Target Cells for Transformation with Avian Leukosis Viruses

T. Graf, B. Royer-Pokora and H. Beug

Max-Planck-Institut für Virusforschung, Tübingen

Introduction

Leukemia is a widespread disorder of the hemopoietic system of vertebrates which has been particularly well analyzed in chickens, mice and recently also in cats (for review see 1). It seems now safe to assume that the majority of the different ypes of leukemias found in animals are caused by infection with or activation of C-type leukemia viruses (1). In fact, it has been known since 1908 that leukemia can be induced by a filterable agent, i.e., a virus (2). With the availability of modern biochemical technology and quantitative biological assays, leukosis-sarcoma viruses have since then been thoroughly analyzed in their structure, mechanism of replication and genetics (1). Little is known, however, about the mechanism of virus-induced leukemogenesis.

The existence of highly oncogenic chicken virus trains which specifically induce certain types of acute leukemias such as myeloblastosis or erythroblastosis stimulated us to attempt to define the corresponding target cells. An important prerequisite for these studies was the availability of *in vitro* transformation assays. While such assays had been developed for two strains causing myeloid leukemia (avian myeloblastosis) (AMV) (3, 4) and avian myelocytomatosis strain MC29 (5, 6) no *in vitro* transformation system was known for viruses causing leukemias of other types of hemopoietic cells.

Here some of the features of a newly developed *in vitro* transformation assay with avian erythroblastosis virus will be described. In addition, data will be discussed which suggest that the target cells for leukemogenesis with chicken erythroid and myeloid leukosis viruses are not pluripotent stem cells but are committed to differentiate along the erythropoietic and granulopoietic series respectively.

In vitro transformation of hemopoietic cells with avian erythroblastosis virus

Avian erythroblastosis virus (AEV) is known to cause the corresponding disease at an incidence of over 50 % in young and adult birds within only a few weeks after infection (for ref. see 7). The picture of a blood smear of a diseased bird is shown in Fig. 1.

Two strains, AEV-R and AEV-ES4, were studied and similar results were obtained with both. They will therefore collectively be referred to as AEV. Infection of freshly prepared bone marrow cell cultures with AEV resulted in the appearance of foci of small refractile, round, rapidly growing cells (Fig. 2). The details of the assay as well as the origin of the virus strains, the methods used for their assay and propagation, and the preparation and culture of bone marrow cells will be described in another communication (8). Here it may suffice to mention

