The viral envelope is a major determinant for the induction of lymphoid and myeloid tumours by avian leukosis virus subgroups A and J, respectively

P. M. Chesters, K. Howes, L. Petherbridge, S. Evans, L. N. Payne and K. Venugopal

Viral Oncogenesis Group, Institute for Animal Health, Compton, Berkshire RG20 7NN, UK

Among the six envelope subgroups of avian leukosis virus (ALV) that infect chickens, subgroups A (ALV-A) and J (ALV-J) are the most pathogenic and widespread among commercial chicken populations. While ALV-A is predominantly associated with lymphoid leukosis (LL) and less frequently with erythroblastosis (EB), ALV-J mainly induces tumours of the myeloid lineage. In order to examine the basis for the lineage specificity of tumour induction by these two ALV subgroups, we constructed two chimeric viruses by substituting the \textit{env} genes into the reciprocal proviral clones. The chimeric HPRS-103(A) virus carrying the subgroup A \textit{env} gene is identical to ALV-J prototype virus HPRS-103 except for the \textit{env} gene, and the chimeric RCAS(J) virus carrying the subgroup J \textit{env} gene is identical to the parent replication-competent ALV-A vector RCAS except for the \textit{env} gene. In experimentally inoculated chickens, HPRS-103(A) virus induced LL and EB similar to ALV-A isolates such as RAV-1, while RCAS(J) virus induced myeloid leukosis (ML) and EB, similar to ALV-J, suggesting that the \textit{env} gene is the major determinant for the lineage-specific oncogenicity. There were genetic differences in susceptibility to tumour induction between line 0 and line 15 chickens, indicating that in addition to the \textit{env} gene, other viral or host factors could also serve as determinants for oncogenicity. Induction of both LL and ML by the two chimeric viruses occurred through the activation of \textit{c-myc}, while the EB tumours were induced by activation of the \textit{c-erbB} oncogene.

Introduction

Avian leukosis viruses (ALV) that infect chickens comprise six envelope subgroups designated A–E and J (Payne, 1998). ALV-J prototype virus HPRS-103, isolated from meat-type chickens, mainly induces myeloid leukosis (ML) after experimental infection of different leghorn and meat-type strains of chickens (Payne et al., 1992a, b). In contrast, ALV subgroups A (ALV-A) and B (ALV-B), including the RCAS-BP replication-competent retroviral vectors derived from them, primarily induce lymphoid leukosis (LL) or erythroblastosis (EB) in susceptible chickens (Tam et al., 2002). Studies on the replication of RAV-1 (ALV-A) and HPRS-103 (ALV-J) in the bursal follicles \textit{in vivo} and in monocyte cultures \textit{in vitro} have suggested that the induction of lymphoid and myeloid tumours by ALV-A and ALV-J, respectively, is associated with their ability to replicate in their respective target cells (Arshad et al., 1997). Both the long terminal repeat (LTR) and \textit{env} genes are known to play important roles in the tissue tropism of ALV, the LTR affecting virus replication in different cell types, while the \textit{env} gene influences the types of cell targets that the virus can infect (Brown et al., 1988).

The genome structure of ALV-J resembles that of other subgroups, with the \textit{gag} and \textit{pol} genes showing over 96% sequence identity and the LTR showing over 90% sequence identity with the equivalents in subgroups A–E (Bai et al., 1995; Benson et al., 1998). However, the sequence of the ALV-J \textit{env} gene shows a much greater similarity to that of a closely
related group of novel chicken endogenous retroviral elements designated EAV-HP (Sacco et al., 2000; Smith et al., 1999), suggesting that env was acquired by recombination (Venugopal, 1999) and raising the possibility that the myeloid lineage-specific oncogenicity of ALV-J could mainly be related to determinants within env.

