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# Susceptibility to *erb*B-Induced Erythroblastosis Is a Dominant Trait of $15_1$ Chickens

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**Rous-associated virus-1 (RAV-1)-induced erythroblastosis results from proviral insertions into or viral transductions of the c-*erb*B region of the epidermal growth factor receptor gene. Most chickens develop low incidences (<5%) of RAV-1-induced erythroblastosis. However, an inbred line of chickens ($15_1$) suffers high incidences (~80%) of RAV-1-induced erythroblastosis. Analysis of $15_1$, K28, and (K28 × $15_1$) × K28 chickens for susceptibility to RAV-1-induced erythroblastosis revealed that susceptibility to RAV-1-induced erythroblastosis is a dominant trait of line $15_1$ chickens. Analysis of $15_1$ × K28 and K28 chicks for susceptibility to the induction of erythroblastosis by two new c-*erb*B-transducing viruses (avian erythroblastosis virus strains AEV-5005 and AEV-5009) revealed that susceptibility to transformation by new c-*erb*B-transducing viruses is also a dominant trait of $15_1$ chickens. We think it is likely that both of these dominant traits are encoded by the same gene or genes. Our hypothesis is that this gene (or genes) potentiates the ability of the transmembrane and cytoplasmic domains of the epidermal growth factor receptor to transform cells.**

The helper viruses for the Bryan high-titer strain of Rous sarcoma virus (Rous-associated viruses [RAVs]) cause erythroblastosis by proviral insertions into or new transductions of the transmembrane and cytoplasmic domains of the receptor for epidermal growth factor (EGF) (6, 11, 20, 22a, 33, 34, 37). This region of the EGF receptor gene is frequently referred to as c-*erb*B (5) since it was first recognized by virtue of its homology to the v-*erb*B oncogene of an avian erythroblastosis virus (AEV) (29, 34).

The ability of RAVs to induce erythroblastosis by proviral insertions into or viral transductions of c-*erb*B is pedigree specific. Random-bred lines of chickens, such as the Robinson subline of K28 and SPAFAS $gs^-$ chickens, as well as most inbred lines of chickens (Hyline SC line, line $6_3$, and $15I_5$ × $7_2$) suffer only a low incidence of RAV-induced erythroblastosis (2, 3, 26; T. Graf, personal communication). However, a line of inbred chickens has a high susceptibility to RAV-induced erythroblastosis. This line is the $15_1$ subline of line 15 chickens (2, 3, 30).

Studies on the unusual susceptibility of $15_1$ chickens to RAV-induced erythroblastosis have demonstrated that the $B^5$ and $B^{15}$ haplotypes of the major histocompatability complex carried in this line (4) is permissive for RAV-induced erythroblastosis (2, 3). The *B* haplotype-associated susceptibility to RAV-induced erythroblastosis is a recessive trait that affects the immune response to infections.

White Leghorn chickens are segregating for at least two different alleles of the EGF receptor gene: c-*erb*$B^I$ and c-*erb*$B^{II}$ (11, 20, 22a). Erythroblastosis-susceptible line $15_1$ chickens carry predominately c-*erb*$B^I$, while erythroblastosis-resistant K28 chickens carry mostly c-*erb*$B^{II}$. Intrigued by the possibility that c-*erb*$B^{II}$ might determine the resistance of K28 chicks to RAV-induced erythroblastosis, we analyzed (K28 × $15_1$) × K28 chicks that had been typed for c-*erb*$B^I$ and c-*erb*$B^{II}$ for susceptibility to RAV-1-induced erythroblastosis. The results of these analyses unambiguously demonstrated that c-*erb*$B^I$ and c-*erb*$B^{II}$ undergo comparable frequencies of erythroblastosis-inducing proviral insertions and viral transductions (20). Thus, the known polymorphisms in the EGF receptor gene do not determine susceptibility to RAV-induced erythroblastosis.

In this paper, we report that $15_1$ chickens have dominant gene(s) for susceptibility to RAV-1-induced erythroblastosis. We also report that $15_1$ chickens have dominant gene(s) for susceptibility to the induction of erythroblastosis by new c-*erb*B-transducing viruses.

## MATERIALS AND METHODS

**Chickens.** The K28 chickens used in this study are a noninbred subline of the K28 line of Kimber Farms (25). The K28 chicken is a hardy chicken that was selected at Kimber Farms for high egg production. The Robinson subline of K28 has been selected for susceptibility to subgroup E avian leukosis viruses (25) as well as for the presence of only one endogenous virus (1). $15_1$ chickens are an inbred line of White Leghorn chickens that are maintained at the Regional Poultry Research Laboratory, East Lansing, Mich. $15_1$ chickens are a subline of line 15 chickens (30). Line 15 chickens were selected for susceptibility to virus-induced cancers. $15_1$ chickens have low fertility, low hatchability, and low viability. Progeny $15_1$ × K28 crosses have high fertility, high hatchability, and high vigor.

