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Characterization of Transplantable Myelomonocytic Leukemia WEHI-3B in Syngeneic BALB/c Mice[1] (40096)

WILLIAM H. MURPHY, HOWARD B. URNOVITZ, JANET L. MARYANSKI, AND GERALD D. ABRAMS

Departments of Microbiology and Pathology (GDA), The University of Michigan School of Medicine, Ann Arbor, Michigan 48109

Clinical trials have shown (1–3) that remission and survival in acute myelomonocytic leukemia (AMML) is increased significantly by immunotherapy. Despite these important clinical findings few well characterized transplantation models of the disease in inbred mice are in use for the analysis of basic immunologic mechanisms. By employing an appropriate model it should be possible to characterize the relative malignancy of the major cell populations that comprise the disease, their relative immunogenicity, whether a protective immune response is serum or cell mediated, and the effects of drugs and immunopotentiating agents on the course of the disease. We are particularly interested in determining whether the immune mechanism recognizes with equal avidity the diverse malignant cell populations that comprise the disease. Warner, Moore and Metcalf (4) originally described the occurrence of AMML in BALB/c mice and derived four sublines from the original parental tumor (WEHI). The A and D sublines were chloromas. The 3B and 3C lines appeared to approximate AMML in humans. We report here the basic characteristics of a transplantation model of WEHI-3B AMML.

Methods. Mice. Inbred BALB/cwm mice (referred to as BALB mice hereafter) were obtained from our breeding stocks. Mice were 6–8 weeks old when used. The lineage of the strain and hematologic properties of the mice were described (5). Female mice were used for all experiments. *WEHI-3B line of AMML.* We obtained the WEHI-3B line from Dr. D. Metcalf of The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia. On receipt the subcutaneous tumor was transplanted immediately into our strain of mice. The methods used to develop the subcutaneous (SQ), splenic (SP) and intraperitoneal (IP) sublines are described under *Results.*

Balanced salt solution. WEHI cells are sensitive to the salt composition and pH of media. A balanced salt solution (MBSS) was used that was optimum for maintaining cell viability. MBSS contained per L of double distilled water: 0.14 g $CaCl_2$; 8.0 g NaCl; 0.4 g KCL; 0.2 g $MgSO_4 \cdot 7H_2O$; 0.2 g $MgCl_2 \cdot 6H_2O$; 0.06 g KH_2PO_4; 0.24 g $Na_2HPO_4 \cdot 2H_2O$; 0.01 g phenol red (Allied Chem. Corp., NY) and 4.5 g Hepes buffer (Schwarz-Mann, Orangeburg, NJ). The solution was sterilized by filtration through a 0.22 μfilter and the pH adjusted to 7.2.

Dose-response assays. Details are presented under RESULTS. The method of Skipper, Schabel and Wilcox (6) was used to determine whether a single viable cell of the IP subline was lethal to mice.

Histopathologic methods. Mice (3/group) inoculated iv or ip with 10^4 viable cells were killed at selected time intervals. Control mice (4/group) received injections of MBSS in place of the cell suspensions and were killed and examined at the end of the experiment. Samples of the thymus, spleen, mesenteric lymph nodes, heart, mediastinum, lung, kidney, adrenal gland, femur (bone marrow), genitourinary organs, gastrointestinal tract, and brain were taken for examination. Tissues were fixed in 10% (v/v) formalin–saline, paraffin-embedded, sectioned, and stained with hematoxylin and eosin. Cell blocks were prepared by centrifugation of cell suspensions, fixed in Bouin's solution, and similarly embedded, sectioned and stained.