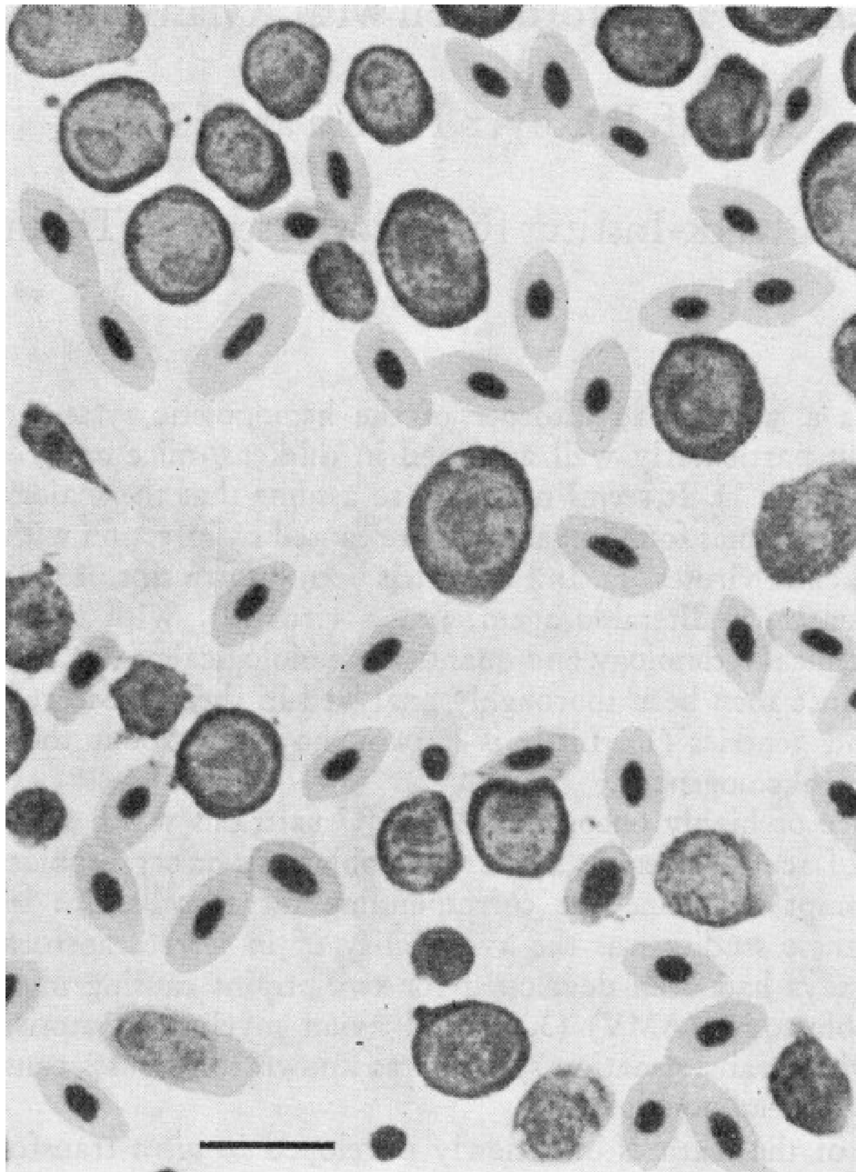

Fig. 1: Smear (stained with Wright-Giemsa) of peripheral blood from a chicken infected with AEV. Note that the chicken erythrocytes are nucleated. Bar represents 20 μ.

that the culture medium contained calf serum and chicken serum and that the addition of 1 % DMSO greatly improved the assay. The *in vitro* transformed cells were indistinguishable in their staining properties from leukemic erythroblasts induced *in vivo* and maintained in culture (Fig. 3).

To determine the proliferative capacity of the *in vitro* transformed cells, 22 randomly selected 7-day-old single foci were isolated and propagated separately. The cells from each culture were then counted and passaged at appropriate intervals. After two weeks of growth (the best cultures duplicating every 15–20 hours), most of the cultures started to accumulate degenerated cell forms and slowed down in their rate of growth. By 4 weeks no more increase in cell number was obtained and the experiment was terminated. From the total number of cells obtained with each clone (the values ranged from $4 \cdot 10^5$ to $7 \cdot 10^8$) the number of population doublings was calculated. As can be seen from Fig. 4, the *in vitro* transformed cells studied were capable of dividing in average for 18–29 generations. These values are