**Viruses.** RAV-1 (27) was grown in cultured turkey cells. It was purified by endpoint dilution before its use in inoculations. AEV-R(RAV-1) was obtained by superinfection of AEV-ES4 nonproducer erythroblast cell lines with our stock of RAV-1. AEV-ES4 and AEV-R appear to be highly related if not the same isolate of an AEV (21). For convenience, we refer to viruses with either of these designations as AEV-R. AEV-R(RAV-1) was grown on cultured turkey embryo fibroblasts. Stocks of AEV-5005(RAV-1) and AEV-5009(RAV-1) (20) were grown in (K28 × $15_1$) × K28 chickens. Sera of chicks developing rapid-onset erythroblastosis after inoculation with AEV-5005(RAV-1) were used as

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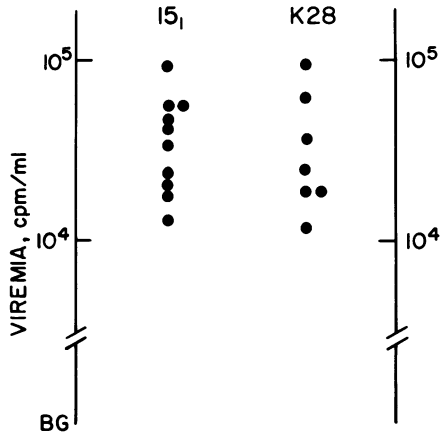


FIG. 1. Viremia in $15_1$ and K28 chicks at 1 month postinoculation with RAV-1. Viremia was tested for by assaying sera for the amount of particulate RNA-directed DNA polymerase. ●, Data from one chicken; BG, background.

stocks of AEV-5005. A filtered tumor homogenate (10% [wt/vol] filtered through a 0.22-μm membrane filter [Millipore Corp., Bedford, Mass.]) was used as a stock of AEV-5009.

**Oncogenicity tests.** Oncogenicity tests of RAV-1 were initiated by the intravenous inoculation of $\sim 10^6$ IU of RAV-1 into day-old chicks. Beginning at 6 weeks postinoculation, the onset of erythroblastosis was monitored by twice-weekly determination of hematocrits and examination of blood smears. Erythroblastosis was recognized by the presence of erythroblasts and polychromatic erythrocytes in blood smears. Most chickens died within 1 week of the detection of erythroblasts in their peripheral blood (20). Some chickens with erythroblastosis underwent an initial rise in hematocrit. As the disease progressed, hematocrits tended to fall. Birds were sacrificed when moribund. At the time of sacrifice, spleens, livers, and bursas were weighed and saved for histological analyses. Spleens, livers, and bursas of recently dead birds were also weighed and saved for histological analyses. Oncogenicity tests of AEV-R, AEV-5005, and AEV-5009 were conducted in a manner similar to those with RAV-1. However, in tests with these viruses, 1-week-old $15_1$ × K28 or K28 chicks were inoculated with virus, and tests for the onset of erythroblastosis were initiated within 2 weeks of inoculation.

**Viremia.** Viremia was determined by assaying sera for particulate RNA-directed DNA polymerase (23). In these assays, virus was collected from serum by pelleting through a 40% glycerol pad. [$^3$H]dGTP was used as a substrate, and poly(rC) · oligo(dG)$_{12-16}$ was used as a template-primer.

***B* haplotypes.** Chickens were typed for *B* haplotypes by incubating erythrocytes with antisera specific for the B5 or B13 histocompatability antigens (4). Incubations were performed in microtiter plates, and agglutination of the erythrocytes was assayed by observing whether the erythrocytes became clumped in the well. In cases of uncertainty, erythrocytes were observed under low-power magnification. The $15_1$ × K28 rooster (3924) used to sire (K28 × $15_1$) × K28 chicks was $B^5/B^{13}$. The K28 hens used in the backcross were $B^{13}/B^{13}$.

**Statistical analyses.** To determine whether differences in the observed incidences of disease were significant, data were analyzed by the chi-square test. An estimate of the expected incidence of disease was obtained by combining the data for the pedigrees being compared (8). The observed incidences of disease were then compared with the expected incidence to determine whether observed differences were significant.

## RESULTS

**$15_1$ and K28 chicks have comparable susceptibilities to RAV-1 infections, and K28 chicks are susceptible to AEV-R-induced erythroblastosis.** To determine whether $15_1$ chicks might have a higher susceptibility than K28 chicks to RAV-1 infections, RAV-1-inoculated $15_1$ and K28 chicks were tested for viremia. At both 1 and 2 months postinoculation, $15_1$ and K28 chicks exhibited viremias that ranged from $10^4$ to $10^5$ cpm/ml of particulate RNA-directed DNA polymerase. At 1 month postinoculation, the median level of viremia in $15_1$ chicks was $3.7 \times 10^4$ cpm/ml, and the median level of viremia in K28 chicks was $2.5 \times 10^4$ cpm/ml (Fig. 1). Of the $15_1$ and K28 chicks tested for viremia at both 1 and 2 months postinoculation, the level of particulate reverse transcriptase dropped more than twofold in one of seven $15_1$ chicks and in one of six K28 chicks. Thus, the difference in susceptibility of $15_1$ and K28 chicks to RAV-1-induced erythroblastosis is not associated with a difference in the susceptibility of these pedigrees to RAV-1 infections.