Hematologic methods. Each of 55 mice received a single ip injection of 10^4 viable cells contained in 1 ml of MBSS. Control mice (14

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animals) received an ip injection of 1 ml of MBSS and were killed either on the day of injection or 18 days later. Methods were described in detail (5). Most animals were bled only once. If mice were bled twice the first bleeding was done on day 13, followed by a second bleeding on day 20 or 21. Peripheral blood was obtained by cutting 1–2 mm off the tail and then letting the blood flow freely. Specimens taken 30–60 sec later were collected directly into hematologic pipettes and heparinized microhematocrit tubes. Total and differential leukocyte counts were made by the method described by Wintrobe (7). Peripheral blood samples were diluted 1:20 in a hematologic pipette with Turk's solution (3% glacial acetic acid (v/v), 1% Gentian violet (v/v), in distilled water). Erythrocytes were allowed to lyse for 5 min and the leukocytes then counted in a hemocytometer. The percent distribution of leukocyte types was determined by counting 100 cells on Wright stained films. The microtechnique was used for hematocrit determinations. Heparin coated capillary tubes were filled approximately halfway and sealed at both ends. Sealed tubes were placed in microhematocrit rotor head and centrifuged for 5 min at 4000 rpm. The percent of packed cells was determined by use of a microhematocrit tube reader.

Plating efficiency of cell lines. Standard procedures were used for passing the tumor cell lines for efficiency of plating tests: Mice were inoculated sq with 2.25×10^5 cells of the SQ line; tumors were harvested 17–21 days later. An inoculum (ip) of 10^5 IP cells was used; tumors cells were harvested 14–16 days later. For the SP line 10^7 tumor cells were injected ip; tumor cells were harvested approximately 18 days later. Under these conditions the cell populations of each transplanted line contained the greatest proportion of tumor cells as determined by microscope observation; viability was 85–90%. Mondisperse preparations of cells were suspended to the desired concentration in RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY) containing 10% fetal calf serum (inactivated at 56° for 30 min). To plate cells serial tenfold dilutions were made in double strength RPMI growth medium containing 20% inactivated fetal calf serum, 200 U/ml of penicillin and 200 μg/ml of streptomycin. Difco agar (Difco Laboratories, Detroit, MI) was made up in double distilled water to a final concentration of 0.6%, melted, maintained at 45°, and added volume:volume to dilutions of cell suspensions. Three ml of each cell suspension was plated in triplicate (3 wells/dilution of cell suspension) in Costar Cluster[6] plates (Costar, Cambridge, MA). Cells were incubated at 37° in a CO_2 incubator (5% CO_2, 95% air) for up to 7 weeks. Cells were fed at approximately 4 day intervals by adding 1–2 ml of semisolid growth medium to each well. An inverted microscope (10× lens) was used to score colonies at approximately 4 day intervals. A discrete cluster of cells was scored as a colony if it contained more than 50 cells (8).

Statistical methods. Statistical methods were described (9). Data from the dose-response assays for the SQ, SP, and IP lines were computer plotted by use of the Michigan Interactive Data Analysis System (MIDAS) which is a subroutine on the Michigan Terminal System. Tests included one-way covariance models for regression analysis, and Student's "t" test.

Results. Derivation of the SQ, SP and IP sublines. The starting material for all three subines was a SQ transplant (360-73) of WEHI-3B.

The SQ subline was maintained by serial passage at 3-week intervals. Tumors were harvested at their peak of growth and minced to 1 cu mm with sterile scalpels in cold (4°) MBSS to yield dispersed cell suspensions. After cell and tissue debris was allowed to settle (4–5 min at 4°) the cells remaining in suspension were washed twice. Viable cell counts were done by the trypan blue dye exclusion technique. Cells were suspended in MBSS to prepare 20% suspensions (v/v) and 0.5 ml was injected SQ between the scapulae of recipient mice. The *splenic (SP) subline* was derived from the SQ line by the ip injection of a 20% suspension of SQ tumor cells. Thereafter spleen cells from mice moribund from leukemia were used for serial passage by the ip route. Because the original sublines of WEHI employed by Warner, Moore and Metcalf (4) underwent significant variation on serial passage, the SP subline was passed serially 25× to adapt it fully to the splenic

growth form. Monodisperse spleen cell suspensions of the SP subline were washed twice in MBSS and viable cell counts done by the trypan blue dye exclusion technique. For routine passage 10^6 viable cells were injected ip. The *IP Subline* was derived from mice that had been inoculated with passage 27 of the SP subline. However, peritoneal cells obtained from mice moribund from leukemia were used for serial passage by the ip route instead of spleen cells. For routine passage 6.0 ml of MBSS were injected ip into mice moribund from AMML, the abdominal cavity gently massaged, and the fluid withdrawn with an 18 gauge needle and syringe. Cells were washed twice and counted. Viability consistently was 95–100%.

