like the closely related subgroup B RSV (Duff & Vogt, 1969).

Virus neutralization

Antisera raised against RSV pseudotypes of subgroups A, B, C and E were used, singly or in combination, in neutralization tests against RSV pseudotypes prepared with the five ALV isolates. The results (Table 3) indicated that the isolates were not neutralized by these antisera, supporting their exclusion from subgroups A, B, C and E. Conversely, antiserum raised against HPRS-100 ALV did not neutralize RSV of subgroups A, B, C and E. Subgroup D virus and antiserum were not included in the tests, but would be expected to give reactions similar to those of subgroup B RSV (Duff & Vogt, 1969).

Host range

The susceptibility of several lines of SPF chickens to RSV(HPRS-103) was studied by inoculation of the chorioallantoic membrane of chick embryos whereas that of QEFs was determined in cell culture (Table 4). All chicken lines, but not the quail line, were susceptible to infection. On the basis of the known susceptibility phenotypes of these lines and hence of the subgroups excluded, it was concluded that RSV(HPRS-103) did not belong to subgroups A, B, C, D or E.

Pock-count assays using RSV(HPRS-103) were also conducted on the five meat-type lines to determine their susceptibility to infection. The natural infection status of the embryos used was not known. In two tests, line 20 was significantly more resistant than lines 21, 22, 23, 24...
Table 1. Interference between five ALV isolates (HPRS-100 to HPRS-104) and RSV pseudotypes in C/E BrL CEFs

<table>
<thead>
<tr>
<th>Interfering virus</th>
<th>Subgroup</th>
<th>RSV(RAV-1)</th>
<th>RSV(RAV-2)</th>
<th>RSV(RAV-49)</th>
<th>RSV(HPRS-100)</th>
<th>RSV(HPRS-101)</th>
<th>RSV(HPRS-102)</th>
<th>RSV(HPRS-103)</th>
<th>RSV(HPRS-104)</th>
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<tbody>
<tr>
<td>None</td>
<td>-</td>
<td>327*</td>
<td>186</td>
<td>185</td>
<td>46</td>
<td>39</td>
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<td>A</td>
<td>0</td>
<td>153</td>
<td>147</td>
<td>41</td>
<td>46</td>
<td>63</td>
<td>55</td>
<td>89</td>
</tr>
<tr>
<td>RAV-2</td>
<td>B</td>
<td>298</td>
<td>0</td>
<td>168</td>
<td>48</td>
<td>31</td>
<td>77</td>
<td>61</td>
<td>73</td>
</tr>
<tr>
<td>RAV-49</td>
<td>C</td>
<td>312</td>
<td>173</td>
<td>0</td>
<td>42</td>
<td>42</td>
<td>71</td>
<td>49</td>
<td>79</td>
</tr>
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<td></td>
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<td>181</td>
<td>173</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<td>282</td>
<td>209</td>
<td>152</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HPRS-102</td>
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<td>164</td>
<td>150</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>346</td>
<td>159</td>
<td>139</td>
<td>0</td>
<td>0</td>
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<td>0</td>
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</tbody>
</table>

* Mean number of foci in two dishes.

Table 2. Lack of interference between HPRS-103 ALV and RSV(RPV)

<table>
<thead>
<tr>
<th>Interfering virus</th>
<th>Challenge virus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>RSV(RPV)</td>
</tr>
<tr>
<td>None</td>
<td>92*</td>
</tr>
<tr>
<td>RPV</td>
<td>0</td>
</tr>
<tr>
<td>HPRS-103 ALV</td>
<td>73</td>
</tr>
</tbody>
</table>

* Number of foci/dish.

and the BrL line (P < 0.05 to P < 0.001) (Table 5); in one test, line 24 was significantly more resistant than line 22 (P < 0.01). Otherwise, lines 21, 22, 23 and 24 did not differ from each other or from the BrL line.

In host range studies with HPRS-103 ALV and RSV(HPRS-103) in six mammalian cell lines, no increase in ALV gs antigen or morphological changes were detected between 7 and 14 days after virus inoculation (data not shown).

Examination of CEFs from commercial meat-type lines for RSV(HPRS-103)

CEF cultures were prepared from six embryos from each of the meat-type lines and supernatant fluids were inoculated into cultures of line 0 CEFs and cultured for 7 days. The cultures were extracted with detergent and tested for gs antigen in an ELISA. The results were negative, providing no evidence for the presence of ALV which had been spontaneously released from the CEF cultures of the five lines.

The cultured CEFs were also fused to R(-)Q cells, using polyethylene glycol, and cultured for 6 days in an attempt to rescue endogenous ALV from the cells. Supernatant culture fluids were inoculated onto line 0 CEFs, which were cultured for 9 days and examined for development of RSV foci. No foci were observed, indicating that HPRS-103 ALV had not been rescued. Thus these results provided no evidence of exogenous or endogenous HPRS-103 ALV in the sample of chick embryos from the five meat-type lines.

