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Growth Pattern of a Transplantable Acute Myeloid Leukemia in the Rat*

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Abstract—*A generalized acute myeloid leukemia was induced in inbred Sprague–Dawley rats by intravenous injection of chloroleukemia cells. In the bone marrow, colonization by blast cells was observed soon after transplantation and at the end of the disease leukemia cells accounted for about* 70% *of the whole cell population. In the peripheral blood, erythrocyte and platelet counts decreased late in the disease, while from* day 5 *onwards there was a progressive increase in the leukocyte count owing to blast cells released into the bloodstream. Spleen, liver and central nervous system were also involved in the disease. The survival time of transplanted animals correlated with the number of cells injected: after transplantation of* 2×10^7 *chloroleukemia cells survival time was* 8.3 days, *and with each* 10*-fold reduction of the inoculum survival time increased by about* 2.5 days. *The data presented show that Shay chloroleukemia mimics some features of human acute myeloid leukemia and possesses a predictable and highly reproducible growth rate, thus providing a further useful experimental model.*

INTRODUCTION

EXPERIMENTAL leukemias provide a valuable model for the development of new therapeutic strategies, and moreover may be used to study from the onset the main changes which take place during the development of the disease, while in man the clinical picture at diagnosis is usually advanced. Experimental myeloid leukemias suitable for such studies are rare [1–4]. Shay chloroleukemia, one of the few rat acute myeloid leukemias available, has been maintained in this laboratory both *in vivo* (K. Stenstrand, P. Foa, W. Paile and T. Rytomaa, in preparation) and *in vitro* as a continuous cell line (W. Paile, P. Foa and T. Rytomaa, in preparation) and has displayed morphological and cytochemical characteristics similar to those of human acute myeloid leukemia (W. Paile and P. Foa, in preparation).

This paper describes the *in vivo* growth pattern of generalized Shay chloroleukemia, the consequent changes in normal hemopoiesis and the main pathological findings. The data presented show that Shay chloroleukemia mimics some clinical features of human acute myeloid leukemia and possesses a highly reproducible growth pattern, thus providing a further attractive model suitable for research on various aspects of this disease.

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MATERIALS AND METHODS

Animals

The investigations were carried out on 8-week-old inbred Sprague–Dawley rats, both male and female.

Leukemia

Shay chloroleukemia, which was originally induced in rats by gastric instillation of 20-methylcholanthrene [5] was classified as an acute myeloid leukemia on the basis of morphological and cytochemical characteristics [6]. While intracellular virus-like particles have been shown to be associated with chloroma cells, it is necessary to inject intact cells in order to transfer Shay chloroleukemia [7, 8]. Clinically, Shay chloroleukemia has three different forms: when transplanted subcutaneously, a local tumor develops; when transplanted intraperitoneally, both an ascites tumor and a generalized leukemia result; when the cells are injected intravenously, a generalized leukemia is induced [9].

Intravenous transplantation of Shay chloroleukemia

A rat bearing a subcutaneous tumor was decapitated. Using sterile techniques, a piece

of tumor was removed, minced in HBSS (Grand Island Biological Co., Grand Island, NY), and gently pressed through a stainless steel sieve. The resulting cell suspension was placed in a glass tube on ice for 15 min to allow the large tissue particles to settle. The cell-rich supernatant was collected and forced through needles of decreasing size in order to obtain a suspension of single cells, which was then centrifuged at 250 ***g*** for 5 min at 4°C, and the cell pellet was resuspended in HBSS. At the end of this procedure, according to the dye exclusion test, more than 95% of cells were viable. The final cell concentration was adjusted so that the appropriate number of chloroleukemia cells would be injected in each animal in a volume of 0.5 ml. Injections were given in a tail vein, under ether anesthesia. In total, 44 recipients were inoculated and killed in groups of 4 every 24 hr.

Growth of leukemia cells in different organs

Blood for hematologic investigations was taken by venipuncture under ether anesthesia. Leukocyte and erythrocyte counts were performed in duplicate by the standard hemocytometer method. Thrombocytes were counted microscopically by Brecher and Cronkite's method [10]. Bone marrow cells for differential counts were obtained by flushing out the femurs from recipient animals with HBSS supplemented with 10% fetal calf serum (Grand Island Biological Co., Grand Island, NY). A single cell suspension was then obtained by forcing the marrow through needles of decreasing size. Slides were prepared with a Shandon cytocentrifuge (90 ***g*** for 10 min) and stained with May–Grunwald–Giemsa. Differential counts were obtained by examining 1000 cells from code numbered slides. Liver and spleen wet weights were recorded daily. Histologic sections from the liver, spleen, brain and spinal cord were prepared according to the usual techniques.