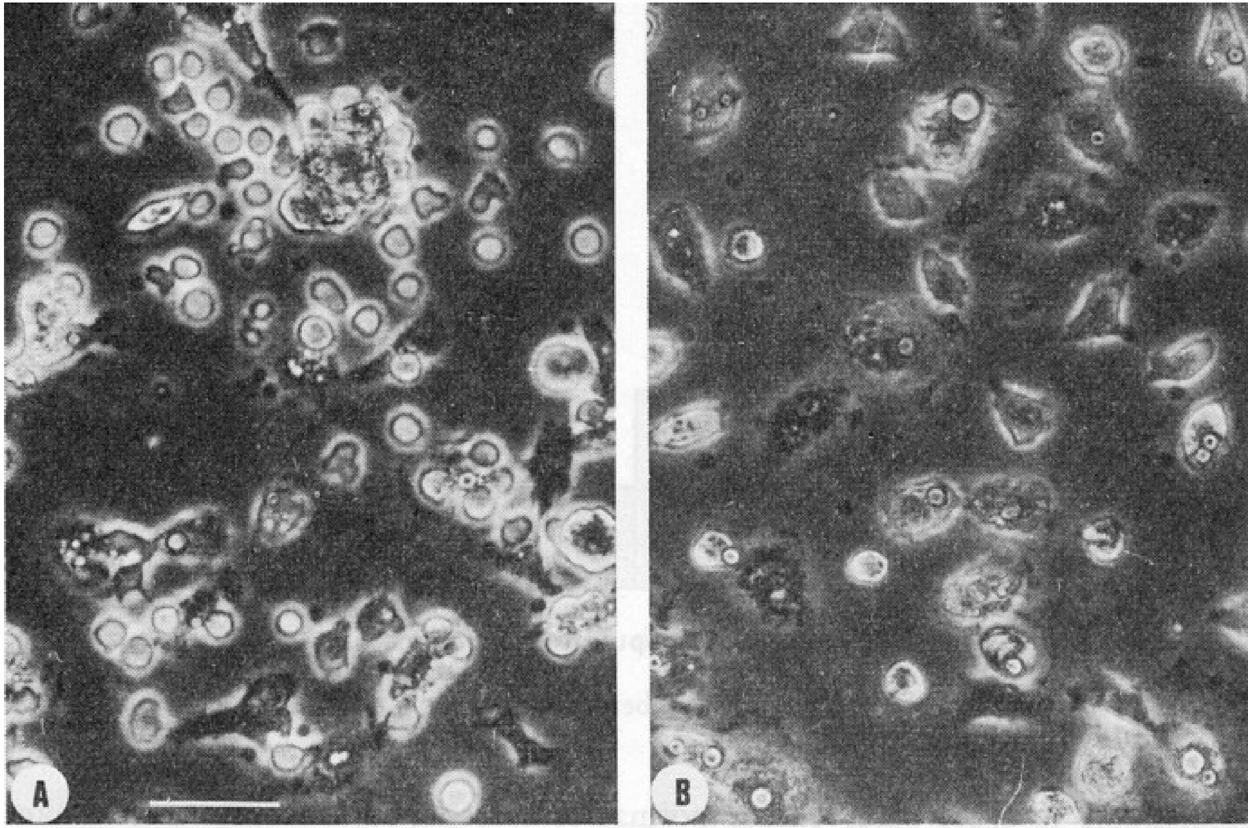

Fig. 2: Phase micrographs of chicken bone marrow cells. (A) Focus of cells transformed *in vitro* 5 days after infection with AEV. (B) Uninfected bone marrow culture. Bar represents 40 μ.