The envelope glycoprotein of ALV, as in other retroviruses, functions mainly as a ligand for receptor binding for virus entry into the susceptible cell (Weiss, 1992). Subgroup-determining sequences in the ALV-A env gene have a significant influence on the lymphomagenic potential (Brown & Robinson, 1988). The ALV-A receptor, encoded by the tva susceptibility allele, contains sequences related to the ligand-binding region of the low-density lipoprotein receptor (Bates et al., 1993). The variation in the distribution of the tva susceptibility allele accounts for the differences in the susceptibility to ALV-A infection among chicken populations. The receptors for the ALV subgroups B, D and E, members of the tumour-necrosis factor receptor family (Adkins et al., 2001), are also not expressed in all lines of chickens. However, unlike the other ALV subgroups, all lines of chickens are susceptible to infection by ALV-J, suggesting that the putative ALV-J receptor, yet to be characterized, would be distinct in terms of its distribution among chicken lines.

It is not known whether the lineage-specific oncogenicity of different ALV subgroups is associated with interactions with the receptor and virus entry into the corresponding cell types or with post-entry events. In order to examine the role of the env gene in the induction of ML by ALV-J, we have made reciprocal chimeric viral constructs by substituting the env regions between HPRS-103 and the replication-competent ALV-A vector RCAS (Hughes et al., 1987) to create HPRS-103(A) and RCAS(J). The oncogenicity of these two chimeric viruses was evaluated in line 0 chickens, which are highly susceptible to ML induction (Payne et al., 1992a) and line 15I chickens, which are selected for susceptibility to LL induction (reviewed by Bacon et al., 2000). Examination of the oncogenicity of HPRS-103(A) is particularly significant because of a recent report of the isolation of three such chimeric ALV-J viruses from albino transgenic chicken cells (Crittenden & Salter, 1992) naturally infected with ALV-J strains (Lupiani et al., 2000).

**Methods**

**Construction of chimeric viruses.** The proviral clones of RCAS and HPRS-103 contain a conserved KpnI restriction site (Fig. 1) between pol and env (Bai et al., 1995; Bieth & Darlix, 1992). For construction of the HPRS-103(A) chimeric virus, the ALV-J env sequence was removed from the full-length infectious proviral clone HPRS-103 by digestion with KpnI and BsaBI. The removal of the KpnI–BsaBI fragment also excised the part of the 3′ non-coding region including the redundant TM (rTM) and direct repeat 1 (DR1) from the HPRS-103 genome (Bai et al., 1995), but left the E (XSR) element and the 3′ LTR intact. The ALV-based replication-competent RCAS(A) vector (Hughes et al., 1987) was digested with BsaAI and treated with Klenow DNA polymerase I to create blunt termini. A second digestion with KpnI was carried out to release the env region together with the splice acceptor site AGGA (nt 6982–6985) and the CIal cloning site (nt 7029) of the RCAS vector used for the expression of foreign genes (Fig. 1a). The agrose gel-purified KpnI–BsaAI blunt-ended fragment was ligated to the KpnI–BsaBI cut HPRS-103 clone to generate the chimeric HPRS-103(A) clone with the ALV-A env gene.

For construction of the RCAS(J) chimeric virus, the single BsaWI site within the env region of the HPRS-103 proviral clone between the KpnI and BsaWI sites (Fig. 1b) was removed without altering the amino acid sequence by the substitution of a PCR product. The resulting construct was digested at the BsaWI site (nt 7032) and blunt-ended with Klenow DNA polymerase. After further digestion with KpnI, the fragment containing the ALV-J env gene was agrose gel-purified and ligated to the RCAS vector cut with KpnI and Shu (Fig. 1b). The resulting construct was designated RCAS(J). The complete sequences of the env genes of both chimeric plasmid clones and the ligation junctions were determined to rule out any errors introduced during PCR and cloning.

**Cell culture, virus propagation and virological assays.** The propagation of the chimeric viruses was initiated by transfection of 1–2 µg of plasmid DNA into primary chicken embryo fibroblasts (CEF) (Payne et al., 1992b) derived from 10-day-old, line 0 (C/E) embryos (Astrin et al., 1979). Production of virus in the transfected cells was monitored by assaying culture supernatants for ALV p27 using ELISA (Smith et al., 1979). Micronuclear tests (Faddy & Witter, 1998) for typing the virus stocks and detecting specific antibodies in the serum sample were carried out using a single dilution of 10 µl of heat-inactivated serum sample and 100 tissue culture infectious units (TCIU) of the virus.