Correlation of survival time with number of cells injected

Four groups of 4 rats each (2 male and 2 female) were injected intravenously with 2×10^4, 2×10^5, 2×10^6 and 2×10^7 chloroleukemia cells, respectively. The number of surviving recipient animals was checked every 8 hr.

RESULTS

Growth pattern of chloroleukemia cells in different organs

A generalized leukemia was induced in rats by intravenous transplantation of Shay chloroleukemia cells. The main clinical findings which became apparent at the end of the disease were skin pallor, weakness and ruffled fur. Progressive paralysis of the hind limbs usually occurred in the animals which survived for longer than 10 days; histologic examination of these animals' central nervous system showed extensive involvement of both brain and spinal cord, consisting of numerous hemorrhages and leukemic infiltration, the latter mostly in the white matter of the spinal cord. Diffuse infiltration of the meninges was also observed.

The growth pattern of chloroleukemia cells was investigated in the bone marrow, peripheral blood, spleen and liver.

Changes in the bone marrow and peripheral blood picture during the course of the disease are shown in Figs. 1–3. In the bone marrow, the percentage of chloroleukemia cells started to increase soon after transplantation, and from day 8 onwards the bone marrow population consisted mainly of blast cells (Fig. 1).

Chloroleukemia cells could be detected in the peripheral blood from day 5 after transplantation. On subsequent days, peripheral leukocyte counts rose sharply (Fig. 2) owing to a dramatic increase in the number of blasts.

Failure of both erythropoiesis and thrombocytopoiesis, as evidenced by peripheral blood counts, occurred in the course of the disease. Erythrocytes remained at a fairly constant level for the first 8 days after transplantation while platelet counts started to fall 2 days earlier (Fig. 3). From then on, counts of both cell types fell rapidly, until on day 10, when

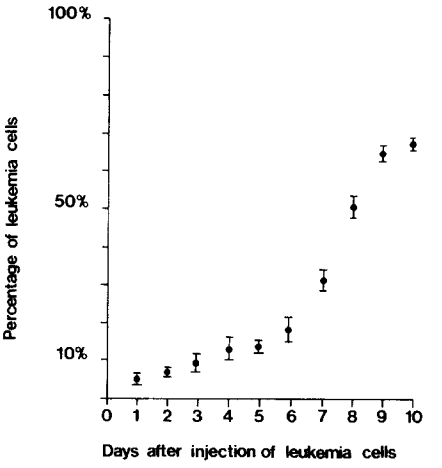

Fig. 1. Percentage of chloroleukemia cells in the bone marrow at different times after intravenous injection of 8×10^6 *chloroleukemia cells. Mean values and standard deviation for four animals in each group are shown.*

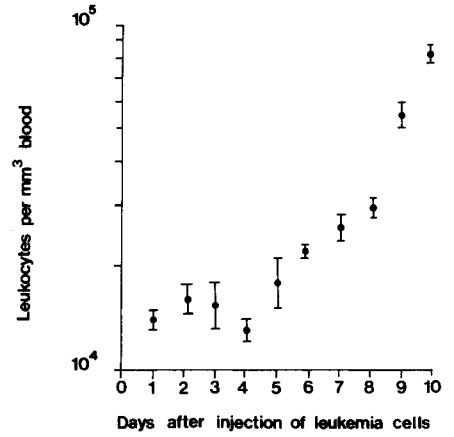

Fig. 2. Leukocyte counts in peripheral blood at different times after intravenous injection of 8×10^6 *chloroleukemia cells. Mean values and standard deviation for four animals in each group are shown.*

