In vitro Transformation of Chicken Bone Marrow Cells with Avian Erythroblastosis Virus

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An *in vitro* transformation of bone marrow cells has been demonstrated for two strains of avian erythroblastosis virus (AEV-R and AEV-ES4). The transformed cells were indistinguishable from *in vivo* transformed erythroblasts in morphology and staining characteristics and could be propagated to large numbers. The transformation efficiency could be greatly increased by the addition of dimethylsulfoxide (DMSO). The number of foci appearing in the presence of DMSO was proportional to the virus concentration.

Avian erythroblastosis virus (AEV) induces an acute erythroleukemia within a few weeks after infection. This is manifested by the appearance of numerous erythroblasts in the peripheral blood followed by the death of a high percentage of inoculated chickens (see ref. 1). Two strains have been described, AEV-R and AEV-ES4, both of which were isolated in the same laboratory [2, 3]. Most of the studies performed so far with these viruses were aimed at the pathology of the disease, and at problems of cell differentiation and of RNA processing using erythroblasts from infected birds. To study the mechanism of transformation by AEV and to identify its target cells it became necessary to investigate the interaction of the virus with hemopoietic cells in culture. Early attempts to induce an *in vitro* transformation with AEV-R, such as has been described for the avian myeloid leukemia strains AMV [4, 5] and MC29 [6], were unsuccesful [7] or erratic [8]. Here an *in vitro* transformation of chick bone marrow cells with avian erythroblastosis virus (strains R and ES4) will be described for the first time.

To determine whether avian erythroblastosis viruses are capable of transforming hemopoietic cells *in vitro*, bone marrow cells were prepared as described earlier [9] from a 3-week-old Spafas chick, seeded at 4×10^6 cells per 35 mm dish and infected with 0.1 ml of undiluted AEV stock. Two to six foci of loosely attached, highly refractile round cells were obtained in the cultures infected with AEV-R and AEV-ES4 which were not observed in uninfected cultures. In subsequent work the properties of both strains were found to be identical and in the following they will therefore be collectively termed as AEV. In some experiments, foci appeared as early as 4 days (Fig. 1 b *) and increased in size until they consisted of several thousand cells after 10 days (Fig. 1 c, d). When suspended and transferred to fresh medium, the AEV-transformed cells could be propagated in suspension into mass cultures, either on petri dishes or in roller bottles. About 10^8 transformed cells were obtained from 5 foci within 3 weeks of culturing. After about 2 months, the transformed cultures started to slow down in growth and to die. Uninfected bone marrow cultures could not be propagated under similar conditions.

The *in vitro* transformed cells were indistinguishable by morphology, growth behaviour and cytological appearance from erythroblasts transformed *in vivo* and maintained in culture (Fig. 2, b). The cultured erythroblasts were more pleomorphic and vacuolated than the erythroblasts from the blood of a diseased chicken (Fig. 2 c). Both *in vitro*- and *in vivo*-transformed cells were hemoglobin negative, as determined by benzidine staining.

In an attempt to improve the efficiency of the assay, the effect of erythropoietin and of DMSO was tested. Sheep erythropoietin as well as DMSO have been found to facilitate colony formation *in vitro* by normal mouse erythroid cells [10], F. Sieber and A. Axelrad, personal communication. The influence of these substances on the efficiency of transformation of bone marrow cells by AEV was therefore investigated. Since no avian erythropoietin was available, serum from an anemic chicken was tested. Such a serum was likely to contain increased amounts of erythropoietin-like activity [11]. Despite of the fact that mammalian erythropoietin has been found to be inactive *in vivo* in avian species [11], sheep erythropoietin was also tested. As shown in Table I, although no significant effect of anemic serum nor of sheep erythropoietin was observed, a drastic increase in the number of AEV-foci was obtained by the addition of DMSO. In contrast, the efficiency of transformation by myeloid leukemia viruses MC29 and AMV was decreased in the presence of DMSO.The number of AEV-foci obtained in DMSO-containing medium was approximately proportional to the virus dilution (Fig. 3). This indicates that one virus particle is sufficient to induce transformation in hemopoietic cells.

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* Figs 1 and 2 see Table on page 848 a.

Table I. Effect of erythropoietin and of DMSO on the *in vitro* transformation of bone marrow cells with AEV [a].

	Virus used for infection [b]				
	AEV-R	AEV-ES4	MC29	AMV	Uninfected control
Normal chicken serum (NChS)	5, 4 [c]	0, 2	75, 65	27, 25	0, 0
NChS + sheep erythropoietin	6, 2	N.T.	N.T.	N.T.	0, 0
Anemic chicken serum	9, 3	N.T.	N.T.	N.T.	0, 0
NChS + 0.5% DMSO	186, 206	62, 55	2, 5	6, 5	0, 0

[a] Except for the results with AMV, data were obtained in one experiment. The assay medium was as described in the legend of Fig. 1. However, instead of chicken serum from GIBCO, fresh serum from Spafas chickens was used. Serum from an anemic chicken was obtained by bleeding an adult rooster for 5 consecutive days. An average of 16 ml of blood was taken each day by heart puncture. Serum from the first bleeding was used as "normal", serum from the last bleeding as anemic" serum. Sheep erythropoietin (step III) was purchased from Connaught Labs., Toronto, Canada. It was used at 3 units/ml. DMSO was from Merck, Germany.

