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Induction of Angiosarcoma by a c-*erb*B Transducing Virus

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Recently, 12 new transductions of c-*erb*B have been identified in a series of Rous-associated virus type 1-induced erythroleukemias. During the passage of these new transducing viruses it has become apparent that the erythroleukemia in chicken 5005 contained two different c-*erb*B transducing viruses. One induces erythroblastosis, whereas the second induces angiosarcoma. The angiosarcoma- and erythroblastosis-inducing viruses appear to have had a common ancestor, since tumors induced by each contain a novel, 4.3-kilobase c-*erb*B-related *Eco*RI fragment. The angiosarcoma-inducing virus has been named avian angiosarcoma virus and is designated for the chicken in which it originated.

c-*erb*B is a cellular gene that contains sequences homologous to the v-*erb*B oncogene of avian erythroblastosis virus (AEV) (20, 22). Presumably v-*erb*B arose from the transduction of c-*erb*B sequences by an avian leukosis virus. Two transductions of c-*erb*B have been characterized for their DNA sequence and their oncogenic potential. One of these is found in the ES4 and R strains of AEV (AEV-R) (2, 4, 7, 15, 17, 19, 23), whereas the second is found in AEV-H (11, 24, 25). The transduced c-*erb*B sequences in AEV-R and AEV-H both encode similar proteins that appear to be a truncated form of the receptor for epidermal growth factor (1, 9, 10, 16–18).

AEV-R and AEV-H have similar oncogenic potentials. The primary disease induced by AEV-R and AEV-H is erythroblastosis. The erythroblastosis induced by AEV-R is more severe than that induced by AEV-H (11). This appears to be due to the presence in AEV-R of a second transduced sequence (v-*erb*A) that enhances the transforming potential of v-*erb*B (4). Both AEV-R and AEV-H also induce fibrosarcoma (7, 11). In AEV-H-infected chickens fibrosarcoma and erythroblastosis have similar times of onset, whereas in AEV-R-infected chickens fibrosarcoma occurs after a longer latency than erythroblastosis (4, 6). Both AEV-R and AEV-H transform erythroblasts present in cultured bone marrow or yolk sac cells (5, 8). Both can also induce foci of cultured chicken embryo fibroblasts and confer on fibroblasts the ability to grow when suspended in soft agar (7, 11).

Recently we have identified 12 new transductions of c-*erb*B in a series of Rous-associated virus type 1-induced erythroleukemias (14). Each of the new transducing viruses was identified by the presence of a spliced form of c-*erb*B sequences in the DNA of an erythroleukemia. Each of these spliced sequences had an *Eco*RI fragment of a different size. Six of the new transducing viruses were tested for their disease potential in 1-week-old chickens. Each induced rapid onset erythroblastosis. In every case, the induced tumor had the same novel fragment as the virus being tested for disease potential. The new erythroblastosis-inducing viruses were named AEVs and were designated for the chickens in which they originated (AEV-5005, AEV-5040, etc.).

In this paper we report the early passage history of four of the new AEVs: AEV-4884, AEV-5005, AEV-5009, and AEV-5040. Our results suggest that each of these AEVs is unlike AEV-R and AEV-H in that each has only a low potential for the transformation of cultured chicken embryo fibroblasts. Our results also indicate that the erythroleukemia in chicken 5005 contained two related, yet different, c-*erb*B-transducing viruses. One of these, AEV-5005, causes erythroblastosis. The second, designated avian angiosarcoma virus 5005 (AAV-5005), causes angiosarcoma.

MATERIALS AND METHODS

Virus stocks. Filtered tumor homogenates, sera from tumor-bearing birds, and the culture medium from infected chicken embryo fibroblasts were used as virus stocks. Tumor homogenates were prepared by homogenizing tumor-containing spleens in tissue culture medium (10%, wt/vol) with a Dounce homogenizer. Homogenates were clarified by low-speed centrifugation and then filtered through a 0.22-μm filter. Viruses were cultured from tumors by cocultivating minced tumor-containing spleens or bone marrow with 15_B *ev* 1 chicken embryo fibroblasts in Dulbecco modified Eagle medium supplemented with 10% tryptose phosphate broth, 4% calf serum, and 1% chicken serum. In each cocultivation, cultures were seeded with two- to threefold dilutions of the tumor suspension. Cultures were examined daily. The cultures that contained the largest amounts of tumor material and growing fibroblasts were passed when the fibroblasts reached confluence. At the first passage, cocultivations were overlaid with soft agar (growth medium plus 0.6% Bacto-agar [Difco Laboratories]). These cultures were fed biweekly (by overlaying the existing soft agar with soft agar that was slightly hypotonic) and passed weekly. At each passage, 2×10^6 cells were tested for their ability to grow when suspended in soft agar (growth medium plus 0.6% Bacto-agar). Both the soft agar overlays and the agar suspension conditions select for the growth of AEV-R-transformed cells. Low levels of dimethyl sulfoxide were used in some assays and agar overlays. However, the presence of dimethyl sulfoxide in the soft agar did not noticeably enhance the transformation of fibroblasts by AEV-R or the new AEVs.