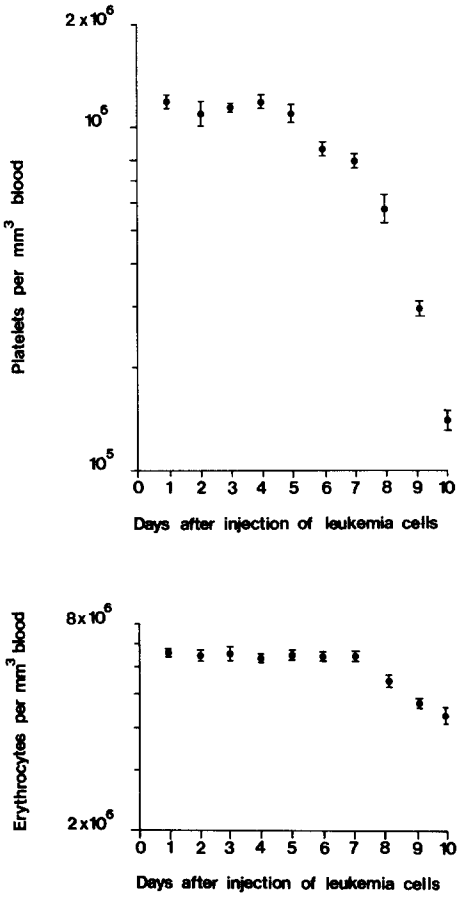

Fig. 3. Erythrocyte and thrombocyte counts in peripheral blood at different times after injection of 8×10^6 *chloroleukemia cells. Mean values and standard deviation for four animals in each group are shown.*

death occurred, the red cell and platelet counts were, respectively, 60 and 10% of their original value.

A progressive increase in wet weight was observed in spleen and liver, which was more pronounced and earlier for the former. At the end of the disease the spleen weighed about 4 times as much as it did originally (Fig. 4), while the liver weighed about 1.2 times as much, an actual increase of about 2 g (Fig. 5). Histologic examination of both these organs showed extensive infiltration of chloroma cells.

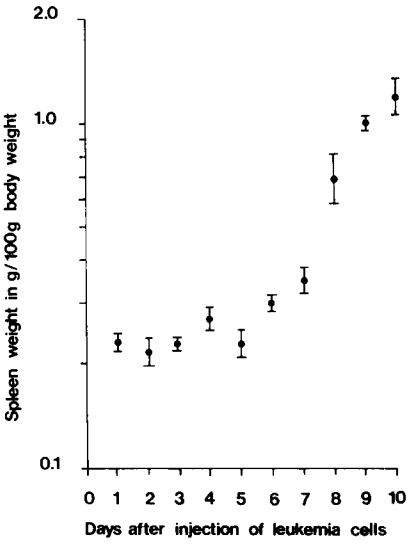

Fig. 4. Wet weight of spleen at different times after intravenous injection of 8×10^6 *chloroleukemia cells. Because of fluctuation in the body weight of recipient animals, spleen weight is expressed as* g/100 g *body weight. The body weight of transplanted animals remained constant during the course of the disease. Mean values and standard deviation for four animals in each group are shown.*

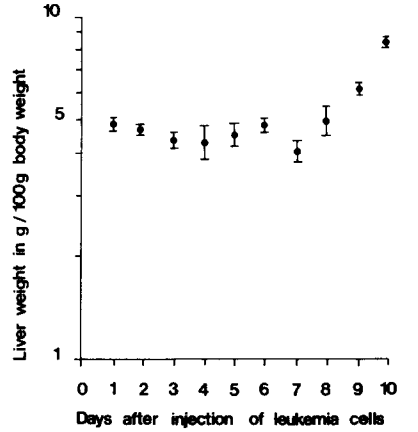

Fig. 5. Wet weight of liver at different times after intravenous injection of 8×10^6 *chloroleukemia cells. Liver weight is expressed as* g/100 g *body weight. Mean values and standard deviation for four animals in each group are shown.*

Dependence of survival time on inoculum size

The mean survival time of 4 groups of rats injected with decreasing numbers of chloroleukemia cells is shown in Fig. 6. It was 8.3 days for animals injected with 2×10^7 leukemia cells. For each 10-fold reduction of the inoculum, the mean survival time was prolonged by about 2.5 days. This finding is

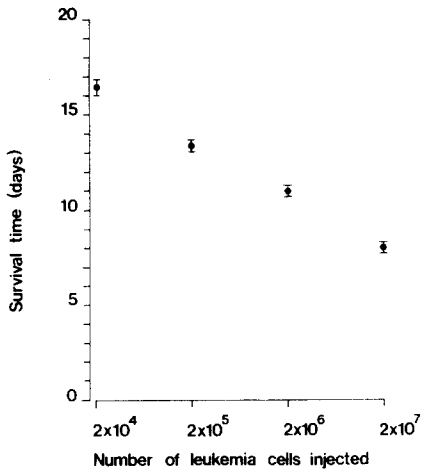

Fig. 6. Relationship between the number of chloroleukemia cells injected intravenously and the survival time of recipient animals. Mean values and standard deviation for four animals in each group are shown. The regression line was fitted by the least squares method.

consistent with an approximate *in vivo* doubling time of the chloroma population of about 19 hr. There was very little variation in survival time in each group. All the transplanted animals developed a typical generalized leukemia, as previously described, regardless of the inoculum size.