Morphologic studies. For morphologic studies Wright-stained films and hematoxylin–eosin stained sections of cell blocks were prepared from the cell suspensions used for routine passage. The morphologic properties of the cells in the SQ tumors were essentially the same as those described by Warner, Moore and Metcalf (4). Figure 1 shows the cell types characteristic of the SP subline as seen in cell block preparations. Many of the cells were large, atypical blasts, characterized by small amounts of cytoplasm and large, pleomorphic, round to irregular nuclei containing two to four nucleoli. Occasional dividing forms were present. Other large atypical cells were monocytoid with indented, lobulated, or folded nuclei or horseshoe-shaped, irregularly lobed, nuclei. Scattered amongst these elements were many lymphocytes, presumably of splenic origin, and occasional megakaryocytes and granulocytes. Cells of the IP subline were more homogeneous morphologically than the SP line because of the absence of admixed splenic elements.

Dose-response assays. Cell suspensions were prepared from each subline as described above. Viable cells in graded $\log_{10}$ dilutions were injected by the appropriate route into separate groups of mice. Mice were observed for 45 days after inoculation and deaths from leukemia were scored. Regression curves were computer analyzed as described previously (9). Data for the SQ subline were obtained for passages 8 and 10, from passages

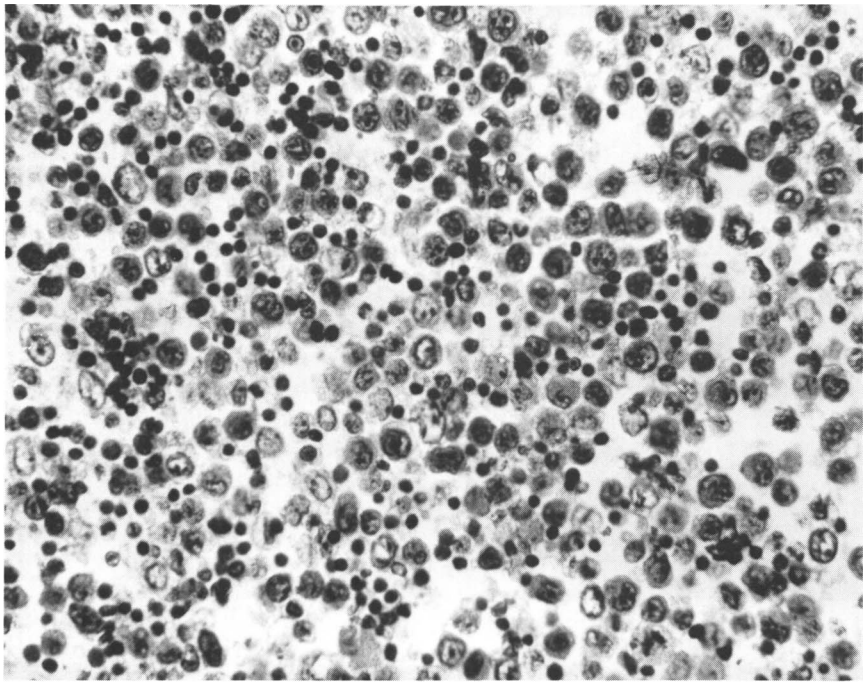

FIG. 1. Spleen cells obtained from a mouse moribund from the SP subline of transplanted WEHI-3B myelomonocytic leukemia were prepared as a cell block and sectioned. Large pleomorphic leukemic cells are mixed with lymphocytes. Magnification × 410; all sections were stained with hematoxylin–eosin.