Virus assays. Virus stocks were tested for their ability to induce foci on monolayers of cultured chicken embryo fibroblasts under our standard assay conditions used to visualize foci of AEV-R-transformed cells. These conditions involved infecting subconfluent monolayers of cells within 18 h of seeding, overlaying the infected cultures with hard agar (medium F10 supplemented with 10% tryptose phosphate broth, 4% calf serum, 1% chicken serum, and 0.9%

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Bacto-agar), and feeding the culture at biweekly intervals with slightly hypotonic hard agar. Foci were counted at 1 to 2 weeks after infection. To facilitate the observation of foci, assay plates were overlaid with tissue culture medium and allowed to stand in the room for at least 24 h. This procedure heightens the differences in the appearance of transformed and untransformed cells. AEV-R foci can be observed without this step; however none of the foci induced by the new AEVs was discernible before this step.

Oncogenicity tests. The new AEVs were tested for their oncogenic potential after intravenous or intramuscular inoculations into 1-week-old $(K28\times15_1)\times K28$ chickens (14). Most inoculations consisted of 0.2 ml of a virus stock. Inoculated chickens were observed daily. Beginning at 2 weeks post-inoculation, chickens that had received intravenous inoculations were examined twice weekly for falling hematocrits and the presence of erythroblasts and polychromatic erythrocytes in blood smears. Chickens that received inoculations in the wing web muscle underwent daily examination of the web beginning at 1 week post-inoculation. Birds that exhibited evidence of disease were sacrificed, and diseased and normal tissues were harvested for histological and biochemical analyses. Samples harvested for histological analyses were fixed in phosphate-buffered Formalin. Samples harvested for biochemical analyses were quickly frozen in liquid nitrogen and stored at −80°C. At the time of harvest, selected tumors were minced and cocultivated with chicken embryo fibroblasts for the recovery of virus. All oncogenicity tests were terminated at 8 weeks post-inoculation.

Analysis of tumor DNA. DNAs extracted from tumors were analyzed by restriction endonuclease digestion, agarose gel electrophoresis, and blotting onto nitrocellulose paper as described by Miles and Robinson (14). When isolating DNA from angiosarcoma, care was taken to remove blood clots from tumor samples. Angiosarcomas (which are remarkably tough) were homogenized with a polytron homogenizer.

Histological studies. Multiple hematoxylin- and eosin-stained microscopic sections of the tumor were examined. For immunohistological studies, frozen sections (4 μm) were dipped in acetone for 4 s and then stored at −20°C. Before use sections were fixed in acetone for 10 min, air dried, and rehydrated in phosphate-buffered saline (pH 7.4). Sections were then incubated at room temperature in 3% hydrogen peroxide to block endogenous peroxidase activity and incubated with swine serum followed by either rabbit anti-human factor VIII or rabbit anti-human placental lactogen at a dilution of 1:100. Finally, slides were incubated with diaminobenzidine and counterstained with 1% methylene blue. The anti-human factor VIII could not be used to identify chicken endothelial cells in Paraffin sections. All reagents were obtained from Dako Laboratories, Santa Barbara, Calif.

TABLE 1. Attempts to cultivate fibroblasts transformed by the new AEVs

AEV	Tumor used in cocultivation[a]	Days in culture	Colonies/ 10^6 cells[b]	Disease
AEV-4884	6079	32	<10	NT[c]
	6091	37	<10	NT
AEV-5005	6085	27	<10	NT
	6093	36	22	None
	6103	34	142	Angiosarcoma
AEV-5009	6117	34	25	NT
AEV-5040	4961	45	73	Erythroblastosis
	6101	27	31	NT

[a] Tumors are designated by the number of the chicken from which they were harvested.
[b] Colonies were formed by cells that could grow when suspended in soft agar.
[c] NT, Not tested.