[b] AEV strains R and ES4 were kindly supplied by Drs. R. Ishizaki and A. Langlois (Duke University Medical Center, Dept. of Surgery, Durham, N.C., USA). The origin of cloned MC29 virus and of AMV has been described earlier [9].

[c] Number of foci per dish. N.T., not tested.

My results demonstrate that it is possible to induce a transformation of hemopoietic cells *in vitro* with erythroleukemia viruses and that a quantitative assay system could be developed with the use of DMSO. Besides morphology, the ability of the altered cells to proliferate to high numbers was taken as the major criterion to call them *transformed*. That they are in fact erythroblasts is suggested by the finding that they were indistinguishable from *in vivo* transformed erythroblasts in morphology, growth behaviour and staining properties. The *in vitro* transformation of avian hemopoietic cells by AEV may thus become a new system to study the mechanism of transformation by C-type leukemia viruses and their interaction with differentiated cells.

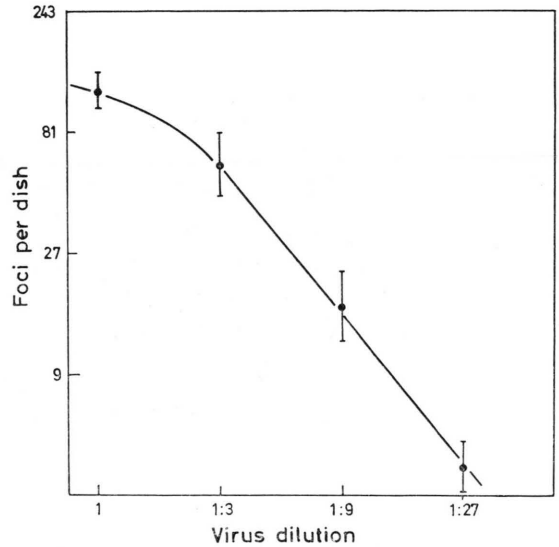

Fig. 3. Dose response curve of transformation of bone marrow cells by AEV-R. The assay was performed in the presence of 0.5% DMSO. Each point represents an average from 4 dishes, the bars indicate the standard deviation. The assay was evaluated 6 days p,i,

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Fig. 1. Chick bone marrow cultures (a) uninfected, (b) 4 days after infection with AEV-R and (c, d) 10 days post infection. The insert shows a colony of transformed cells grown in the presence of 0.5% DMSO. Figs 1 a, b, c are phase micrographs; the bars represent 40 μm. The bar in Fig. 1 d represents 160 μm. The medium employed consisted of 70 parts medium F10 (GIBCO), 10 Dulbecco's modified Eagle's medium, 10 tryptose phosphate broth, 5 calf serum (Roth KG, Karlsruhe) and 2.5 parts of heat inactivated chicken serum (GIBCO). Before infection, 2 μg/ml polybrene (EGA-Chemie, Steinheim, W.-Germany) was added to enhance virus infectivity [12]. The medium also contained 50 μg/ml streptomycin and 50 U/ml penicillin. Cells were obtained from chickens of the Spafas flock, provided through the courtesy of Dr. R. Luginbuhl, University of Connecticut, Storrs, Conn., USA. For the origin of the virus see legend of Table I.

Fig. 2. Smears of hemopoietic cells transformed by AEV-R (a) *in vitro*. (b) Erythroblasts transformed *in vivo* and cultured for 10 days *in vitro*. The culturing medium was as described in the legend of Fig. 1. (c) Erythroblasts in the blood of a chick 12 days after inoculation with AEV-R. Note the nucleated chicken erythrocytes. Smears were stained with Wright-Giemsa. Bar represents 10 μm.