**Infection of chickens.** Specific-pathogen-free (SPF) line 0 and line 15I chickens were infected as 11-day-old embryos by intravenous inoculation via a chorioallantoic vein with 50 µl of virus stock containing 5 × 105 TCIU of HPRS-103(A) or 5 TCIU of RCAS(J) viruses. Lower doses of RCAS(J) virus were used, since higher-titre virus stocks could not be generated, in spite of repeated transfection attempts with the DNA constructs. Control birds belonging to the uninfected group were injected with tissue culture medium from CEF. As the oncogenic characteristics of both subgroup A and subgroup J ALV, in terms of their lymphoid and myeloid leukaemogenicity, respectively, are well documented through several previous studies (Payne et al., 1991; 1992a; Purchase et al., 1977), we did not include control groups of birds infected with these viruses to reduce the unnecessary usage of animals. Virus-infected and control chicks of each line were incubated, hatched and reared in separate incubators and rooms in the experimental animal house. In addition, 1-day-old chicks from both lines were also infected by intraperitoneal inoculation of 200 µl of the above two virus stocks, and blood samples were collected 12 weeks later to obtain specific antisera against the virus. All experiments were carried out in accordance with UK Home Office Guidelines, and birds that showed signs of disease were euthanized, autopsied and examined for gross and microscopic lesions. Experiments were terminated at 150 days post-infection (p.i.) for birds infected with HPRS-103(A) and 220 days p.i. for birds infected with RCAS(J). All birds from the infected and control groups were examined for lesions.

**DNA extraction, Southern blotting and hybridization.** Frozen tumour tissue was collected during post-mortem examination from five birds from the HPRS-103(A) virus-infected group, four (nos 1261, 1262, 1285 and 1302) diagnosed as LL and one (no. 1303) diagnosed as EB, and from two birds from the RCAS(J) virus-infected group (nos 1335 and 1346) diagnosed as ML by histopathological examination. Birds 1261, 1262, 1335 and 1346 belonged to line 0 and birds 1285, 1302 and 1303
integration junctions were agarose gel-purified and sequenced, either by agarose gel electrophoresis, transferred to nylon membranes and hybridized with a $^{32}$P-labelled probe derived from c-myc (kindly provided by Dr Don Ewert, Wistar Institute, Philadelphia, USA) or c-myb (Klempnauer et al., 1982) DNA.

- **PCR amplification.** Two types of PCR tests were carried out on tumour DNA samples (2 µg) using a long template PCR kit (Roche Molecular Biochemicals). The first test, using sense primers that annealed to the ALV-A env sequences at positions between nt 5420 and 5776 of RCAS and antisense primers that annealed to HPRS-103 sequences at positions between nt 7386 and 7438, specifically detected the chimeric HPRS-103(A) DNA. The second PCR test was designed to distinguish between the two chimeric and parent viruses based on the Clal restriction profile of the PCR products, which gives bands of sizes 2 kb and 0·4 kb for RCAS, 2 kb and 0·5 kb for HPRS-103(A), 1·4 kb and 1·1 kb for HPRS-103 and 1·4 kb, 0·6 kb and 0·4 kb for RCAS(J). In this PCR, the upstream primer annealed at nt 5258–5277 or 5033–5052, and the downstream primer annealed at nt 7722–7749 or 7374–7401 in HPRS-103 and RCAS, respectively. The amplifications were performed as follows: one cycle at 94°C for 5 min, 14 cycles consisting of 94°C for 10 s, 60°C (−1°C/cycle) for 30 s and 68°C for 4 min, followed by 30 cycles of 94°C for 10 s, 58°C for 30 s and 68°C for 4 min, and one cycle of 68°C for 6 min. The second-round PCR was performed under the same conditions using 10 µl of a 1:500 dilution of the first-round product. Amplification of the proviral/c-myc or c-erbB junction sequences from the tumour DNA was carried out using a nested PCR with primers that annealed to the LTR U5 region and exon 2 of c-myc or exon 15 of c-erbB, as described previously (Gong et al., 1998).