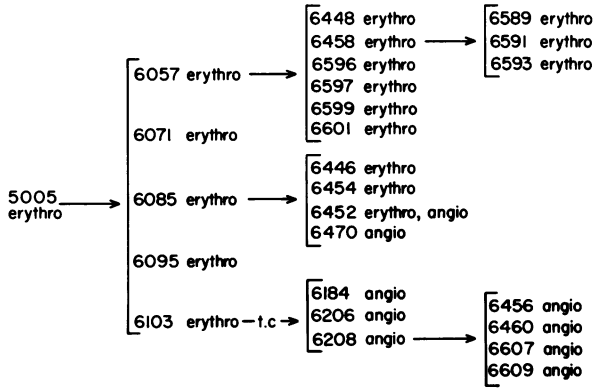

FIG. 1. Disease potential of the c-*erb*B transducing viruses originating in chicken 5005. Numbers refer to individual chickens. Symbols: →, use of a tumor homogenate or serum for a virus stock; ——t.c.→, use of medium harvested from the cocultivation of a tumor with chicken embryo fibroblasts as a virus stock.

RESULTS

Isolation of an angiosarcoma-inducing virus. To obtain high-titer stocks of the new c-*erb*B transducing viruses, eight erythroleukemias were cocultivated with chicken embryo fibroblasts. Cocultivations were selected for transformed cells by overlaying them with soft agar under conditions that select for AEV-R-transformed fibroblasts. At weekly intervals cultures were assayed for the number of transformed cells by testing 2×10^6 cells from the cocultivation for their ability to grow when suspended in soft agar. After 3 weeks of culture, the cocultivations from chicken 6103 (inoculated with AEV-5005) and chicken 4691 (inoculated with AEV-5040) contained small numbers of cells that grew when suspended in soft agar (Table 1, Fig. 1). Colonies were harvested from the soft agar suspension and seeded onto fresh fibroblasts. Approximately 24 h after seeding, these cultures were overlaid with soft agar. After 2 weeks, cultures were passed and maintained in growth medium until the cultures were confluent. At this point the culture medium was harvested for a virus stock. Neither the cultures under soft agar nor those in liquid medium contained cells that appeared transformed. However, stocks from the cocultivations of chickens 6103 and 4691 induced small numbers of foci (10^2 to 10^3 focus-forming units per ml), which could be detected by an experienced observer. These foci were unlike those caused by AEV-R in that the cells in the foci were both more fusiform and less refractile than cells in AEV-R-induced foci.

The stock cultured from the erythroleukemia in chicken 6103 was inoculated intravenously into three 1-week-old chickens and into the wing web muscle of two 1-week-old chickens. All of the chickens inoculated intravenously developed angiosarcoma (Fig. 1). Neither of the birds inoculated in the wing web developed disease. The diagnosis of the angiosarcoma as well as the description of further

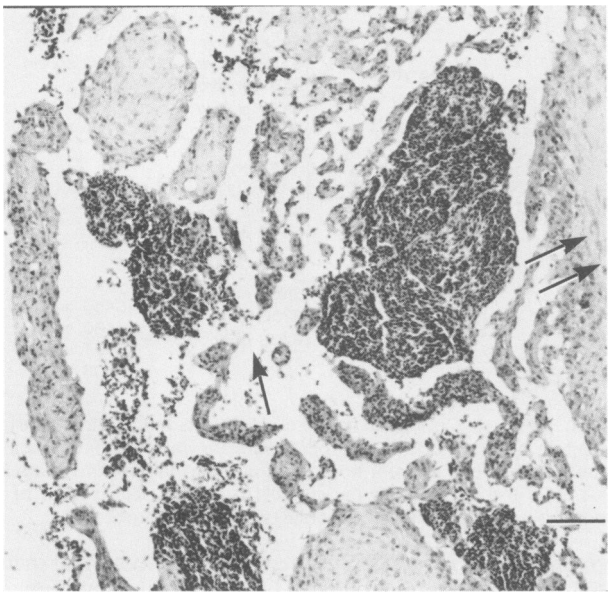

FIG. 2. Pathological patterns in AAV-5005-induced angiosarcoma. The micrograph shows an angiosarcoma composed of freely anastomosing vascular channels (single arrow). A spindle cell component is seen at the periphery of the lesion (double arrows). Bar, 40 μm.

passages of this stock (containing the virus we now designate AAV-5005) is presented below. The stock cultured from the erythroleukemia in chicken 4691 was inoculated intravenously into seven 1-week-old chickens and into the wing web muscles of five 1-week-old chickens. Five of the seven chickens inoculated intravenously developed erythroblastosis. None of the chickens inoculated in the wing web developed tumors.

Diagnosis of AAV-5005-induced angiosarcoma. Three chickens (6206, 6208, 6184) were inoculated intravenously with the virus stock cultured from the erythroleukemia in chicken 6103 (Fig. 1). At 7 weeks post-inoculation the hematocrits of these chickens began to decline. Although cells typical of erythroblastosis were not readily discerned in blood smears, the chickens were sacrificed and examined for disease. On autopsy many sites of internal bleeding were evident. The spleens were enlarged and filled with large hemorrhagic masses. Hemorrhagic tumors were also found embedded in muscle, skin, and thymus.

