Estimation of Prevalence Rates of Radiogenic Leukemias in RFM/U Mice (37489)

ROBERT M. ELASHOFF, FREDERIC C. LUDWIG, AND PAMELA HEMPHILL
(Introduced by A. C. Upton)

Research Systems Division, University of California in San Francisco, California 94122, and Department of Pathology, College of Medicine, University of California at Irvine, Irvine, California 92664

Murine leukemia can be diagnosed only when the mouse is moribund. Pallor of ears, feet, or nose, ruffled fur, palpable spleen, and shortness of breath indicate terminal leukemia. As a result, no data exist on the prevalence rates of murine leukemias. This lack contrasts strangely with the widespread use of these diseases in experimental cancer research.

The morphology of preterminal lesions in irradiated mice has been studied extensively (1), as well as various component processes of the preleukemic state (2); but the available observations do not allow inferences regarding the prevalence rates of overt leukemia.

An accidental finding during an earlier experiment with murine leukemia suggested a new approach to this problem.

In that experiment (3), we studied correlations between late radiation injury in blood-forming tissues and the percent cumulative probability of leukemia. The latter was determined in groups of mice allowed to live out their natural life span. Out of these groups, clinically nonleukemic mice were randomly selected and sacrificed. Thirty-five of the 296 mice sacrificed revealed early leukemic change when examined histologically, and could not be used for the study of nonmalignant late radiation injury.

This finding suggested the design of the experiment described here. A group of several hundred mice received one single leukemogenic X-ray exposure. Starting with Day 70 after irradiation, randomly selected groups of mice were sacrificed at seven preselected time intervals, and examined for early histopathological evidence of leukemia. This enabled us to estimate the point-prevalence rates of leukemia at the time periods selected for sacrifice.

Materials and Methods. Mice. A total of 524 mice of both sexes, of the RFM/U strain, were used. At the time of irradiation they were 80–120 days old. The original stock was supplied by Dr. Arthur C. Upton, Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee (now at the Health Sciences Center, University of New York at Stony Brook). The mice were subsequently inbred by the Simonsen laboratories, Gilroy, California. The animals received purina chow and tap water *ad libitum* after weaning.

X-ray exposure. All mice received a single radiation dose of 300 rads to the entire body (full scatter). The radiation factors were 250 KVP X-rays; HVL 1.5 mm Cu; absorbed dose rate in soft tissue, approximately 55 rads/min.

Sequential sacrifices and design of experiment. About 70 mice were sacrificed at Days 70, 90, 110, 130, 150, 170 and 200 after irradiation (Table I). Since the 524 animals used could not be made available simultaneously at the outset, they were randomly assigned to the various sacrifice groups, at a rate determined by the output of the breeding colony. This required a time period of about 6 months. In addition, spontaneous deaths occurring in the surviving colony were recorded (Table II).

Histopathological examination. Each mouse, whether sacrificed at a preselected time or because it was found moribund, was autopsied and microscopically examined. The tissues were fixed in Bouin's solution, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin-eosin. Slides from the following

organs were prepared: thymus; spleen; liver; axillary, inguinal, and retroperitoneal lymph nodes; left lung; left kidney; brain; left adrenal; a longitudinal section of the left decalcified femur. Except for some early cases of thymic leukemia, we were unable to detect leukemia in clinically intact mice by means other than microscopic examination. Early thymic lymphoma, if it was not identified on the basis of an enlarged thymus, involved replacement of the normal thymic architecture by large lymphoid cells which had sometimes begun to invade the mediastinum. In none of the clinically intact, sequentially sacrificed mice was thymic leukemia observable in organs remote from the thymus. This is consistent with the classical concept that this form of murine leukemia remains limited to the thymus before it spreads to other organs (4). In this respect, the myeloid and the stem cell variety are different. Besides replacement of the femoral marrow by densely packed leukemic cells, which was present in all cases observed, small colonies of leukemic cells could be seen in various organs. In contrast to some of the published observations on myeloid leukemias in mice (1), infiltration of Glisson's triangles in the liver was frequently seen in these early cases. Likewise, involvement of the interstitia of the renal epithelium enabled us to establish the diagnosis of incipient leukemia. The stem cell forms were sometimes limited to the lymph nodes alone. Involvement of the lung, brain or adrenals never occurred alone but always in conjunction with hepatic and renal infiltrations. We were unable to discriminate between earlier and more advanced forms among the leukemias discovered in clinically intact mice. But it is conceivable that the bone marrow is affected first, followed by emergence of colonies in liver and kidney, whereas lung, adrenals and brain show such changes only in more advanced cases. The leukemias found in the moribund mice resembled anatomically those described in the literature (1).