To determine whether K28 chicks were susceptible to the induction of erythroblastosis by AEV-R (a c-*erb*A- and c-*erb*B-transducing virus) (34), day-old and week-old K28 chicks were inoculated intravenously with $\sim 2 \times 10^4$ focus-forming units of AEV-R(RAV-1). Within 2 to 3 weeks postinoculation, all of the chicks had succumbed to erythroblastosis (Table 1) (24). This result indicates that day-old and week-old K28 chicks contain cells that are targets for the induction of erythroblastosis.

**Susceptibility to RAV-1-induced erythroblastosis is a dominant trait of $15_1$ chickens.** At the time our work on the susceptibility of $15_1$ chickens to RAV-1-induced erythroblastosis was undertaken, it was known that the $B^5$ haplotype of $15_1$ was permissive for RAV-1-induced erythroblastosis and that this permissiveness was a recessive trait (3). To determine whether susceptibility to RAV-1-induced erythroblastosis might also be encoded by dominant genes carried in $15_1$, ($15_1$ × K28) × K28 chicks were tested for susceptibility to RAV-1-induced erythroblastosis ($15_1$ × K28 chicks were not tested because our one $15_1$ hen was only sporadically fertile). Within the same time frame (a 3-year period) and with the same stock of RAV-1, $15_1$ chicks and K28 chicks were tested for their susceptibility to RAV-1-induced erythroblastosis. Table 2 shows that 79% of the $15_1$, 1% of the K28, and 24% of the backcross chicks developed RAV-1-induced erythroblastosis. The 24% incidence of RAV-1-induced erythroblastosis in the (K28 × $15_1$) × K28

TABLE 1. Susceptiblity to transformation by c-*erb*B-transducing viruses is a dominant trait of $15_1$ chickens[a]

| Virus | Transduced sequences | Susceptibility of inoculated chicken (no. susceptible/no. tested) | |
|---|---|---|---|
| | | $15_1$×K28 | K28 |
| AEV-5005 | c-*erb*B | 10/10 | 0/10 |
| AEV-5009 | c-*erb*B | 5/5 | 0/6 |
| AEV-R | c-*erb*A, c-*erb*B | NT | 7/7 |

[a] One-week-old chicks were inoculated intravenously with the indicated viruses. NT, Not tested.

chicks demonstrates that susceptibility to RAV-1-induced erythroblastosis is a dominant trait of $15_1$ chickens.

In the pathogenicity tests, $15_1$ chickens developed erythroblastosis almost exclusively, while K28 chickens developed B-cell lymphoma almost exclusively. (K28 × $15_1$) × K28 chickens developed high incidences of both erythroblastosis and B-cell lymphoma (Table 2). The median latency for morbidity due to erythroblastosis occurred at 7 to 9 weeks postinoculation (Fig. 2). In contrast, the median latency for morbidity due to lymphoma was 16 to 17 weeks postinoculation (Fig. 2). Thus, RAV-1-induced erythroblastosis occurred after a 1- to 2-month-shorter latency than RAV-1-induced B-cell lymphoma.

Since none of the $15_1$ birds survived long enough to be at risk for the development of lymphoma, our data do not address the susceptibility of this line to RAV-1-induced lymphoma. Interestingly, however, (K28 × $15_1$) × K28 chicks that did not succumb to erythroblastosis developed a higher incidence of lymphoma ($P < 0.01$) and kidney tumors ($P < 0.05$) than K28 chicks (Table 2, Fig. 3). These results indicate that $15_1$ chickens carry dominant gene(s) that confer an unusually high susceptibility to RAV-1-induced lymphoma and kidney tumors. Whether these gene(s) are the same as those that confer susceptibility to RAV-1-induced erythroblastosis is a subject of current study.

**The $B^5$ haplotype of $15_1$ chickens is not dominant for susceptibility to RAV-1-induced erythroblastosis.** To rule out the possibility that the $B^5$ haplotype is a dominant gene for susceptibility to RAV-1-induced erythroblastosis, nine of the (K28 × $15_1$) × K28 chicks that developed erythroblastosis were tested for the B5 histocompatability antigens of $15_1$ and the B13 histocompatability antigens of K28. Seven of these were found to have B5 and B13 antigens, while two were found to have only B13 antigens. Thus, the $B^5$ haplotype is not dominant for susceptibility to RAV-1-induced erythroblastosis.