Histological evaluation of the lesions in chickens 6206, 6208, and 6184 and lesions found in subsequent passage of AAV-5005 (Fig. 1) showed tumors with various morphological patterns. Some of the well-differentiated lesions showed blood-filled spaces lined by flat to cuboidal endothelial cells. The lining cells formed short, delicate, papillae that projected into the lumina. Other tumors (Fig. 2) were composed of freely anastomosing vascular channels lined by elongated endothelial cells. Tufted papillar projections lined by atypical, hyperchromic endothelial cells were often seen (Fig. 3). These tumors contained variable amounts of spindle cells that merged with the vascular component (Fig. 2). Some tumors were composed predominantly of spindle cells with slitlike spaces containing extravasated erythrocytes (Fig. 4). This histological pattern is similar to that described in Kaposi's sarcoma (21). The tumors also contained components composed of polygonal cells embedded in an abundant extracellular matrix resembling immature mesenchymal elements (Fig. 5). Thus, the histological features of these tumors were similar to those described for angiosarcoma, a malignant tumor of endothelial cell origin (3).

Endothelial cells are known to synthesize the blood-clotting protein factor VIII. Consequently the presence of factor VIII in a cell can be used to confirm the endothelial nature of a cell (13). Using immunohistological techniques on frozen sections of various chicken organs, we determined that

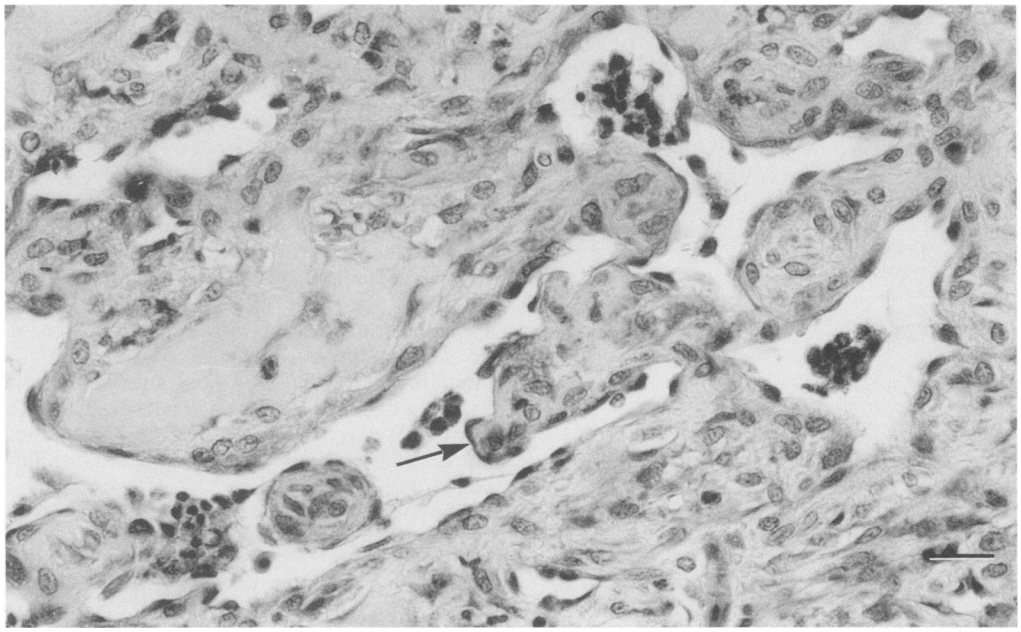

FIG. 3. Pathological patterns in AAV-5005-induced angiosarcoma. The micrograph shows intravascular tufted papillary projections lined by hyperchromatic, atypical endothelial cells (arrow). Bar, 20 μm.