- **DNA sequencing.** PCR products representing the c-myc and c-erbB integration junctions were agarose gel-purified and sequenced, either directly or after cloning into the pGEM-T vector (Promega), using insert- or vector-specific primers. The sequences were analysed using the Genetics Computer Group version 10 software.

## Results

### Characterization of the chimeric viruses

Chimeric HPRS-103(A) virus stock obtained from culture supernatants of transfected CEF gave similar titres ($10^5$ TCIU/ml) to the wild-type viruses derived from cells transfected with infectious proviral clones of HPRS-103 and RCAS viruses (not shown). However, the titres of RCAS(J) virus stocks from similar transfections were much lower ($10^2$ TCIU/ml). High-titre virus stocks could not be obtained from repeated transfections of RCAS(J) constructs into CEF. The antigenicity of the chimeric virus stocks was characterized by a series of neutralization tests using antisera prepared against various ALV subgroups and with serum samples collected from birds infected with the chimeric viruses. A single dilution of each serum was used, and the results were expressed as complete neutralization (+) or no neutralization (−) (Table 1) based on a negative or positive ELISA reading.

Serum samples from line 0 or 15 chickens, collected 12 weeks after infection with HPRS-103(A) virus, completely neutralized both RCAS and HPRS-103(A) viruses but not HPRS-103, demonstrating that the chimeric virus induced specific neutralizing antibodies against the subgroup A envelope protein. Furthermore, while the RAV-1 (ALV-A)-specific serum neutralized the chimeric HPRS-103(A) virus, the antisera against ALV subgroups B (RAV-2), C (RAV-49) and J
(HPRS-103) failed to neutralize the chimeric HPRS-103(A) virus, confirming the subgroup A specificity of the chimeric virus. The chimeric RCAS(J) virus was neutralized only by the ALV-J (HPRS-103)-specific serum, confirming its J-specific antigenicity. None of the serum samples collected from birds 12 weeks after infection with RCAS(J) neutralized either HPRS-103 or RCAS(J) virus (data not shown), demonstrating that the chimeric RCAS(J) virus failed to induce any antibodies in these birds, probably due to low levels of replication.

**Oncogenicity of HPRS-103(A) and RCAS(J) viruses**

Chimeric HPRS-103(A) virus was highly oncogenic for both lines of birds, as shown by its ability to induce tumours in a large proportion of birds during the 150-day experimental period. Line 0 birds were more susceptible to the induction of tumours by the HPRS-103(A) virus than line 15I birds, as 24/32 (75%) of the former developed tumours detected by gross or histological examination. In comparison, only 13/32 (40.6%) line 15I chickens infected in a similar manner developed tumours during the same period. Chimeric RCAS(J) virus was not highly oncogenic, and even after 220 days, only 3/31 (9.7%) line 0 chickens were shown to have any type of tumour by gross or histological examination. None of the line 15I chickens infected with RCAS(J) virus and none of the birds from the control group developed tumours detectable by gross or histological examination.