**Susceptibility to transformation by new c-*erb*B-transducing viruses is a dominant trait of $15_1$ chickens.** As a by-product of our work on susceptibility to RAV-1-induced erythroblastosis, we had acquired 18 new c-*erb*B-transducing viruses (20; work in progress). As we began to analyze the pathogenicity of these new viruses, we came to appreciate that most had pathogenic potentials that were different from those of AEV-R and AEV-H (7, 16). Both AEV-R and AEV-H induce an easily recognized transformation of cultured cells (13, 16); our new AEVs did not (32). Both AEV-R and AEV-H cause fibrosarcoma (12, 13, 16); our new AEVs have not induced a single fibrosarcoma (32). In the initial passages of the new AEVs, (K28 × $15_1$) × K28 chicks were used for inoculations. Since some but not all of these chicks had developed rapid-onset erythroblastosis and since the new AEVs had pathogenic potentials that were different from those of AEV-R and AEV-H, it was decided to test whether the new AEVs could cause erythroblastosis in K28.

TABLE 2. Susceptibility to RAV-1-induced erythroblastosis is a dominant trait of $15_1$ chickens[a]

| Pedigree | No. inoculated | % Survivors | Deaths from[b]: | | | |
|---|---|---|---|---|---|---|
| | | | Ery | Lym | Kid | Misc |
| $15_1$ | 14 | 0 | 79 | 0 | 0 | 21 |
| K28 | 87 | 43 | 1 | 39 | 0 | 17 |
| (K28×$15_1$)×K28 | 82 | 4 | 24 | 46 | 6 | 19 |

[a] Data are for the first six months of the oncogenicity tests. Backcross chicks were the progeny of $15_1$×K28 rooster 3924 mated with eight different K28 hens. Homogeneity chi-square analyses showed that the incidence of erythroblastosis and lymphoma in K28 and (K28×$15_1$)×K28 chickens differed significantly ($P < 0.1$). These analyses also indicated that the difference in the incidence of kidney tumors in K28 and (K28×$15_1$)×K28 are significant ($P < 0.05$).

[b] Abbreviations: Ery, erythroblastosis; Lym, lymphoma; Kid, kidney tumors; Misc, deaths due to miscellaneous causes.

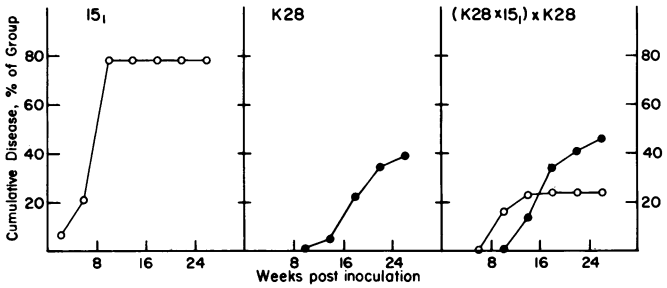


FIG. 2. Time course of development of erythroblastosis and lymphoma in RAV-1-inoculated chicks. All pedigrees were inoculated intravenously at 1 day of age with ~1 × $10^6$ IU of RAV-1. Symbols: ○, erythroblastosis; ●, lymphoma.

Week-old $15_1$ × K28 and week-old K28 chicks were inoculated intravenously with two of the new AEVs, AEV-5005 and AEV-5009. Within 4 to 6 weeks, all of the $15_1$ × K28 chicks but none of the K28 chicks had developed erythroblastosis (Table 1). This experiment dramatically demonstrated that new isolates of c-*erb*B-transducing viruses cause erythroblastosis in $15_1$ × K28 but not K28 chicks. Thus, $15_1$ chickens appear to have a dominant gene (or genes) that confers susceptibility to the induction of erythroblastosis by c-*erb*B sequences.

## DISCUSSION

Evidence has been presented that the unusual susceptibility of $15_1$ chickens to RAV-1-induced erythroblastosis is a dominant trait (Table 2). Evidence is also presented that $15_1$ chickens have dominant gene(s) for susceptibility to the induction of erythroblastosis by new c-*erb*B-transducing viruses (Table 1). Our current hypothesis is that both of

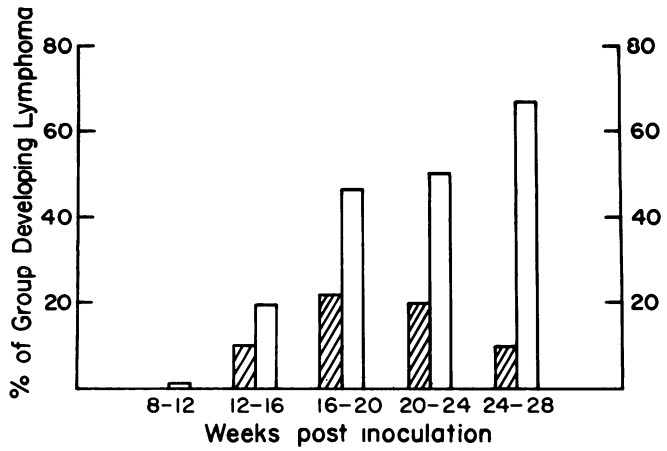


FIG. 3. Susceptibility of K28 and (K28 × $15_1$) × K28 chicks to RAV-1-induced lymphoma. For each interval, the incidence of lymphoma = (the number of chickens developing lymphoma)/(the number of chickens alive at the beginning of the interval) × 100. Symbols: ▨, K28 test groups; ▭, (K28 × $15_1$) × K28 test groups.