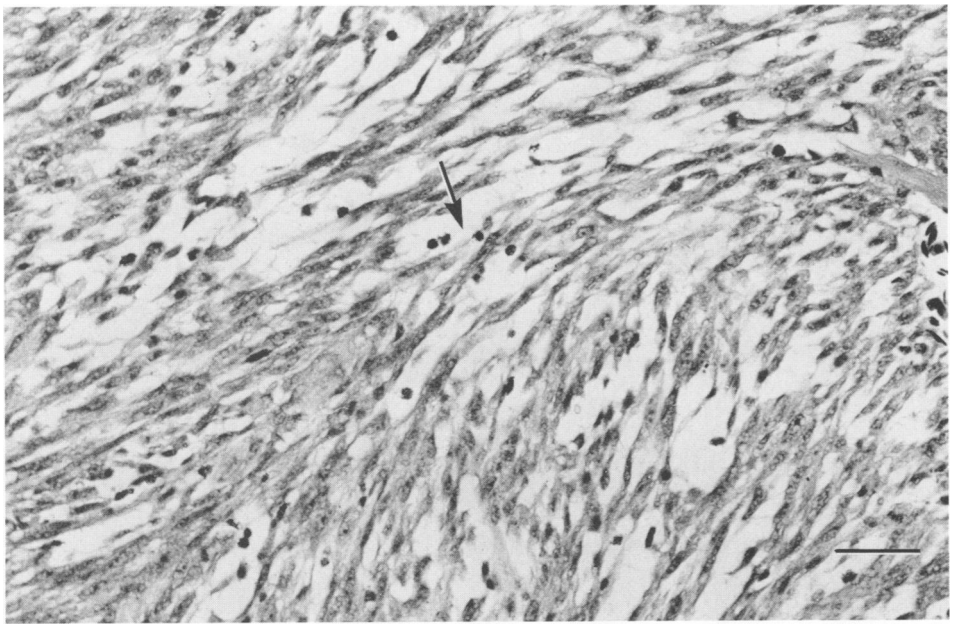

FIG. 4. Pathological patterns in AAV-5005-induced angiosarcoma. Some tumors showed a prominent spindle cell component. These tumors contained slitlike spaces that contained erythrocytes (arrow). Bar, 20 μm.

factor VIII could be easily localized in chicken endothelial cells. This finding indicated that human and chicken factor VIII were antigenically cross-reactive and that the anti-human factor VIII could be used to identify chicken endothelial cells. In the well-differentiated endothelial tumors, factor VIII was localized in the cells lining the vascular spaces (Fig. 6B). These cells were not stained by a control antibody (Fig. 6C). Interestingly, the anti-factor VIII did not stain the poorly differentiated areas of the tumor that contained spindle cells. Thus the less differentiated areas of the tumor appeared to not express factor VIII.

Production of a high-titer stock of AAV-5005 and testing of this stock for disease potential. A filtered homogenate of the angiosarcoma in chicken 6208 was inoculated intravenously into three 1-week-old chickens (Fig. 1). Each of these chickens succumbed to rapid-onset angiosarcoma. The angiosarcoma in chicken 6456 (Fig. 1) was minced and cocultivated with fibroblasts. The stock from this cocultivation had a high titer of focus-forming units (5×10^6/ml). These foci had a similar appearance to the foci caused by the stock harvested from the cocultivation of the erythroleukemia in chicken 6103 (Fig. 1). The high-titer stock cultured from the angiosarcoma in chicken 6456 was tested for its disease potential in 1-week-old chickens. In these tests, $\sim 10^6$ focus-forming units of virus were inoculated either intravenously or into the wing web muscle of 1-week-old chickens. Each of the four chickens inoculated intravenously succumbed to angiosarcoma between 34 and 39 days after inoculation. Thus a latency period of 4 to 6 weeks appears to be a minimum latency period for the onset of angiosarcoma. Three chickens received wing web inoculations; two of these succumbed to angiosarcoma at 31 and 34 days post-inoculation, and one did not develop disease. Thus no evidence could be found for AAV-5005 having the potential to induce fibrosarcoma.

Each of the 19 chickens that received an intravenous inoculation of a virus stock containing AAV-5005 has developed rapid-onset angiosarcoma. Angiosarcoma has occurred at multiple sites in each afflicted chicken. These sites have included the spleen, muscle, mesenteries, skin, thymus, liver, and pericardium. Lesions have varied in size from <1 by 1 cm to >2 by 5 cm. The histological analyses of these lesions have revealed that each has been a vascular lesion with the histological characteristics of angiosarcoma.

Blood smears of birds succumbing to angiosarcoma have been scrutinized for cells that are characteristic of erythroblastosis. Each of the 19 chickens that succumbed to angiosarcoma has had large numbers of reticulocytes in its blood. We think that the large number of reticulocytes

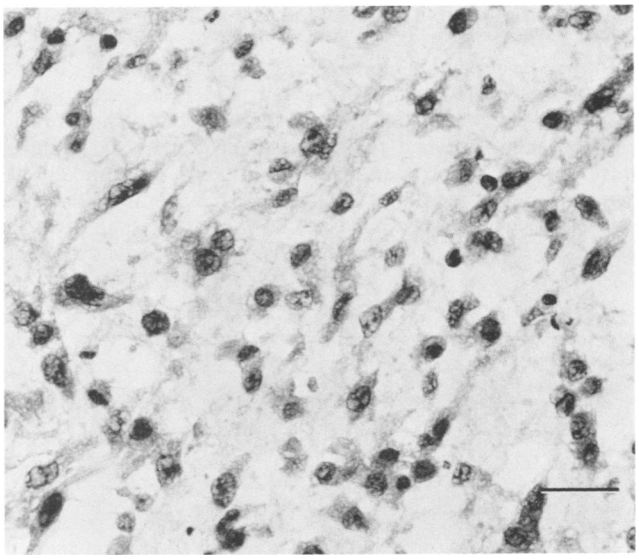

FIG. 5. Pathological patterns in AAV-5005-induced angiosarcoma. The micrograph shows a portion of tumor composed of immature mesenchymal elements. The tumor cells are large and polygonal and are embedded in an abundant extracellular matrix. Bar, 20 μm.