The tumours were classified into LL, EB or ML, based on the typical gross characteristics of the tumour, tumour tissue distribution and the histological phenotype of the transformed cells. Most of the LL tumours included involvement of the bursa of Fabricius, where the tumour appeared to be localized to single [Fig. 2a(I)] or multiple lymphoid follicles. Histologically, the tumour cells appeared to be lymphoblastic and mainly showed extravascular infiltration [Fig. 2a(2)] in the affected tissues. The two RCAS(J) virus-induced ML tumours showed massive infiltration of the characteristic myelocytes [Fig. 2a(3)] in the various organs. The EB tumours could be differentiated by the morphology of the erythroblasts and the typical intravascular accumulation in the sinusoidal spaces of the liver [Fig. 2a(4)]. In line 0 birds infected with HPRS-103(A) virus, 87.5% of the tumours were LL, while the remaining 12.5% were EB. In a single case in line 0 birds, the tumours consisted of both lymphoid and myeloid cells. In line 15I chickens, 53.8% of the tumours were diagnosed as LL, while 46.2% consisted of EB tumours. The time of detection varied between the types of tumours and the lines of birds (Fig. 2b). The shortest time to detection of LL in line 0 was 71 days with a mean period of 101.2 days. In line 15I birds, the first case of LL was seen 117 days after infection, with a mean period of 141.6 days. The onset of EB in both lines of birds was faster with a mean latency period of 82.7 days in line 0 and 84.7 days in line 15I. RCAS(J) virus induced tumours of slow onset, both ML tumours being detected 200 days after infection and the single case of EB 126 days after infection (Fig. 2b).

**Chimeric virus-induced LL tumours show rearrangement in the c-myc locus**

The majority of cases of LL induced by pathogenic ALV subgroups such as A and B show virus integration within the c-myc locus (Hayward et al., 1981; Robinson & Gagnon, 1986). Such integration events can be detected by digestion of genomic DNA from tumour tissues with EcoRI and BamHI, which detect smaller integration junction fragments in addition to the characteristic 6.5 kb c-myc DNA fragment (Fynn et al., 1992). Southern blot hybridization of genomic DNA extracted from three LL tumours and one EB tumour with a c-myc probe detected a 6.5 kb band representing the intact c-myc locus in all the DNA samples, similar to the uninfected control tissue (Fig.
ALV env determines cell-specific oncogenicity

Fig. 2. Induction of LL, EB or ML by the chimeric viruses. (a) H&E-stained sections of tissues from birds infected with the chimeric viruses. (1) LL lesion showing a single neoplastic lymphoid follicle in the bursa of Fabricius. Bar, 200 µm. (2) Metastatic LL lesion showing accumulation of lymphoblasts in the extravascular spaces in the liver. Bar, 25 µm. (3) ML lesion showing accumulation of myelocytes. Bar, 25 µm. (4) EB lesion showing accumulation of erythroblasts in the intravascular spaces in the liver. Bar, 25 µm. (b) Comparison of the time taken for the detection of tumours in line 0 and line 15I, chickens. LL (▲) and EB (△) induced by HPRS-103(A), and ML (●) and EB (○) induced by RCAS(J) are shown.

Fig. 3. Southern blot of DNA extracted from normal or tumour tissues of experimental birds infected with HPRS-103(A) virus. DNA samples, digested with EcoRI and BamHI, were resolved on agarose gels and Southern blots were hybridized with c-myc or c-myb probes. (a) The higher-sized bands (approximately 6.5 kb), detected with the c-myc probe in lanes 1–5 including the control, represent the germ-line c-myc, while the smaller-sized bands of 3–4 kb (lanes 1, 2 and 3) represent proviral–c-myc junction fragments. (b) The c-myc probe detected only the single germ-line c-myc band (lanes 1–5) indicating no integration at this locus. Bird numbers and the types of tumours (LL or EB) are indicated against each lane.