Survey of serum antibodies against RSV(HPRS-103) from meat-type and layer lines

Serum samples from adult birds from the five meat-type lines and also from seven layer breeder lines were examined for the presence of neutralizing antibodies against RSV(HPRS-103). Antibodies were detected in meat-type lines 20, 22 and 23 only (Table 6).

Pathogenicity tests

Pathogenicity tests were conducted by inoculating ALV isolates HPRS-100 and HPRS-104 into 1-day-old line 151 chicks, and ALV isolate HPRS-103 into 11-day-old line 151 chick embryos. HPRS-100 and HPRS-104 ALV showed evidence of low level oncogenicity, with occurrence of tumours in four of 28 birds after long latent periods (Table 7); no tumours occurred in chicks inoculated with HPRS-103 ALV. Of the 12 birds that died from other causes in the two experiments, two had telangiectasia of the liver.

Discussion

The novel virus described in this paper, of which isolate HPRS-103 is the prototype, is a member of the ALV class of avian retroviruses (Porterfield, 1989). It is a typical C-type virus (Dalton & Haguenau, 1973), with p27 gs antigen and an envelope glycoprotein similar to gp85. Preliminary studies indicate that it has high DNA identity to RAV-1 ALV and a linear DNA form of
Table 3. Virus neutralization tests between RSV pseudotypes of the new isolates and of known subgroups and their antisera

<table>
<thead>
<tr>
<th>Virus</th>
<th>Subgroup</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>E</th>
<th>A + B</th>
<th>A + C</th>
<th>A + E</th>
<th>B + C</th>
<th>B + E</th>
<th>C + E</th>
<th>HPRS-100</th>
<th>Negative serum</th>
</tr>
</thead>
<tbody>
<tr>
<td>RSV(RAV-1)</td>
<td>A</td>
<td>0</td>
<td>101</td>
<td>99</td>
<td>107</td>
<td>0</td>
<td>0</td>
<td>117</td>
<td>92</td>
<td>106</td>
<td>115</td>
<td>111</td>
<td></td>
</tr>
<tr>
<td>RSV(RAV-2)</td>
<td>B</td>
<td>88</td>
<td>0</td>
<td>92</td>
<td>101</td>
<td>0</td>
<td>0</td>
<td>97</td>
<td>90</td>
<td>0</td>
<td>98</td>
<td>103</td>
<td>99</td>
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<td>RSV(RAV-49)</td>
<td>C</td>
<td>93</td>
<td>98</td>
<td>2</td>
<td>92</td>
<td>107</td>
<td>0</td>
<td>102</td>
<td>0</td>
<td>89</td>
<td>1</td>
<td>100</td>
<td>103</td>
</tr>
<tr>
<td>RSV(RAV-01)†</td>
<td>E</td>
<td>91</td>
<td>82</td>
<td>78</td>
<td>1</td>
<td>79</td>
<td>84</td>
<td>2</td>
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<td>91</td>
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<td>83</td>
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<td>93</td>
<td>2</td>
<td>102</td>
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* All antisera tested at final dilution of 1/10.
† Mean number of foci in two dishes.
‡ Tested on QEFs.

Table 4. Host range of RSV(HPRS-103) in chicken and quail lines*

<table>
<thead>
<tr>
<th>Line</th>
<th>Phenotype</th>
<th>No. of embryos</th>
<th>Pock or focus count</th>
<th>No. of embryos</th>
<th>Pock or focus count</th>
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<tbody>
<tr>
<td>BrL</td>
<td>C/E</td>
<td>8</td>
<td>40-5</td>
<td>10-90</td>
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<tr>
<td>C</td>
<td>C/AE</td>
<td>15</td>
<td>88-0</td>
<td>44-162</td>
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<tr>
<td>67</td>
<td>C/E</td>
<td>13</td>
<td>35-3</td>
<td>0-65</td>
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<tr>
<td>72</td>
<td>C/ABDE</td>
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<td>93</td>
<td>64-132</td>
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<tr>
<td>0</td>
<td>C/E</td>
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<td>70-5</td>
<td>20-141</td>
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<td>Quail</td>
<td>Q/BC</td>
<td>3</td>
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* Cell cultures were challenged with 3-4 × 10² f.f.u. RSV(HPRS-103)

Table 5. Susceptibility of meat-type chicken lines to RSV(HPRS-103)