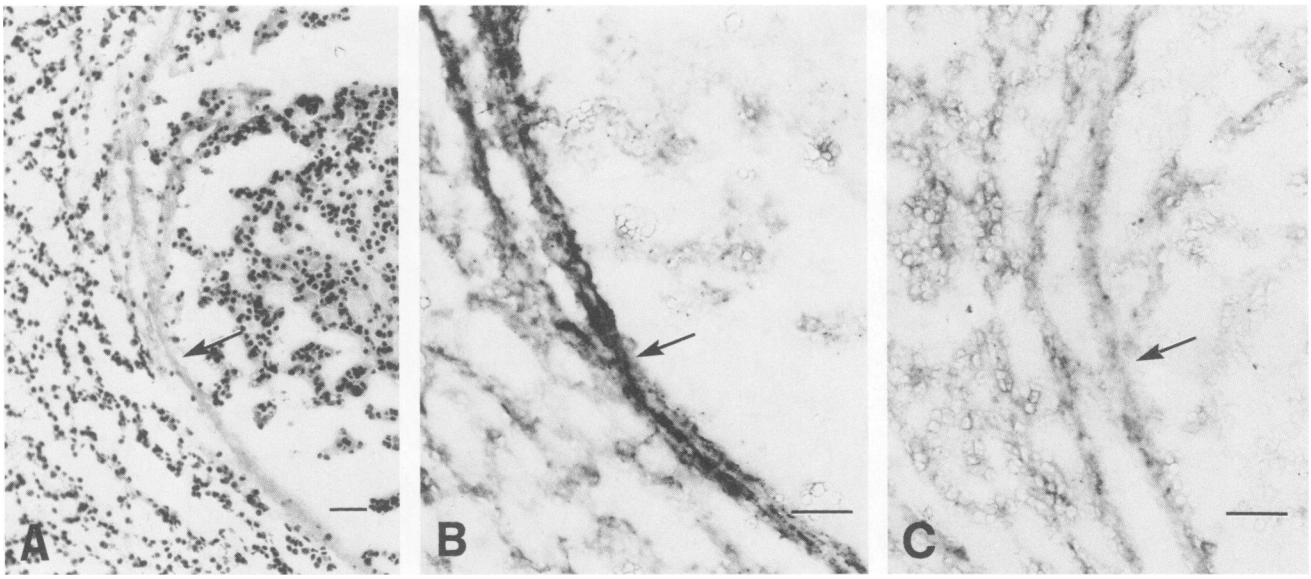

FIG. 6. Immunohistological studies of AAV-5005-induced angiosarcoma. (A) Frozen section stained with hematoxylin and eosin showing a blood-filled space lined with endothelial cells (arrow). (B) Frozen section stained with anti-factor VIII showing the localization of factor VIII to the lining cells (arrow). (C) Frozen section stained with a control antibody, anti-human placental lactogen. Note the lack of staining of the endothelial lining cells (arrow). Bar, 20 μm.

reflects a response of the chicken to the loss of blood from the many lesions in its vascular system. Blood smears from 3 of the 19 birds with angiosarcoma have displayed low numbers of polychromatic erythrocytes and occasional erythroblasts. The presence of even slightly elevated levels of polychromatic erythrocytes and erythroblasts in blood smears raises the possibility that AAV-5005 can cause erythroblastosis. Alternatively, the low number of primitive erythroid cells in the blood of chickens with AAV-5005-induced angiosarcoma could (i) be a response to bleeding, (ii) reflect the presence of low titers of AEV-5005 in stocks of AAV-5005 (AAV-5005 has not been cloned), or (iii) represent the generation of erythroblastosis-inducing viruses from AAV-5005.

Induction of erythroblastosis and angiosarcoma by chicken 5005-derived viruses that have not undergone selection in culture. c-*erb*B transducing viruses that had originated in chicken 5005, but had not been selected in tissue culture, were inoculated intravenously into 1-week-old chickens to determine whether these viruses could cause angiosarcoma as well as erythroblastosis (Fig. 1). Serial passage of these viruses (with filtered spleen homogenates or sera as inocula) resulted in 28 chickens developing erythroblastosis, 3 chickens developing erythroblastosis and angiosarcoma, and 2 chicken developing only angiosarcoma (Table 2). The ability of stocks of AEV-5005 to induce a low incidence of angiosarcoma suggests that AAV-5005 (or viruses like AAV-5005) was present at low titers in the erythroleukemia in chicken 5005.