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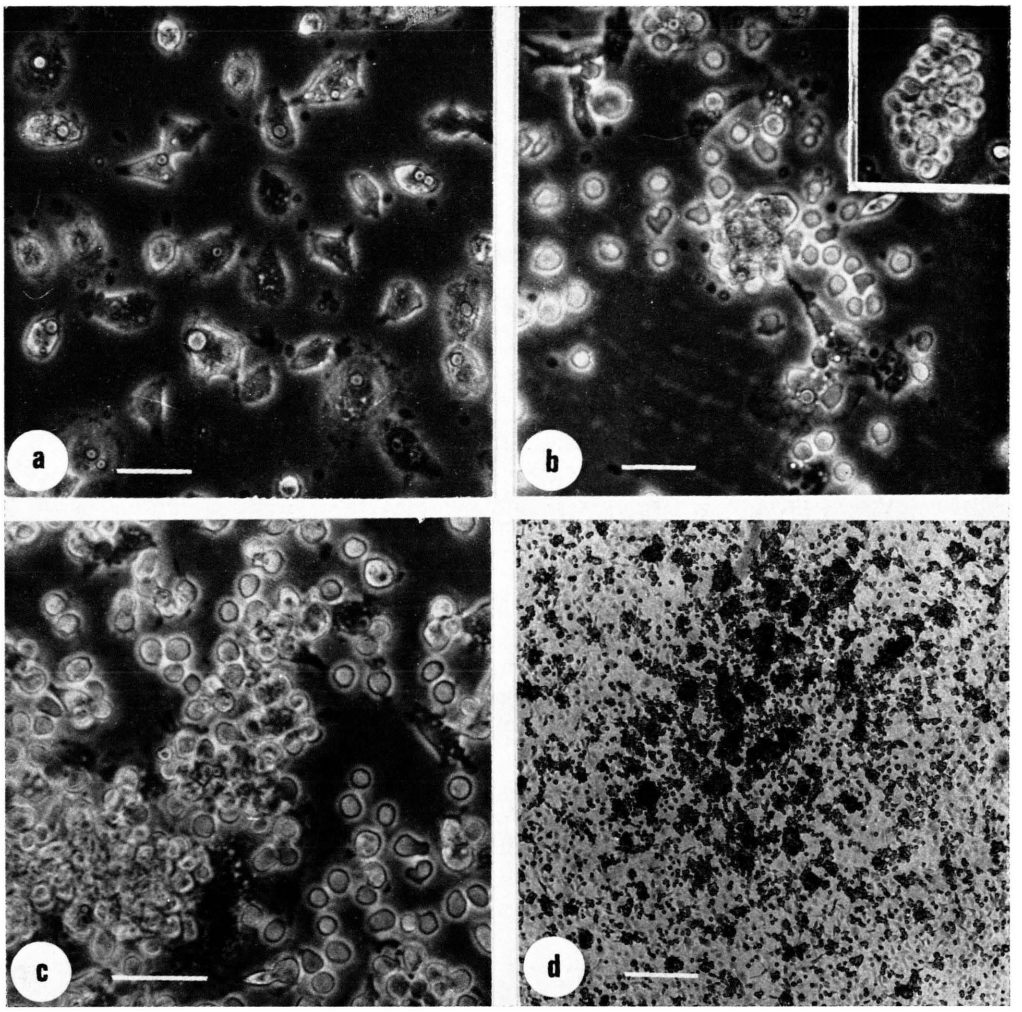

Fig. 1. (Legend see page 848)

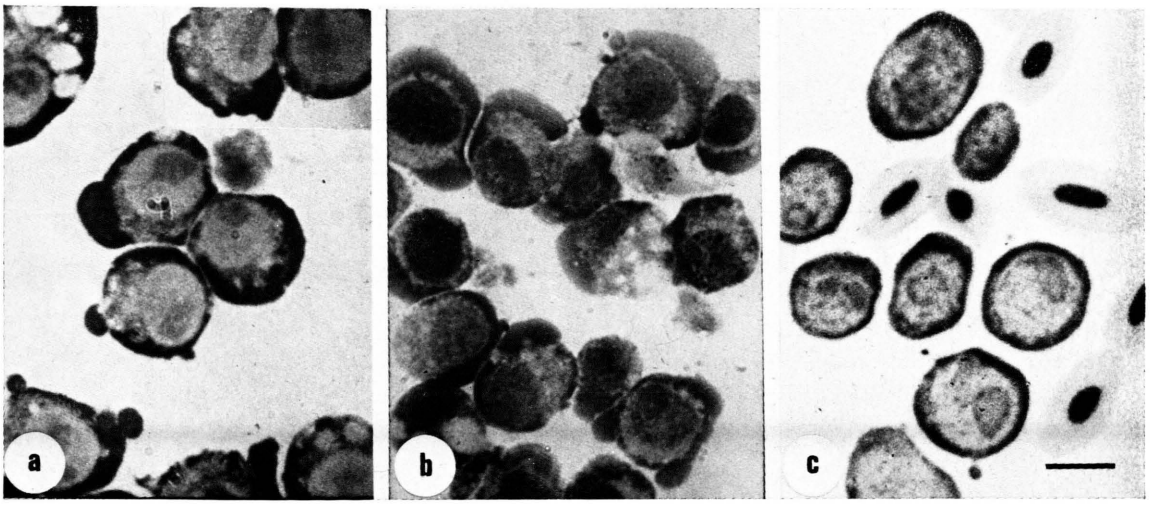

Fig. 2.

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