Chimeric viruses are integrated in the c-myc or c-erbB locus

In ALV-induced bursal lymphomas, proviral integration, most often upstream of c-myc exon 2 (Robinson & Gagnon, 1986), can be specifically detected by PCR amplification of the integration junctions with primers designed to anneal to the LTR (L1, L2) and c-myc sequences (M1, M2) (Gong et al., 1998). Using this approach, we carried out a nested PCR on the DNA from the four LL tumours (1261, 1262, 1285 and 1302) and one EB tumour (1303) from HPRS-103(A) virus-infected birds and from two ML tumours (1335 and 1346) from RCAS(J) virus-infected birds. PCR products of sizes ranging from 0.2–0.8 kb were obtained from the four LL and the two ML tumour DNA samples, indicating that the viral integration sites were within intron 1 of the c-myc locus. This was further confirmed by determining the sequence of the PCR products from each of the six tumour DNA samples. The sequence of the c-myc integration junctions from the four LL tumours (1261, 1262, 1285 and 1302) and one ML tumour (1346) showed that the 3′ terminal nucleotide 7841 of HPRS-103 was joined to the c-myc intron 1 sequence (Fig. 4). In the PCR product from the second ML tumour (1335), nucleotide 389 in the viral gag gene of RCAS was fused to c-myc exon 2 (Fig. 4), a structure suggesting oncogene transduction in acutely transforming viruses.
PCR tests on these DNA samples using primers that specifically amplify the c-erbB junction fragments (L1/E1 and L2/E2) gave no evidence of integration in the c-erbB locus. However, the DNA from the EB tumour (1303) produced three distinct bands of approximate sizes 0–8, 2–5 and 0–45 kb (Fig. 4), indicating the involvement of the c-erbB locus. Sequence analysis showed that the 0–8 kb product represented viral integration upstream of the known c-erbB intron 14 sequences [EB 1303(a)], while the 2–5 and 0–45 kb products [EB 1303(b) and EB 1303(c), respectively] contained the viral genome with transduced c-erbB sequences. The 0–8 kb and 2–5 kb fragments showed sequences with a high degree of similarity (only two nucleotide differences) to the c-erbB intron 14 up to nt 1245 in avian erythroblastosis virus (AEV) (Bruskin et al., 1990) and the corresponding sequence in ALV carrying transduced erbB. In addition, a 135 bp sequence upstream from this position showed only one nucleotide difference from the transduced ALV erbB intron sequences (Raines et al., 1988). Remaining upstream sequences in the 0–8 kb product [Fig. 4, EB 1303(a)], thought to be from another intron, do not match any sequences in the database except for a 14 bp region matching the HPRS-103 gag gene (except for a single nucleotide) between nt 2184 and 2197 (Bai et al., 1995). Sequences identical to parts of the unmatched region in the 0–8 kb product were also seen in the 2.5 kb fragment. However, these sequences were fused to the 3′ end of the gag gene and showed significant deletion compared with those seen in the 0–8 kb fragment. The 0–45 kb fragment [EB 1303(c)] had a deletion point in the gag gene at nt 623, which was fused directly to exon 15 of c-erbB at nt 1436, where there is a common three-nucleotide sequence (gag nt 620–622 and c-erbB nt 1433–1435). These positions correspond to known splice junctions in gag and erbB. PCR tests also demonstrated the presence of the respective chimeric proviral molecules in the tumour DNA samples, but not in the uninfected tissues from the control bird (data not shown).