9, 13, and 33, respectively for the SP subline, and for passages 1, 4, 6, and 20, respectively, of the IP subline. The slopes of the regression curves for the serial passages of any single cell line (data not shown) did not differ significantly. When the data were pooled (Fig. 2) it was found that the three sublines did not differ significantly in their lethality for mice. Within any single experiment the standard deviations in survival data were of the range ±0.1–3.8. In practice, the spread in time in days until death at any given cell dose with any of the three transplantation lines did not exceed 1–5 days.

Single cell kill for the IP subline. The statistical approach employed previously by us (9) and by Skipper, Schabel and Wilcox (6) was used to test whether a single tumor cell of the IP subline caused fatal AMML. A detailed discussion of the theoretical basis for the analysis is presented by Skipper, Schabel and Wilcox (6). In brief, the fraction of mice that die from transplanted leukemia, when they receive an inoculum of two or less cells, is predictable from the Poisson distribution series where $P(m) = a^m \cdot e^{-a}/m!$ In this equation $P(m)$ is the fraction of mice that receive "m" viable cells when the average number of viable cells injected per mouse is "a". Cell suspensions are diluted so that some mice receive 0 cells, some one cell, some two cells, etc. One can estimate the number of cells injected into each mouse by applying the Poisson distribution to evaluate $P(0)$, *i.e.*, when some mice survive because they receive

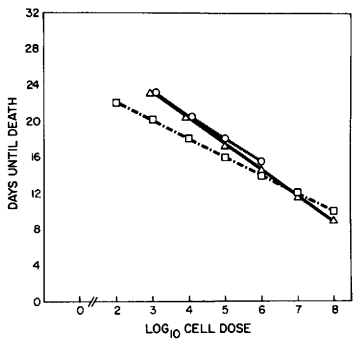

FIG. 2. Dose response of BALB/cwm mice to graded $\log_{10}$ doses of the SQ (○· · · ·○), SP (△——△) and IP (□- - -□) sublines of WEHI-3B myelomonocytic leukemia. Each point on each regression curve represents the data from pooled results (two to four experiments, 20–25 mice per point).

0 cells. Under these assay conditions the equation simplifies to $P(0) = e^{-a}$. In our experiments we simply sought to determine whether the lethality of tumor cell suspensions was consistent with single cell kill. Thus, a suspension of IP cells (10^7 viable cells/ml) was diluted to contain (statistically speaking) one viable cell/ml of inoculum. In the experiment 68 mice received a 1 ml ip injection of the diluted preparation. Of these 7 died from transplanted AMML whence $P(0) = 61/68$ or $P(0) = 0.9$. Substituting 0.9 for $P(0)$ yields $0.9 = e^{-a}$ whence "a" becomes 0.11. Thus, under the test conditions the average number of leukemic cells injected per mouse was 0.11. The computed probability distribution is as follows: $P(0) = 61$ mice received no leukemic cells; $P(1) = 7$ mice received one leukemic cell; $P(2) = 0.005$ mice received 2 malignant cells. The actual experimental results were in excellent accord with the computed probability distribution. Thus, the findings provide evidence that a single malignant cell of the IP subline caused fatal AMML. Since the dose-response curves for all 3 sublines were so similar it is reasonable to assume that they all approximated single cell kill.

Hematologic studies. Detailed hematologic studies were done with the IP subline (passage 49). Each of 55 mice received a 1 ml injection of 10^4 viable cells ip. The experiment was terminated at 21 days because cumulative mortality reached 80–90% by this time and values were so variable in such moribund mice. The data summarized in Table I are based on the following definitions: Lymphocytes were assigned to a single category irrespective of size. Cells were scored as mature granulocytes if they had distinctly segmented nuclei and specific cytoplasmic granules. Granulocytes with slender, ringlike, but nonsegmented nuclei were classified as bands while those with heavier ringlike nuclei, or round to oval nuclei, were defined as metamyelocytes and myelocytes, respectively. These last three categories are classified under the single heading "immature granulocytes." Monocytes were identified by their abundant gray-blue cytoplasm and indented, lobulated, or folded nuclei. "Blast cells" were identified by their blue cytoplasm and large nuclei, a fine chromatin pattern, and usually multiple nucleoli. Some blast cells were only

slightly larger than the other circulating leukocytes while others were giant forms with a two to threefold larger volume. "Immature monocytes" were intermediate morphologically between blasts and monocytes.