TABLE 2. Oncogenic potential of AEV-5005 and AAV-5005[a]

Virus	No. inoculated	% Diseased			
		Erythroblastosis only	Erythroblastosis angiosarcoma	Angiosarcoma only	Erythroblastosis, other[b]
AEV-5005	34	76	9	6	3
AAV-5005	19	0	16	84	0

[a] The data summarize disease observed in a number of different groups of chickens inoculated intravenously with uncloned stocks of AEV-5005 or AAV-5005. AEV-5005-infected chicken that had erythroblastosis as well as angiosarcoma succumbed to erythroblastosis. AAV-5005-infected chickens that exhibited angiosarcoma as well as possible erythroblastosis (see the next) succumbed to angiosarcoma.

[b] Undiagnosed growth in the spleen.

Transduced c-*erb*B sequences in angiosarcomas. DNAs extracted from the angiosarcomas in chickens 6206, 6184, 6456, and 6460 were analyzed for novel c-*erb*B-related *Eco*RI fragments that could be visualized on autoradiographs of Southern blots. These analyses revealed that each contained a novel 4.3-kilobase fragment. This fragment was indistinguishable in size from the novel c-*erb*B-related *Eco*RI fragment present in the erythroleukemias in chickens 5005, 6103, and 6458 (Fig. 1 and 7A). Thus, AEV-5005 and AAV-5005 appear to have had a common ancestor. This ancestor could have been a proviral insertion that generated erythroblastosis and angiosarcoma-inducing transductions of c-*erb*B. Alternatively, an erythroblastosis-inducing transduction of c-*erb*B could have undergone a mutation such that it became an angiosarcoma-inducing virus.

AAV-5005-induced angiosarcomas are polyclonal. Southern blots of *Eco*RI-digested DNAs can be used to determine whether an avian leukosis virus-induced tumor is clonal or polyclonal. Avian leukosis viruses integrate at many sites in the chromosomal DNA of their host (12). If the tumor is clonal, junctions of proviral and host sequences that were present in the founder cell exist in each cell of the tumor. These junctions are easily detected as novel long terminal repeat-related fragments in blot analyses of tumor DNAs. However, if the tumor is polyclonal, individual junctions of proviral and host sequences are not present at high enough frequencies to be detected on autoradiographs of Southern blots.

Hybridization of a Southern blot of *Eco*RI-digested DNA from the angiosarcoma in chicken 6460 with a long terminal repeat probe did not reveal junctions of the inducing virus

and host sequences. Rather, the long terminal repeat probe revealed junctions of endogenous virus and host sequences as well as the internal 4.3-kilobase fragment of the inducing virus (Fig. 7B). Thus, the angiosarcoma in the spleen of chicken 6460 appears to be polyclonal. Similar analyses of erythroleukemias induced by AEV-5005 have revealed that many cases of AEV-5005-induced erythroblastosis are polyclonal (14).

DISCUSSION

AAV-5005, a virus that induces rapid-onset angiosarcoma, has been isolated from an erythroleukemia in chicken 5005 (Fig. 1–6, Tables 1 and 2). The erythroleukemia in chicken 5005 was induced by an erythroblastosis-inducing transduction of c-*erb*B (AEV-5005) that was generated during the replication of Rous-associated virus type 1 in chicken 5005. AAV-5005 and AEV-5005 appear to be closely related in that both have indistinguishable c-*erb*B-related *Eco*RI fragments (Fig. 7A). AAV-5005-induced angiosarcomas were polyclonal outgrowths of endothelium-like, mesenchyme-like, and fibroblast-like cells (Fig. 2–6, and 7B). AAV-5005 was found to have little or no potential to induce erythroblastosis or fibrosarcoma (Table 2).

Origin of AAV-5005. AAV-5005 was isolated from an erythroleukemia induced by AEV-5005 in chicken 6103 (Fig. 1). The erythroleukemia in chicken 6103 was cocultivated with chicken embryo fibroblasts, and the cocultivation was selected for cells that would grow when suspended in soft agar (Table 1). A virus stock was then obtained by cocultivating cells from soft agar colonies with chicken embryo fibroblasts. This stock, now termed AAV-5005, has induced a 100% incidence of angiosarcoma (Table 2). Inoculation of chickens with chicken 5005-derived viruses that had not been selected in culture resulted in the vast majority of chickens developing erythroblastosis (Fig. 1, Table 2). However, occasional chickens developed angiosarcoma as well as erythroblastosis, and one developed angiosarcoma in the absence of erythroblastosis (Fig. 1, Table 2). Thus we believe that the erythroleukemia in chicken 5005 contained relatively high titers of AEV-5005 and low titers of AAV-5005. At this point we do not know whether the enrichment for AAV-5005 that occurred during culture was fortuitous or the result of the selection of colonies that would grow in soft agar.