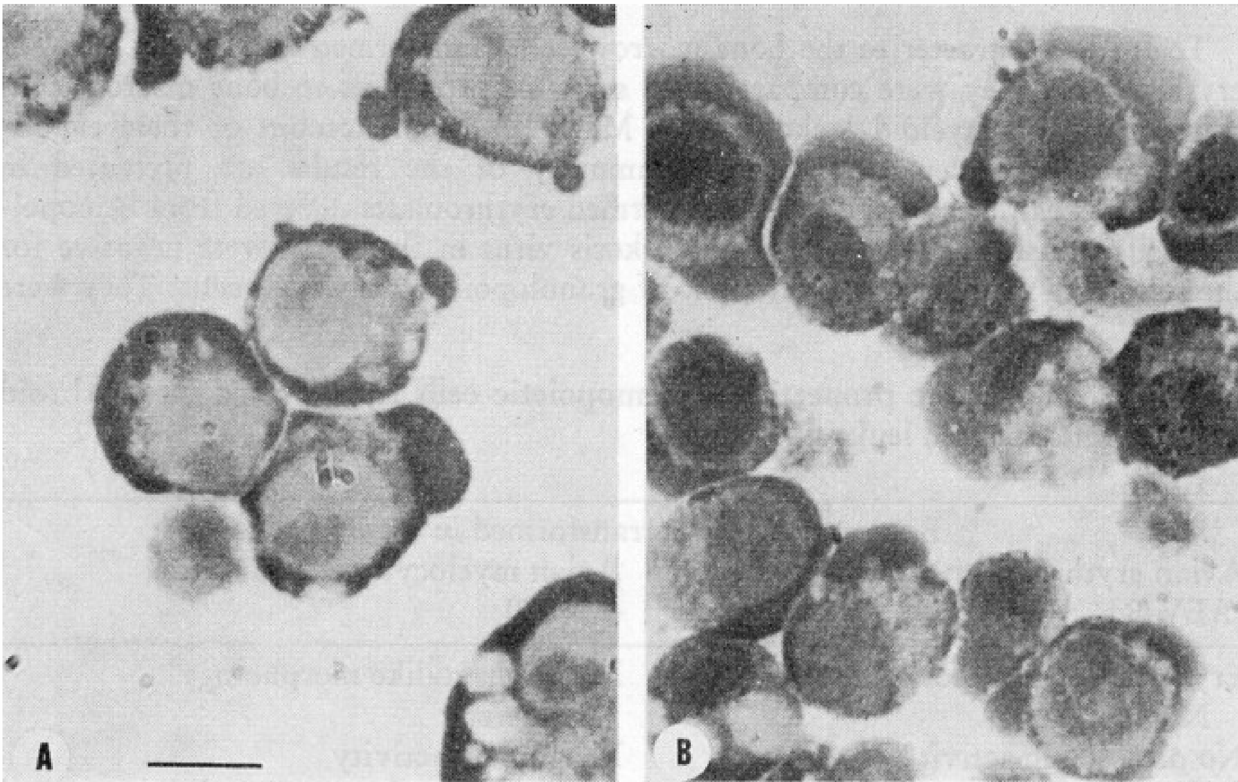

Fig. 3: Smears (stained with Wright-Giemsa) of erythroblasts transformed *in vitro* (A), and transformed *in vivo* and maintained in culture for 10 days (B). Bar represents 10 μ.