Table I shows that hematocrits remained normal for 18 days but decreased approximately 20% during the last 2 to 3 days of life. Similarly, total leukocyte counts were essentially normal through day 17. Thereafter, elevation of total counts was pronounced with median values at days 18, 19, 20 and 21 of approximately 22,000, 28,000, 42,000 and 73,000 WBC/mm^3, respectively. This leukocytosis could be attributed largely to an increase in cells in the granulocyte compartment (mostly neutrophils, an occasional eosinophil, but no basophils). An increase in mature granulocytes was evident by 12 days after incubation with approximately a tenfold increase immediately before death. There was an fourfold increase in immature granulocytes by day 18 with a 16-fold increase before death. An increase in mature monocytes was evident by 18 days with approximately a tenfold increase before death. Immature monocytes and blast cells were detected as early as 8 to 16 days after inoculation and either remained elevated or increased in number up to death.

Histopathologic studies. 10^4 viable IP cells were injected ip and mice were killed at 4, 8, 12 and 16 day intervals. At 4 days malignant cells were not detectable in tissues. By 8 days there were neoplastic infiltrates in the intra-abdominal adipose tissue. At 12 days these infiltrates were larger and malignant cells were evident in the liver. Neoplastic cells were present in mediastinal and retroperitoneal lymph nodes but were not detected in the spleen or bone marrow. By 16 days infiltrates were more extensive and had begun to penetrate into the pelvic and abdominal viscera. Although the splenic red pulp was infiltrated by neoplastic cells, similar leukemic infiltrates could not be detected in the marrow. However, there were small, ill-defined foci in the marrow in which less mature forms seemed more numerous than is normal. Figures 3 and 4 show typical histopathologic changes. When mice were inoculated with the SP subline the results were essentially the same. When IP or SP cells were inoculated by the iv route no remarkable differences were found compared with the ip route.

Plating efficiency of cells. Although the three transplanted cell lines were essentially equal in their lethality for mice, the possibility existed that the proportions of tumor cells of different types comprising each line might differ as a result of selective pressures exerted during serial passage. The histologic studies described above indicated either qualitative or quantitative differences in the cell popu-

TABLE I. HEMATOLOGIC CHANGES IN MICE INOCULATED WITH THE IP SUBLINE OF WEHI-3B MYELOMONOCYTIC LEUKEMIA.