DISCUSSION

The main object of this study was to investigate the growth characteristics of generalized Shay chloroleukemia in rats after i.v. transfer.

As in other experimental leukemias [2], colonization of blast cells occurred from the outset in the bone marrow where, according to the [^{3}H]-thymidine uptake (data not shown), chloroleukemia cells were already proliferating 24 hr after transplantation. During the last 3 days of the disease, blast cells accounted for more than 50% of the whole bone marrow population. Interestingly enough, according to the [^{3}H]-thymidine uptake and the chromosome analysis (data not shown), from day 8 onwards all normal hemopoietic cells were in a quiescent state. This is consistent with a previous demonstration that, in animals bearing experimental rat leukemia L5222, there are fewer CFU-C in the cell cycle than in normal rats [11].

How leukemia cells suppress normal hemopoiesis is still unclear; cell–cell interactions between normal and leukemic marrow cells have been reported to give rise sometimes to inhibition of colony growth in agar culture [12–14]. Moreover, leukemia cells might also act on normal hemopoiesis by means of humoral inhibitors of cell proliferation [15, 16].

During the development of the leukemia, anemia and thrombocytopenia occurred in the recipient animals. A decrease in the red cell count was detectable 8 days after the transplantation of chloroleukemia cells. Mechanisms inducing the anemia are not yet clearly understood. There is accumulating evidence for depressed marrow erythropoiesis in rats after Shay chloroleukemia transfer [17–20]. Nevertheless, since the rat erythrocyte life span is about 50 days [21], it seems reasonable to conclude that anemia was not only due to suppression of erythropoiesis but also to peripheral factors. It is interesting to note that there was a relationship between the degree of anemia and the increase in spleen size, so that trapping and lysis of red cells by the spleen could be a cause of the anemia. Splenomegaly might also contribute to the thrombocytopenia, but the fall in platelet counts in peripheral blood should be attributed mainly to the decrease in megakaryocyte numbers; in fact, in normal rats about 50% of platelets are destroyed daily [22], so that suppression of megakaryocyte production would soon bring about a decrease in the number of platelets in the peripheral blood. According to the histologic findings, the liver and spleen were also invaded by chloroleukemia cells. Splenomegaly was a constant finding in the late stage of the disease, and histologic investigation showed an almost complete replacement of normal lymphocytes by leukemic myeloblasts. Proliferation of leukemia cells in the spleen was from the onset uniformly spread through the whole organ.

As previously reported in L5222 leukemia [23], blast cell infiltration in the central nervous system was a common finding in recipient animals with neurologic symptoms, so that Shay chloroleukemia seems to provide a useful model also for the study of central nervous system leukemia which has been reported to occur in some patients suffering from myeloid leukemia [24–26].

An essential feature of experimental leukemias is predictable growth. Based on the above data it has been calculated that the death of recipient animals occurred when the total number of chloroleukemia cells reached 10^{11}. Since the approximate *in vivo* doubling time is about 19 hr, it is easy to predict the survival time of recipient animals according to the number of injected cells. Moreover, since survival time varied so little in animals injected with the same number of chloro-

leukemia cells, it should be possible to evidence even slight variations of this parameter caused, for example, by therapy.

As mentioned above, the approximate *in vivo* doubling time of chloroma cells is about 19 hr, much shorter than usually reported in the human disease and very close to the *in vitro* generation time of chloroma cells as calculated by a percentage-labelled mitoses curve, which is about 16 hr (H. Toivonen, P. Foa, W. Paile and T. Rytomaa, in preparation). It appears, therefore, that the chloroleukemia cells grew *in vivo* with little cell loss, possibly owing to a poor immunological reaction of the host animal. This hypothesis seems to be supported by the fact that even an inoculum as small as 10^2 chloroleukemia cells was not rejected, and induced a generalized leukemia. Thus Shay chloroleukemia could also be useful as a model in the study of immunostimulating procedures.

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