these dominant traits are encoded by a single gene that potentiates the transformation of erythroid cells by the transmembrane and cytoplasmic domains of the EGF receptor. Interestingly, the role of the $15_1$ chicken gene (or genes) in potentiating the induction of erythroblastosis may be able to be superseded by sequences transduced from a second proto-oncogene (c-*erb*A) (Table 1). In view of these findings, we suggest that the $15_1$ gene for susceptibility to c-*erb*B-induced erythroblastosis is a cancer-predisposing mutation in a proto-oncogene.

**Susceptibility to RAV-1-induced erythroblastosis and the frequency of proviral insertions in c-*erb*B.** When we undertook the study of why $15_1$ chickens are susceptible to RAV-1-induced erythroblastosis, our tacit assumption was that susceptibility to erythroblastosis reflected a high frequency of proviral insertions in the EGF receptor gene of $15_1$ chicks. We now believe that the frequency of RAV-1 insertions in the EGF receptor genes of erythroblastosis-susceptible and -resistant chicks does not determine differences in susceptibility to RAV-1-induced erythroblastosis. Two lines of evidence support this belief.

First, $15_1$ and K28 chickens appear to be equally susceptible to RAV-1 infections. Under our experimental conditions, comparable levels and frequencies of persistent viremias were established in RAV-1-inoculated $15_1$ and K28 chickens (Fig. 1). Second, the most frequent allele of the EGF receptor gene in $15_1$ chickens (c-*erb*$B^I$) has a polymorphism that can be distinguished by *Eco*RI restriction endonuclease digests from the most frequent allele of the EGF receptor gene of K28 chickens (c-*erb*$B^{II}$) (11, 20, 22a). The backcross reported in Table 2 was done with a (K28 $\times$ $15_1$) rooster heterozygous for c-*erb*$B^I$ and c-*erb*$B^{II}$ crossed with K28 hens homozygous for c-*erb*$B^{II}$. In the backcross, susceptibility to RAV-1-induced erythroblastosis did not segregate with c-*erb*$B^I$ (20). Thus, the frequency of proviral insertions in the EGF receptor genes of $15_1$ and K28 chickens does not determine the >10-fold difference between these two pedigrees in susceptibility to RAV-1-induced erythroblastosis.

**Susceptibility to RAV-1-induced erythroblastosis and susceptibility to transformation by c-*erb*B.** Under conditions of a high multiplicity of infection, approximately 50% of the cases of RAV-1-induced erythroblastosis contain new erythroblastosis-inducing transductions of c-*erb*B (20). Two of these new viruses (AEV-5005 and AEV-5009) caused rapid-onset erythroblastosis in $15_1$ $\times$ K28 chicks but not in K28 chicks (Table 1).

Differences in the number of target cells for erythroblastosis induction could account for the relative susceptibilities of $15_1$ $\times$ K28 and K28 chickens to erythroblastosis induction by AEV-5005 and AEV-5009. However, K28 chicks are highly susceptible to AEV-R-induced erythroblastosis (Table 1). The susceptibility of K28 chicks to AEV-R-induced erythroblastosis demonstrates that K28 chicks have at least some target cells for the induction of erythroblastosis. Also, tests of AEV-5005 for transformation of bone marrow cells cultured from chickens that are resistant to RAV-1-induced erythroblastosis have been negative (T. Graf, personal communication). These bone marrow cultures contained many cells that are targets for transformation by AEV-R. In these cultures, cells that are targets for AEV-R transformation most likely underwent infection by the new AEVs, yet no transformation was observed. Therefore, we think that cells that are targets for the induction of erythroblastosis by AEV-R are not necessarily targets for transformation by the new AEVs.

Alternatively, the $15_1$ gene for susceptibility to erythroblastosis could determine whether c-*erb*B sequences are able to transform erythroid cells. Two lines of circumstantial evidence support this possibility. First, AEV-R, an avian erythroblastosis virus that induces erythroblastosis in virtually all chickens, contains sequences transduced from two cellular genes: c-*erb*A and c-*erb*B (34). The transduced c-*erb*B sequences appear to determine the ability of AEV-R to induce erythroblastosis, while the transduced c-*erb*A sequences appear to enhance this potential (9, 28). Thus, an AEV that has been highly selected for its ability to induce erythroblastosis has acquired a cellular gene that potentiates the ability of c-*erb*B to induce erythroblastosis. By analogy, might not chickens that have been selected for susceptibility to viral-induced cancers also have acquired a gene that potentiates the ability of c-*erb*B sequences to cause erythroblastosis?