AAV-5005 appears to be closely related to AEV-5005 in that both have 4.3-kilobase c-*erb*B related *Eco*RI fragments (Fig. 7A). Each new transduction of c-*erb*B has had a c-*erb*B-related *Eco*RI fragment of a unique size (14). Therefore the observation of novel fragments of the same size in two viruses that originated in one chicken suggests that the two transductions had a common ancestor. Whether this ancestor was a proviral insertion that initiated several independent transductions of c-*erb*B or an erythroblastosis-inducing transduction that mutated to an angiosarcoma-inducing virus is not clear at this time.

Oncogenic potential of AEV-5005 and AAV-5005. In our attempts to clone AEV-5005 and AAV-5005 (as well as AEV-4884, AEV-5005, and AEV-5040) we have found that conditions suitable for the assay and cloning of AEV-R were not suitable for the assay and cloning of the new AEVs. Consequently, all of our oncogenicity tests have been done with uncloned stocks of AEV-5005 and AAV-5005. The results of the tests with uncloned stocks indicate that AEV-5005 causes primarily erythroblastosis and that AAV-5005 causes primarily angiosarcoma (Fig. 1, Table 2). Furthermore, our data suggest that neither causes fibrosarcoma or an easily detected transformation of cultured fibroblasts (Tables 1 and 2).

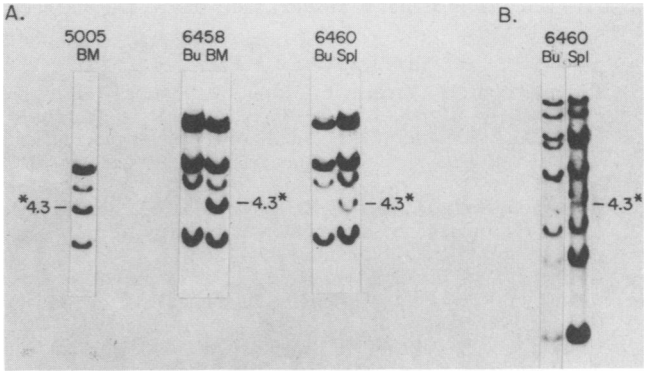

FIG. 7. Novel c-*erb*B and viral sequences in AEV-5005- and AAV-5005-induced tumors. Shown are autoradiographs of a Southern blot of *Eco*RI-digested DNAs. Blots were hybridized with the BAM probe (14) for v-*erb*B sequences (A) or with a probe for viral long terminal repeat sequences (B) (14). The numbers over the lanes indicate the chickens from which the DNA was prepared. BM, DNA from an erythroleukemia (bone marrow); spl, DNA from an angiosarcoma (spleen); bu, DNA from normal tissue (bursa). *4.3, 4.3-kilobase, tumor-associated *Eco*RI fragment.

Do other new c-*erb*B transducing viruses cause angiosarcoma? Interestingly, our tests for the disease potential of the new AEVs suggest that angiosarcoma-inducing transductions of c-*erb*B frequently accompany erythroblastosis-inducing transductions of c-*erb*B. The data in Fig. 1 through 6 and Tables 1 and 2 indicate that the erythroleukemia in chicken 5005 contained angiosarcoma-inducing as well as erythroblastosis-inducing viruses. Preliminary studies on the disease potential of c-*erb*B transducing viruses in the erythroleukemia in chicken 4890 (14) indicate that this erythroleukemia also harbors angiosarcoma-inducing as well as erythroblastosis-inducing transductions of c-*erb*B (work in progress). Thus two of six erythroleukemias induced by the generation of new transductions of c-*erb*B have had angiosarcoma-inducing as well as erythroblastosis-inducing viruses.

Oncogenic potential of c-*erb*B. In the experiments reported here, we have shown that a c-*erb*B transducing virus can cause angiosarcoma. Both angioblasts and erythroblasts arise from the differentiation of mesenchymal hemangioblasts. Thus, angioblasts are closely related to erythroblasts, which have long been known to be affected by transductions of c-*erb*B. However, despite their close relationship, angioblasts and erythroblasts appear to be affected by different transductions of c-*erb*B. AEV-5005 causes erythroblastosis, whereas AAV-5005 causes angiosarcoma. Thus our results suggest that cells derived from hemangioblasts are uniquely susceptible to transformation by c-*erb*B sequences, with some combinations of c-*erb*B sequences resulting in the malignant growth of erythroblasts and others resulting in the malignant growth of angioblasts.

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