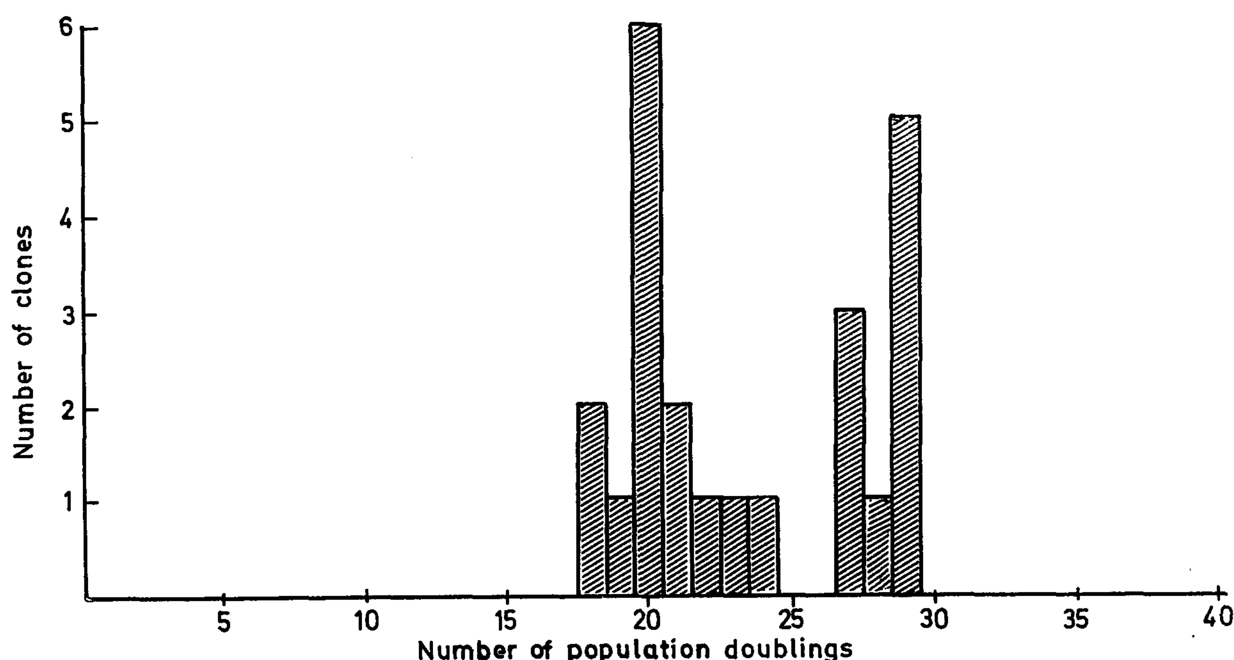

Fig. 4: Frequency distribution of the number of population doublings obtained with 22 clones of *in vitro* transformed erythroblasts.

comparable to those observed for normal or sarcoma virus-transformed chicken fibroblasts (9).

Comparative properties of bone marrow cells transformed in vitro by erythroblastosis and myeloid leukosis viruses

To further characterize the bone marrow cells transformed *in vitro* by AEV as erythroblasts, they were compared in a series of properties to bone marrow cells transformed by myeloid leukosis virus MC29. A fuller account of these experiments will be given elsewhere. A summary of the results are presented in Table 1. As can be seen, *in vitro* transformed erythroblasts differed from hemopoietic cells transformed by a myeloid leukosis virus in that they were negative for the following properties characteristic of granulopoietic (myeloid) cells: They were

Table I: Comparative properties of hemopoietic cells transformed by erythroid and myeloid leukosis viruses

Bone marrow cells transformed *in vitro* by	
Avian erythroblastosis virus (AEV-R)	Avian myelocytomatosis virus (MC29)
Erythroblast-like morphology	Macrophage-like morphology
Non adherent	Adherent
No phagocytic activity	Phagocytic activity
Colony formation in semisolid media not CSF dependent	Colony formation in semisolid agar is CSF dependent

not adherent, did not phagocytize bacteria, and were not dependent on colony stimulating factor (CSF) for colony formation in semisolid agar. Other more specific markers of erythroid differentiation could not yet be determined. Like the *in vivo* induced leukemic erythroblasts, *in vitro* transformed erythroblasts were hemoglobin negative (as determined by benzidine staining) and could not be induced to synthesize hemoglobin by the addition of DMSO, as it is possible with mouse erythroid cells transformed by Friend leukemia virus (10). Neither could the effect of erythropoietin on the growth of these cells be evaluated since avian erythropoietin was not available and mammalian erythropoietin has been found to be inactive in birds (11).

Models explaining the transformation specificity of avian leukosis viruses

Two basic models will be discussed to account for our findings. In accordance with current notions about normal hemopoiesis it is assumed that all differentiated hemopoietic cells arise from pluripotent stem cells by a series of maturation steps (12). In the drawings of the models in Fig. 5 only the erythropoietic and granulopoietic (or myeloid) series of differentiation were included. The terms "transformation of hemopoietic cells" or "leukotransformation" are defined as the process by which certain hemopoietic cells are morphologically altered and induced to proliferate by infection with a leukosis virus.