Second, the dominant gene(s) of $15_1$ for susceptibility to RAV-1-induced erythroblastosis may also confer unusual susceptibility to RAV-1-induced lymphoma and RAV-1-induced kidney tumors (Table 2, Fig. 3). The action of proto-oncogenes in the control of cell growth appears to be interactive (14, 17–19). If $15_1$ chickens contain a nonlethal yet cancer-predisposing mutation in a proto-oncogene, one might predict that they would be unusually susceptible to more than one cancer. At present, the possibility exists that $15_1$ chickens do indeed carry a dominant gene that predisposes them to several cancers.

**Susceptibility and latency.** Chickens develop RAV-1-induced erythroblastosis after a much shorter latency than is required for the development of RAV-1-induced lymphoma (Fig. 2). However, only a few pedigrees of chickens are susceptible to RAV-1-induced erythroblastosis while many pedigrees are susceptible to RAV-1-induced lymphoma. If the latency for RAV-1-induced erythroblastosis is so short, why don't more chickens suffer RAV-1-induced erythroblastosis?

Both RAV-1-induced erythroblastosis and RAV-1-induced lymphoma are caused by proviral insertions in proto-oncogene sequences: c-*erb*B in the case of erythroblastosis (11, 20, 22a) and c-*myc* in the case of lymphoma (10, 15, 22). Interestingly, the longer latency for the induction of c-*myc*-associated disease by proviral insertions is also observed for infections with c-*myc*-transducing viruses. When K28 chicks are inoculated with AEV-R or MC29 (a c-*myc*-transducing virus), AEV-R-induced disease precedes MC29-induced disease by 4 to 6 weeks (24). Similarly, inoculation of $15_1$ $\times$ K28 chicks with new transductions of c-*erb*B (AEV-5005, AEV-5009) or c-*myc* (MYC-3475) results in the occurrence of AEV-5005- or AEV-5009-induced erythroblastosis 4 to 8 weeks before the occurrence of MYC-3475-induced myelocytomatosis (work in progress).

An explanation for both the short latency and pedigree-specific susceptibility to c-*erb*B-induced erythroblastosis is that the relatively short life span of erythroid cells (35) restricts the occurrence of erythroblastosis to pedigrees in which one event, the abnormal expression of c-*erb*B, is sufficient to cause disease. If this is so, then most chickens may be resistant to RAV-1-induced erythroblastosis because the abnormal expression of c-*erb*B is not sufficient (in and of itself) to transform their erythroid cells. Accordingly, the rare pedigrees that are susceptible to RAV-1-induced erythroblastosis may be pedigrees that have acquired a mutation that potentiates the ability of c-*erb*B to transform erythroid cells. In contrast, the relatively long life spans of lymphatic cells (36) may allow c-*myc*-associated disease to

occur in hosts in which more than one rare event is required for disease induction. Consistent with this notion is the observation that c-*erb*B-transducing viruses cause polyclonal tumors (20), while c-*myc*-transducing viruses are associated with clonal or oligoclonal tumors (work in progress).

**$B^5$ haplotype of $15_1$ and susceptibility to RAV-1-induced erythroblastosis.** The $B^5$ haplotype of $15_1$ has been shown to be permissive for the induction of erythroblastosis by RAV-1. We do not think that $B^5$ is the dominant gene of $15_1$ that influences the frequency of the development of RAV-1-induced erythroblastosis because (i) $B^5$ is recessive for the development of erythroblastosis (3), and (ii) $B^5$ did not segregate with susceptibility to erythroblastosis in (K28 × $15_1$) × K28 chicks.

**Genes that affect the susceptibility of mice to viral-induced erythroleukemias.** At least eight genes affect the susceptibility of mice to the induction of erythroleukemias by murine retroviruses (for review, see reference 31). Of these, seven are recessive for susceptibility to virus-induced erythroleukemias. Three of these (*Fv-1*, *Fv-4*, and *Fv-6*) affect the spread of virus in infected hosts, two (*H-2* and *Rfv-3*) influence the immune response of the host to the infection, and two (*Sl* and *W*) appear to affect erythropoiesis. One, *Fv-2*, is dominant for susceptibility to viral-induced erythroleukemia. The product of the *Fv-2* allele appears to affect both virus replication in hematopoietic cells as well as the differentiation of hematopoietic cells. We think it unlikely that the $15_1$ gene for susceptibility to erythroblastosis is a homolog of *Fv-2*. The *Fv-2* allele is found in most mice (both inbred and wild). In contrast, the $15_1$ gene(s) for susceptibility to erythroleukemia occurs only in some inbred chickens.