Discussion

Avian leukosis viruses are mainly associated with the induction of tumours in cells of the haemopoietic lineage. They include viruses such as RAV-1 (ALV-A) and RAV-2 (ALV-B), which have been repeatedly shown to induce LL or EB, and HPRS-103 (ALV-J), which is mostly associated with ML (Payne et al., 1992a). Although the viral determinants of oncogenesis and the different mechanisms of induction of tumours by ALV are known (reviewed in Kung & Liu, 1997), the bases for cell lineage-specific oncogenicity are not completely understood. Both the LTR and env gene have an influence on the oncogenicity for lymphoid cells (Brown et al., 1988), and studies with chimeric viruses carrying env genes from different subgroups have demonstrated that the viral envelope has a large influence on tissue tropism (Brown & Robinson, 1988). Since the retroviral envelope mainly acts as a ligand for receptor binding and virus entry, the envelope-
mediated tropism of ALV subgroups for cells of different lineages might be related to the distribution of the specific receptors on different cell types. This hypothesis is supported by the observation that the ML-inducing HPRS-103 strain (ALV-J) replicates well in monocyte cultures in vitro and poorly in the lymphoid follicles of the bursa of Fabricius in vivo, in contrast to the LL-inducing RAV-1 strain of ALV-A, where the reverse is true (Arshad et al., 1997). However, the envelope–receptor interaction alone is unlikely to be the single determinant in the lineage-specific oncogenicity, since the RAV-2 strain (ALV-B), which also grows in monocyte cultures (Arshad et al., 1997), mainly induces LL (Gazzolo et al., 1975). Furthermore, it is also true that not all of the cell types that support ALV replication develop tumours, suggesting that other virus- and/or cell lineage-specific determinants are also important for oncogenicity. As HPRS-103 has a distinct env gene that shows only 40% homology (Bai et al., 1995) to that of other subgroups, it would suggest that the determinants for myeloid tropism are within the env gene. However, ALV-J also has other distinct features, such as the deletion of an EFII region in the 3' non-coding region (Bai et al., 1995). We examined the effects of various viral determinants on the lineage-specific induction of tumours, by constructing two chimeric ALV, substituting the env region of subgroups A and J. These chimeric viruses, HPRS-103(A) and RCAS(J), were identical to the parents HPRS-103 and RCAS, respectively, except for their env regions. HPRS-103(A) virus was highly oncogenic and, in the majority of cases, induced LL and, less commonly, EB in both lines of chickens. Since LL is usually associated with ALV-A infection, these data indicate that the subgroup A env region is the major determinant for cell lineage-specific oncogenicity of the lymphoid lineage. Similarly, the substitution of the ALV-A env region of RCAS virus with the corresponding region of ALV-J shifted its tropism from lymphoid to myeloid cells, since two out of the three tumours induced by RCAS(J) virus in line 0 birds were ML. Although the oncogenicity of RCAS(J) was considerably lower than that of HPRS-103(A), the induction of ML in two cases further implicated the viral env region as the major determinant for cell lineage-specific oncogenicity. Since the induction of EB has been shown to be associated with both ALV-A (Fung et al., 1983) and ALV-J (Venugopal et al., 2000) subgroups, the low incidence of EB in line 0 chickens infected with both chimeric viruses was not unexpected.

The reasons for the lower oncogenicity of RCAS(J) are thought to be directly related to the poor replication, since the virus titres obtained from CEF transfected with the RCAS(J) construct were at least 10^3-fold less than those of the parent viruses and the HPRS-103(A) chimeric virus. This was further demonstrated by the negative antibody response in all the birds infected with the virus after hatching, as well as the small proportion of birds developing viraemia after embryonic infection. However, it has been shown that viruses of subgroup A are the most consistently pathogenic, even at relatively low doses (Purchase et al., 1977). Furthermore, a virus dose response has been demonstrated, with high virus dosage inducing death by haemorrhages, erythroblastosis and sarcomas, and low virus doses inducing lymphoid leukosis (Payne et al., 1977). Thus, the shift to a myeloid tropism by RCAS(J) most probably cannot be ascribed to the low infectious dose. The induction of ML in two line 0 birds and the demonstration of virus integration in the c-myc locus confirmed that the ALV-J env region is the major determinant for the myeloid lineage-specific oncogenicity.

Cell lineage-specific oncogenicity of ALV is also dependent on host genetic factors. We have previously demonstrated that while strain HPRS-103 can infect several inbred and commercial lines of chickens, a greatly variable incidence of ML was shown among chicken lines. Commercial meat-type lines of chickens and, among the inbred lines, line 0 chickens were the most susceptible to the development of ML by HPRS-103, with the incidence in line 0 birds often close to that seen in some of the meat-type lines (Arshad et al., 1997; Payne et al., 1992a). On the other hand, line 15_I, selected for increased susceptibility to the induction of LL (reviewed by Bacon et al., 2000), showed increased resistance to the induction of ML tumours (Payne et al., 1992a). Line 0 birds were highly susceptible to the induction of LL by chimeric virus (similar to the high incidence of ML induced by HPRS-103 virus) with 75% of the infected birds developing tumours, compared with only 40% in line 15_I birds. The lower incidence of LL induced by the chimeric virus in line 15_I also correlates with their increased resistance to the induction of ML by HPRS-103 (Payne et al., 1992a). These differences in tumour susceptibility between inbred lines are thought to be related to the differences in the LTR between ALV-A and ALV-J viruses, which could affect the interaction with various enhancer binding factors (Ruddell et al., 1989). The results from this present study indicate that the two major determinants of oncogenicity, the env gene and the LTR (Brown et al., 1988), act in concert as the factors associated with lineage specificity and tumour induction, respectively.