Model 1. Transformation of specific committed progenitor cells. Avian erythroblastosis virus (AEV) transforms *erythroid* cells at an early stage of maturation whereas avian myeloblastosis virus (AMV) and myelocytomatosis virus (MC29) transform immature *myeloid* cells. The target cells for the latter two viruses differ

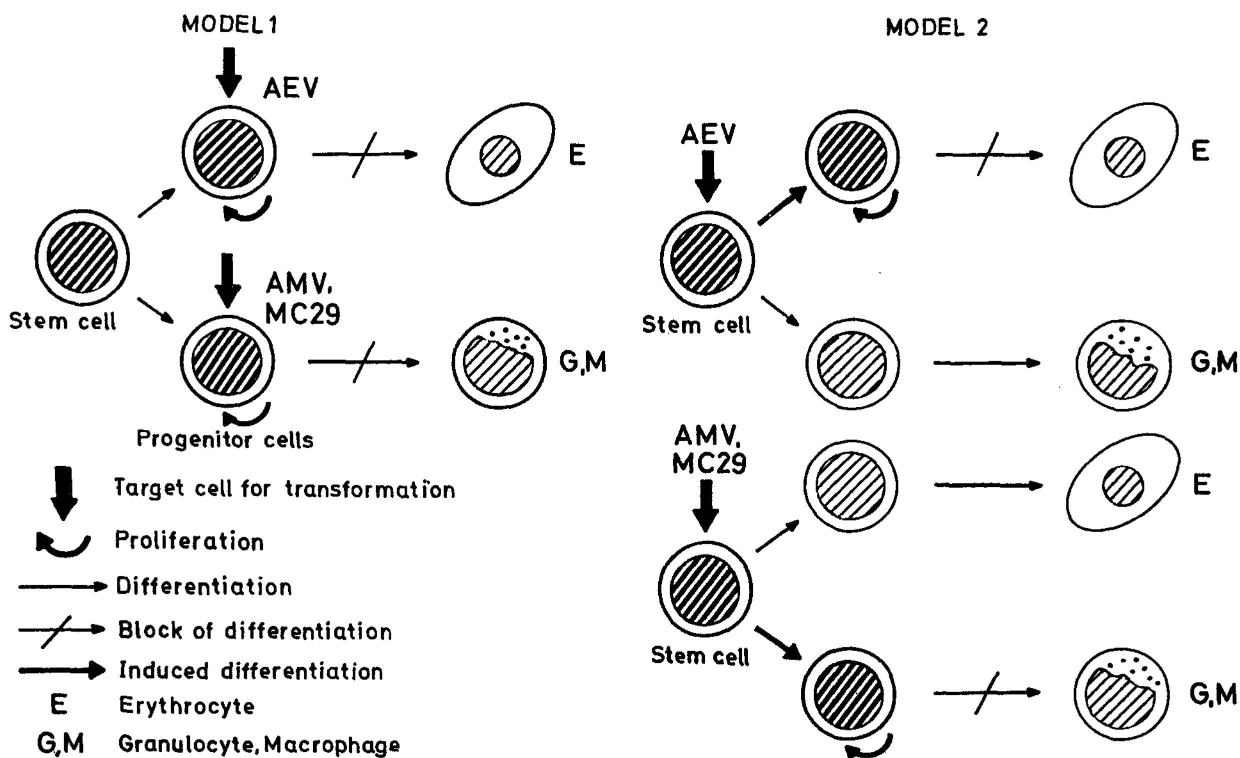

Fig. 5: Two basic models concerning the involvement of hemopoietic cells in leukemogenesis.

in their degree of maturation. Experimental observations indicate that concomitantly with leukotransformation normal differentiation is arrested. The model also allows room for the possibility that leukosis viruses induce a limited maturation or dedifferentiation of their target cells. A testable prediction arising from this model is that the target cells for erythroid and myeloid virus strains can be physically separated or selectively eliminated prior to infection.

Model 2. Involvement of stem cells. In this case, AEV induces pluripotent stem cells to differentiate into transformed erythroblasts. Conversely, AMV and MC29 viruses induce the formation of transformed myeloid cells of different stages of maturation. A similar specificity could also be achieved if it is assumed that these viruses are capable of selectively blocking the differentiation of hemopoietic cells belonging to series other than those transformed. As in model 1, once the cells are transformed they do not usually differentiate anymore. If this model is correct it should not be possible to separate or selectively eliminate target cells for erythroid and myeloid leukosis viruses.