Computation of point prevalence rates of leukemias. At each of the days selected for sacrifice times, the number of the leukemic mice in the sample was determined. This figure was used to estimate the number of leukemias in the pool from which the sample was taken. The point-prevalence rate is then obtained by dividing this estimated number of leukemic mice in the pool by N, the total number of leukemic mice in the pool at a given time point. To be able to estimate this unknown number of leukemic mice we will assume at each time point that the number of leukemic mice in the sample follows a hypergeometric distribution:

$$\text{Prob}\left(\begin{matrix}\text{number of leukemic mice}\\ \text{in the sacrifice sample}\end{matrix}\right) = \frac{\binom{L}{I}\binom{N-L}{n-I}}{\binom{N}{n}},$$

where L = Total unknown number of leukemic mice,
I = Number of leukemic mice found in the sacrifice sample,
N = Total number of mice in the pool before sampling,
n = Number of mice taken in the sacrifice sample.

Using this assumption of a hypergeometric distribution we may now estimate L, the actual number of leukemic mice in the pool, by maximum likelihood methods. This estimate, L, is the greatest integer not exceeding $\frac{I(N+I)}{n}$ where I, N, and n are defined before. When L is divided by the total number of mice N at a time point, the estimate of the point-prevalence rate is obtained for that time point.

Results and Discussion. Table I summarizes the results of the sequential sacrifices; Table II, the findings from the animals dying spontaneously.

Approximately 30% of the nonleukemic deaths occurred shortly after radiation exposure. Death from radiogenic leukemias occurred essentially not earlier than 3 months after irradiation and increases thereafter until the end of the 200-day period. As expected, the incidence of the disease is higher in the sequentially sacrificed animals than in the

TABLE I.

Days	No. sacrificed (n)	No. leukemic (I)
70	70	1 (Th)[a]
90	75	1 (M)
110	77	2 (Th; M)
130	70	6 (S; 2 Th; 3 M)
150	72	5 (2 S; 3 Th)
170	70	10 (3 S; 4 Th; 2:M; N)
200	59	10 (2 S; 2 T; 5 M; H)

[a] Th, thymic; M, myeloid; S, stem cell; H, not identified.

TABLE II.

Time period (days)	Nonleukemic deaths	Leukemic deaths
0–29	5 (4 at 0 days)	0
30–49	0	1 (S)
50–69	3	0
70–89	1	0
90–109	0	2 (S, Th)
110–129	5	2 (2 S)
130–149	0	3 (S)
150–169	0	5 (S; 3 Th; M)
170–199	1	2 (S M)

spontaneously dying animals.

Both myeloid leukemia and thymic lymphoma are seen in the sequentially sacrificed animals before they occur in those found moribund. In contrast, stem cell leukemia is seen in the moribund mice before it occurs in those sacrificed at a preselected time. This is consistent with the assumption that stem cell leukemia is very short in duration, which is indeed well known from classical pathology.

The point-prevalence rates are given in Table III, as are L and N for each time point. These rates are variable due to random sampling. Some measure of this variability may be given in a 95% confidence interval on the true point-prevalence rate. These intervals are included in Table III.

During the period of 100–200 days there is a steady increase of the point-prevalence rates. The computed confidence intervals are based on large sample statistical theory. The limits reflect the same increase.

In an earlier study we have postulated the existence of a preleukemic state, distinct from overt leukemia (2). This preleukemic state does not entail the presence of malignancy but critically high numbers of virus particles and/or virus susceptible cells. It is only the probabilistic interaction between both which initiates the leukemic process. Since this event must occur some time before leukemia becomes histologically detectable, the prevalence rates suggested by the present studies are underestimated. The same applies to estimates of the duration of murine leukemias based on such data. The time difference between the appearance of leukemia in the sequentially sacrificed animals and its appearance as a cause of spontaneous death is a minimum value. It should be noted, however, that this is in principle not different from what we know about human leukemia. The actual disease process is present before it becomes observable.

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TABLE III.

Days	L	N	Prevalence rate (%)	95% Confidence interval
70	7	514	1.36	0.26– 6.87
90	5	443	1.13	0.20– 6.05
110	9	366	2.46	0.74– 7.77
130	24	282	8.51	4.33–15.92
150	14	209	6.70	3.31–12.96
170	19	132	14.39	9.55–20.83
200	10	59	16.95	