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## LITERATURE CITED

1. **Astrin, S. M., and H. L. Robinson.** 1979. *Gs*, an allele of chickens for endogenous avian leukosis viral antigens, segregates with *ev* 3, a genetic locus that contains structural genes for virus. J. Virol. **31**:420–425.
2. **Bacon, L. D., L. B. Crittenden, R. L. Witter, A. Fadly, and J. Motta.** 1983. $B^5$ and $B^{15}$ are associated with progressive Marek's disease, Rous sarcoma, and avian leukosis virus-induced tumors in inbred $15I_4$ chickens. Poult. Sci. **62**:573–578.
3. **Bacon, L. D., R. L. Witter, L. B. Crittenden, A. Fadly, and J. Motta.** 1981. *B* Haplotype influence on Marek's disease, Rous sarcoma, and lymphoid leukosis virus-induced tumors in chickens. Poult. Sci. **60**:1132–1139.
4. **Briles, W. E., N. Bumstead, D. L. Ewert, D. G. Gilmour, J. Gogusev, K. Hala, C. Koch, B. M. Longenecker, A. W. Nordskog, J. R. L. Pink, L. W. Schierman, M. Simonsen, A. Toivanen, P. Toivanen, O. Vainio, and G. Wick.** 1982. Nomenclature for chicken major histocompatibility (B) complex (English). Immunogenetics **15**:441–447.
5. **Coffin, J. M., H. E. Varmus, J. M. Bishop, M. Essex, W. D. Hardy, Jr., G. S. Martin, N. E. Rosenberg, E. M. Scolnick, R. A. Weinberg, and P. K. Vogt.** 1981. Proposal for naming host cell-derived inserts in retrovirus genomes. J. Virol. **40**:953–957.
6. **Downward, J., Y. Yarden, E. Mayes, G. Scrace, N. Totty, P. Stockwell, A. Ullrich, J. Schlessinger, and M. D. Waterfield.** 1984. Close similarity of epidermal growth factor receptor and v-*erb*B oncogene protein sequences. Nature (London) **307**:521–526.
7. **Engelbreth-Holm, J., and A. Rothe-Meyer.** 1932. Bericht über neue Erfahrungen mit einem Stamm Hubner-Erythroleukose. Acta Pathol. Microbiol. Scand. **9**:293–312.
8. **Freund, J. E.** 1970. Differences among proportions, p. 245–249. *In* Statistics, a first course. Prentice-Hall, Inc. Englewood Cliffs, N.J.
9. **Frykberg, L., S. Palmieri, H. Beug, T. Graf, M. J. Hayman, and B. Vennstrom.** 1983. Transforming capacities of avian erythroblastosis virus mutants deleted in the *erb*A or *erb*B oncogenes. Cell **32**:227–238.
10. **Fung, Y.-K. T., A. M. Fadly, L. B. Crittenden, and H.-J. Kung.** 1981. On the mechanism of retrovirus-induced avian lymphoid leukosis: deletion and integration of the provirus. Proc. Natl. Acad. Sci. U.S.A. **78**:3418–3422.
11. **Fung, Y.-K. T., W. G. Lewis, L. B. Crittenden, and H.-J. Kung.** 1983. Activation of the cellular oncogene c-*erb*B by LTR insertion: molecular basis for induction of erythroblastosis by avain leukosis virus. Cell **33**:357–368.
12. **Graf, T., D. Fink, H. Beug, and B. Royer-Pokora.** 1977. Oncornavirus-induced sarcoma formation obscured by rapid development of lethal leukemia. Cancer Res. **37**:59–63.
13. **Graf, T., B. Royer-Pokora, G. E. Schubert, and H. Beug.** 1976. Evidence for the multiple oncogenic potential of cloned leukemia virus: *in vitro* and *in vivo* studies with avian erythroblastosis virus. Virology **71**:422–433.
14. **Greenberg, M. E., and E. B. Ziff.** 1984. Stimulation of 3T3 cells induces transcription of the c-*fos* proto-oncogene. Nature (London) **311**:433–438.
15. **Hayward, W. S., B. G. Neel, and S. M. Astrin.** 1981. ALV-induced lymphoid leukosis: activation of a cellular *onc* gene by promoter insertion. Nature (London) **290**:475–480.
16. **Hihara, H., H. Yamamoto, H. Shimohira, K. Avai, and T. Shimizu.** 1983. Avian erythroblastosis virus isolated from chick erythroblastosis induced by avian lymphatic leukemia virus subgroup A. J. Natl. Cancer Inst. **70**:891–897.
17. **Kelly, K., B. H. Cochran, C. D. Stiles, and P. Leder.** 1983. Cell-specific regulation of the c-*myc* gene by lymphocyte mitogens and platelet-derived growth factor. Cell **35**:603–610.
18. **Land, H., L. F. Parada, and R. A. Weinberg.** 1983. Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature (London) **304**:596–602.
19. **Land, H., L. F. Parada, and R. A. Weinberg.** 1983. Cellular oncogenes and multistep carcinogenesis. Sciences **222**: 771–778.
20. **Miles, B. D., and H. L. Robinson.** 1985. High-frequency transduction of c-*erb*B in avian leukosis virus-induced erythroblastosis. J. Virol. **54**:295–303.
21. **Nishida, T., S. Sakamoto, T. Yamamoto, M. Hayman, S. Kawai, and K. Toyoshima.** 1984. Comparison of genome structures among 3 different strains of avian erythroblastosis viruses. Gann **75d**:325–333.
22. **Payne, G. S., J. M. Bishop, and H. E. Varmus.** 1982. Multiple arrangements of viral DNA and an activated host oncogene (c-*myc*) in bursal lymphomas. Nature (London) **295**:209–213.