Preliminary data supporting model 1

Most of freshly seeded chicken bone marrow cells die within a few days of incubation and a population of adherent cells survive. Cultures of these cells consist in their majority of macrophage-like cells (13). They can be passaged by trypsinization and maintained with a net increase in cell number for several months (unpublished observations). To determine the incidence of target cells for transformation in freshly prepared cultures of bone marrow and in cultures of adherent cells at different passages, they were infected with AEV and MC29 viruses and the number of foci obtained with each virus scored. As can be seen from Table 2, AEV-induced erythroblast foci were only obtained in freshly prepared cultures and not in the cultures passaged up to 3 times. This suggests that the target cells for AEV are non adherent cells or that they have been otherwise eliminated during the transfer procedure. In contrast, MC29 induced foci in all cultures. The much

Table II: Incidence of bone marrow cells transformed by avian erythroid and myeloid leukosis viruses[a]

	Fresh cultures	Passaged cultures (number of passages)		
		1	2	3
Avian erythroblastosis virus (AEV)	55[b]	0	0	0
Avian myelocytomatosis virus (MC29)	160	15.000	5.400	4.400

a) Cultures were prepared from a 4-week-old chicken and passaged at 5-day intervals.
b) Average number of transformed foci. Duplicate cultures in 35 mm dishes were seeded with 10^3, 10^4, 10^5 and 10^6 cells and infected with 10^4–10^5 transforming units of virus per dish.

higher efficiency of transformation with this virus in cultures which had been transferred at least once can be explained by a selection of a population of myeloid cells particularly susceptible to transformation with myeloid leukosis virus. These results demonstrate that the target cells for AEV can be selectively eliminated and are therefore probably not identical to the target cells for MC29. Cell separation experiments devised to further test this interpretation are currently being performed.

Concluding Remarks

Chromosomal studies of human leukemic cells strongly suggest that chronic myeloid leukemia (CML) and possibly also acute myeloid leukemia (AML) are stem cell diseases (for review see 14). The data presented show that other mechanisms probably exist in a model animal system. A diversity of target cells as suggested for chicken leukosis viruses, however, does not rule out the possibility that stem cells are also affected.

In drawing parallels to C-type virus induced leukemogenesis in animals it is assumed that human leukemia is induced by similar agents. Recently, evidence has accumulated demonstrating the presence of C-type viruses or some of their components in human leukemic cells (see articles by Spiegelman, Gallo, Todaro, Bentvelzen and Till in this volume). It is not clear, however, whether these viruses are cause or consequence of the disease. And, even if the former is assumed, it remains to be determined whether they are endogenous agents that become activated, as postulated by the oncogene theory (15), or whether they represent infectious viruses as suggested by the work of Spiegelman, Gallo and their coworkers (16, 17). It appears as if both of these possible mechanisms of virus-induced leukemogenesis are realized in animals (1).

Studies performed with another highly oncogenic strain, the murine leukemia virus FLV, indicate that transformation of *committed* hemopoietic cells is a mechanism not restricted to avian viruses (18). It is, however, questionable whether the so-called lymphoid leukosis viruses, the C-type virus strains found most frequently under natural conditions in the chicken and which possess an extensive period of latency (19), also act on committed hemopoietic cells as proposed here for highly oncogenic strains. So far, because of the lack of *in vitro* transformation assays, the mechanism of leukemogenesis by these "weakly" oncogenic viruses has not been well analyzed.

The isolation of infectious human leukemia viruses might allow studying the question of whether or not all leukemias are stem cell disorders by an approach such as described here in a model system. Besides its academic interest the answer to this question may have implications for the therapy of the disease.

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