The incidence of LL and EB induced by HPRS-103(A) virus varied between the two lines. In line 0 birds, the incidence of these tumours was 87.5% and 12.5%, respectively, compared with 53.8% and 46.2% in line 15_I. Line 0 birds also appeared to be more susceptible than line 15_I based on the shorter time for the detection of LL (Fig. 2b). The slower progression of LL tumours in line 15_I could be a reflection of genetic resistance to the induction of tumours by the chimeric virus. Genetic resistance to tumour development is manifested as a failure of the cells with c-myc integration to hyperproliferate in the bursal environment (Bird et al., 1999). The slow expansion of cells with proviral c-myc genes has also been attributed to the delay of onset of tumours in line 0, another line genetically resistant to the development of ALV tumours (Bacon et al., 2000). However, as line 15_I is highly susceptible to the
induction of LL by RAV-1 (Bacon et al., 2000), the degree of resistance to the induction of LL by the chimeric virus is likely to be related to determinants in the HPRS-103 LTR and the interaction with cell type-specific transcription factors (Curristin et al., 1997).

Induction of LL or EB tumours by ALV-A strains such as RAV-1 occurs by integration of the virus in the c-myc (Hayward et al., 1981) or c-erbB (Fung et al., 1983) loci, respectively. Induction of ML by ALV-J strains also occurs through the involvement of the c-myc oncogene (Chesters et al., 2001). Using a PCR assay (Gong et al., 1998), we showed here that LL and ML induced by the chimeric HPRS-103(A) and RCAS/J viruses, respectively, also resulted from proviral integration within the c-myc locus. Similarly, we also showed that the c-erbB locus was involved in the induction of EB by the chimeric HPRS-103(A) virus. These results indicate that activation of oncogenes is a common pathway of induction of tumours, regardless of cell type or virus subgroup. However, one of the major differences between LL tumours induced by ALV-A or ALV-B and ML tumours induced by ALV-J is the high frequency of generation by ALV-J of acutely transforming viruses with transduction of v-myc (Chesters et al., 2001; Payne et al., 1993). High levels of transduction of erbB sequences have also been reported in EB tumours induced by activation of c-erbB (Miles & Robinson, 1985). We were not able to isolate any acutely transforming viruses from any of the tumours induced by the chimeric viruses. However, sequencing PCR products from the EB tumour (1303) and one of the ML tumours (1335) showed that the 5’ LTR and part of the gag gene had fused with the c-erbB and c-myc sequences, respectively. These structures resemble those of acutely transforming viruses with transduced oncogene sequences. Compared with this, sequencing PCR products from all the four LL tumours showed 3’ LTR integration in c-myc intron 1, a feature of ‘insertional activation’ (Kung & Liu, 1997). The reasons for the changes in frequency of transduction of c-myc between LL and ML tumours are not clear. However, it is possible that the interaction between the LTR and cell-type specific factors in myeloid and lymphoid cells could influence the frequency of the events involved in the transduction of v-myc and the generation of acutely transforming viruses.

Although the chimeric viruses described here were derived by molecular manipulation of the HPRS-103 and RCAS proviral clones, three chimeric viruses that probably arose naturally by recombination between exogenous subgroup J virus and a recombinant-defective endogenous virus with subgroup A env gene have recently been described (Lupiani et al., 2000). Although the tropism and oncogenicity of these viruses have yet to be described, the data from our studies suggest that they are likely to cause LL or EB rather than ML tumours.

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