<table>
<thead>
<tr>
<th>No. of embryos</th>
<th>Expt. 1</th>
<th>Median</th>
<th>No. of embryos</th>
<th>Expt. 2</th>
<th>Median</th>
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<td>21</td>
<td>29</td>
<td>555c</td>
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<td>155b</td>
<td></td>
</tr>
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<td>22</td>
<td>27</td>
<td>61a</td>
<td>15</td>
<td>121b</td>
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<td>202b</td>
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<td>28</td>
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<tr>
<td>BrL</td>
<td>NT†‡</td>
<td>NT</td>
<td>45</td>
<td>189b‡</td>
<td></td>
</tr>
</tbody>
</table>

* Different virus stocks were used for Expt. 1 and 2
† Median counts with a common superscript within experiments do not differ significantly.
‡ NT, Not tested.

approximately 8 kb, smaller than that of replication-competent transforming RSV (9.5 kb) (Weiss et al., 1982). The virus belongs, however, to a new subgroup for chickens.

The five known subgroups, A to E, of ALV which occur in chickens were reported between 1965 and 1970 (Vogt, 1977), so the discovery of an additional subgroup was surprising and unexpected. The novel virus is unusual because it has a wide host range within the strains of chickens tested, a low oncogenicity, and yet it is transmitted apparently as an infectious exogenous ALV. Low oncogenicity has hitherto been associated only with endogenous subgroup E viruses. The virus was found in three of five meat-type chicken lines, but not in seven layer lines, examined. In general, fewer
studies of the prevalence of ALVs in meat lines have been made compared to those in egg lines and further studies in other meat lines would be justified. No evidence was found for replication of and/or transformation by HPRS-103 ALV or RSV(HPRS-103) in mammalian cell lines.

The novel ALV appears to be distinct from viruses of subgroups A, B, C, D, E and F on the basis of viral interference, neutralization and host-range patterns, indicating unique properties of the env gene and the gp85 gene product. The new virus does not belong to subgroup G, which is characterized by a pheasant virus which belongs to a virus group quite distinct from that of the other subgroups and the new virus (Hanafusa et al., 1976). We have not yet been able to exclude the possibility that the virus belongs to subgroups H or I, although this is perhaps unlikely as viruses of these other subgroups and the new virus (HPRS-100) was isolated also from a case of myeloid leukemia but this tumour was not induced in the pathogenicity tests. It is noteworthy that one of the isolates (HPRS-104) came from a case of myeloid leukemia and related isolates need to be studied. Low oncogenicity of subgroup E ALV produced osteopetrosis, in contrast to subgroup E viruses which have little or no pathogenicity (Motta et al., 1975; Purchase et al., 1977). Okazaki et al. (1982) found that 42 of 43 field isolates of ALV (mainly subgroup A) were oncogenic and that attempts to isolate non-oncogenic recombinant clones from mixtures of subgroup A or B viruses with poorly oncogenic RAV-60 (subgroup E) virus were unsuccessful. The basis for the low oncogenicity of HPRS-103 ALV and related isolates needs to be studied. Low oncogenicity of subgroup E ALV is believed to depend on the weak enhancer properties of the long terminal repeat (LTR) region of the viral genome (Kung & Maihle, 1986), so that comparison of LTRs from the new and other subgroups would be of interest. It is noteworthy that one of the isolates (HPRS-104) came from a case of myeloid leukemia but this tumour was not induced in the pathogenicity tests.

The new virus (isolate HPRS-100) was isolated also from heart and other tissues provided by Dr M. H. Maxwell from a bird with the ascites syndrome; this virus is probably that illustrated in his paper (Maxwell et al., 1986). The occurrence of a retrovirus in cases of ascites may be coincidental, although it is of interest that extensive replication of ALV in myocardium has been observed in chickens naturally infected with ALV (Gilka & Spencer, 1985) and a chronic circulatory syndrome has

Table 7. Pathogenicity tests on ALV isolates HPRS-100, HPRS-103 and HPRS-104

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Age at inoculation</th>
<th>Inoculum</th>
<th>No. of chickens</th>
<th>No. with neoplasms</th>
<th>Other deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-day-old chicks</td>
<td>HPRS-100</td>
<td>19</td>
<td>3*</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HPRS-104</td>
<td>9</td>
<td>1†</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diluent</td>
<td>16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>11-day-old chick embryos</td>
<td>HPRS-103</td>
<td>17</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Diluent</td>
<td>12</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

* Lymphoid leukemia (273 days p.i.), myxosarcoma (336 days p.i.) and haemangioma (353 days p.i.).
† Myxofibroma (502 days p.i.).
been induced experimentally with ALV (Gilka & Spencer, 1990). The possible role of retroviral infection
in avian ascites merits further study.

We are indebted to Mrs A. Barrow and Mrs S. Sanderson for
technical assistance.

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(Received 5 July 1990; Accepted 7 January 1991)