22a. **Raines, M. A., W. G. Wynne, L. B. Crittenden, and H.-J. Kung.** 1985. c-*erb*B activation in avian leukosis virus-induced erythroblastosis: clustered integration sites and the arrangement of provirus in the c-*erb*B alleles. Proc. Natl. Acad. Sci. U.S.A. **82**:2287–2291.

23. **Robinson, H. L.** 1976. Intracellular restriction on the growth of induced subgroup E avian type C viruses in chicken cells. J. Virol. **18**:856–866.
24. **Robinson, H. L.** 1982. Retroviruses and cancer. Rev. Infect. Dis. **4**:1015–1025.
25. **Robinson, H. L., and W. F. Lamoreux.** 1976. Expression of endogenous ALV antigens and susceptibility to subgroup E ALV in three strains of chickens. Virology **69**:50–62.
26. **Robinson, H. L., M. N. Pearson, D. W. DeSimone, P. N. Tsichlis,**

**and J. M. Coffin.** 1979. Subgroup E avian leukosis virus associated disease in chickens. Cold Spring Harbor Symp. Quant. Biol. **44**:1133–1142.

27. **Rubin, H., and P. K. Vogt.** 1962. An avian leukosis virus associated with stocks of Rous sarcoma virus. Virology **17**:184–194.
28. **Sealy, L., M. L. Privalsky, G. Moscovici, C. Moscovici, and J. M. Bishop.** 1983. Site-specific mutagenesis of avian erythroblastosis virus—*erb*B is required for oncogenicity. Virology **130**:155–178.
29. **Sergeant, A., S. Saule, D. LePrince, A. Begue, C. Rommens, and D. Stehelin.** 1982. Molecular cloning and characterization of the chicken DNA locus related to the oncogene *erb*B of avian erythroblastosis virus. EMBO J. **1**:237–242.
30. **Stone, H. A.** 1975. Use of highly inbred chickens in research. U.S. Dep. Agric. Agric. Res. Ser. Tech. Bull. **1514**:1–22.
31. **Teich, N. A., J. Wyke, T. Mak, A. Bernstein, and W. Hardy.** 1984. Pathogenesis of retrovirus-induced disease, p. 871–876. *In* R. Weiss, N. Teich, H. Varmus, and J. Coffin (ed.), RNA tumor viruses. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
32. **Tracy, S. E., B. A. Woda, and H. L. Robinson.** 1985. Induction of angiosarcoma by a c-*erb*B transducing virus. J. Virol. **54**:304–310.
33. **Ullrich, A., L. Coussens, J. S. Hayflick, T. J. Dull, A. Gray, A. W. Tam, J. Lee, Y. Yarden, T. A. Lieberman, J. Schlessinger, J. Downward, E. L. V. Mayes, N. Whittle, M. D. Waterfield, and P. H. Seeburg.** 1984. Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature (London) **309**:418–425.
34. **Vennstrom, B., and J. M. Bishop.** 1982. Isolation and characterization of chicken DNA homologous to the two putative oncogenes of avian erythroblastosis virus. Cell **28**:135–143.
35. **Wintrobe, M. M., G. R. Lee, D. R. Boggs, T. C. Bithell, J. Foerster, J. W. Athens, and J. N. Lukens.** 1981. Origin and development of the blood and blood forming tissues, p. 35–74. *In* Clinical hematology. Lea & Febiger, Philadelphia.
36. **Wintrobe, M. M., G. R. Lee, D. R. Boggs, T. C. Bithell, J. Foerster, J. W. Athens, and J. N. Lukens.** 1981. The lymphatic system, p. 271–289. *In* Clinical hematology. Lea & Febiger, Philadelphia.
37. **Yamamoto, T., H. Hihara, T. Nishida, S. Kawaii, and K. Toyoshima.** 1983. A new avian erythroblastosis virus, AEV-H, carries *erb*B gene responsible for the induction of both erythroblastosis and sarcomas. Cell **34**:225–232.