Days[a] after inoculation	Cells per cu mm × 10^{-3}							
	Hematocrit	Total WBC	Lymphocytes	Granulocytes[b]	Immature Granulocytes[c]	Monocytes	Immature monocytes	Blasts[d]
4	55.6 ± 2.0	11.3 ± 2.4	9.1 ± 2.3	1.7 ± 0.3	0.1 ± .1	0.2 ± 0.1	0.0 ± 0.0	0 ± 0
8	56.4 ± 5.7	13.2 ± 3.6	10.3 ± 3.3	2.4 ± 0.8	0.2 ± .3	0.3 ± 0.3	0.0 ± 0.0	.1 ± .1
12	57.1 ± 9.8	17.6 ± 3.7	12.6 ± 4.3	3.9 ± 2.5	0.2 ± .2	0.2 ± 0.3	0.0 ± 0.1	.1 ± .2
16	50.9 ± 3.1	13.8 ± 2.9	7.4 ± 2.6	5.1 ± 1.4	0.3 ± .3	0.4 ± 0.2	0.2 ± 0.2	.4 ± .2
17	53.4 ± 1.9	13.6 ± 3.0	6.9 ± 1.7	5.6 ± 2.1	0.2 ± .2	0.2 ± 0.2	0.2 ± 0.1	.3 ± .2
18	51.7 ± 5.5	21.8 ± 7.8	10.0 ± 5.5	8.3 ± 4.0	0.4 ± .1	0.7 ± 0.4	0.7 ± 0.5	.4 ± .3
19	47.3 ± 3.8	28.5 ± 13.1	11.5 ± 7.0	14.4 ± 5.7	0.8 ± .9	1.4 ± 1.5	0.1 ± 0.2	.2 ± .3
20	43.9 ± 5.4	41.7 ± 14.6	17.6 ± 7.9	19.2 ± 5.8	1.0 ± .8	2.2 ± 1.1	0.4 ± 0.5	1.2 ± 1.5
21	41.6 ± 3.3	72.8 ± 46.0	35.0 ± 20.0	30.0 ± 23.8	1.6 ± 1.7	3.9 ± 2.8	0.5 ± 0.7	1.8 ± 2.0
18[e]	54.1 ± 2.1	14.3 ± 3.3	11.1 ± 3.1	2.7 ± 0.7	0.1 ± .2	0.4 ± 0.3	0.0 ± 0.0	0 ± 0

[a] 10^4 viable IP cells were injected ip into separate groups of mice so that most animals were bled only once. Each data point represents the results from 7 animals.

[b] Polymorphonuclear neutrophils and eosinophils.

[c] Myelocytes, metamyelocytes, and bands.

[d] Undifferentiated stem cells.

[e] Control mice inoculated with MBSS, and killed either on the day of injection or 18 days later. Since counts were essentially the same for both sets of mice, data are presented only for the 18 day animals.

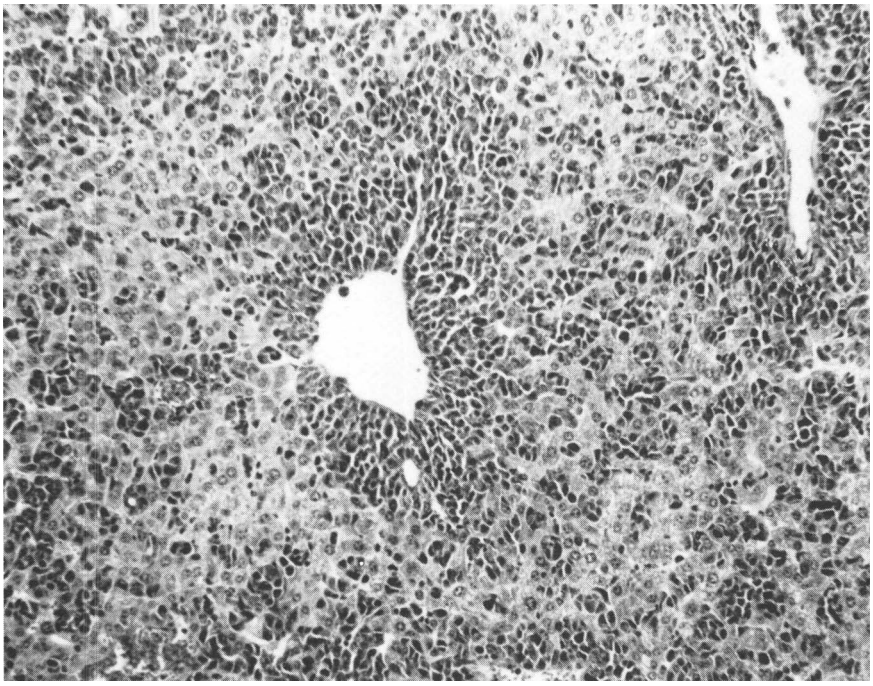

FIG. 3. Liver from a mouse moribund from the IP subline of transplanted myelomonocytic leukemia. The hepatic parenchyma is partly replaced by deeply staining leukemic cells that are particularly dense perivascularly (× 160).

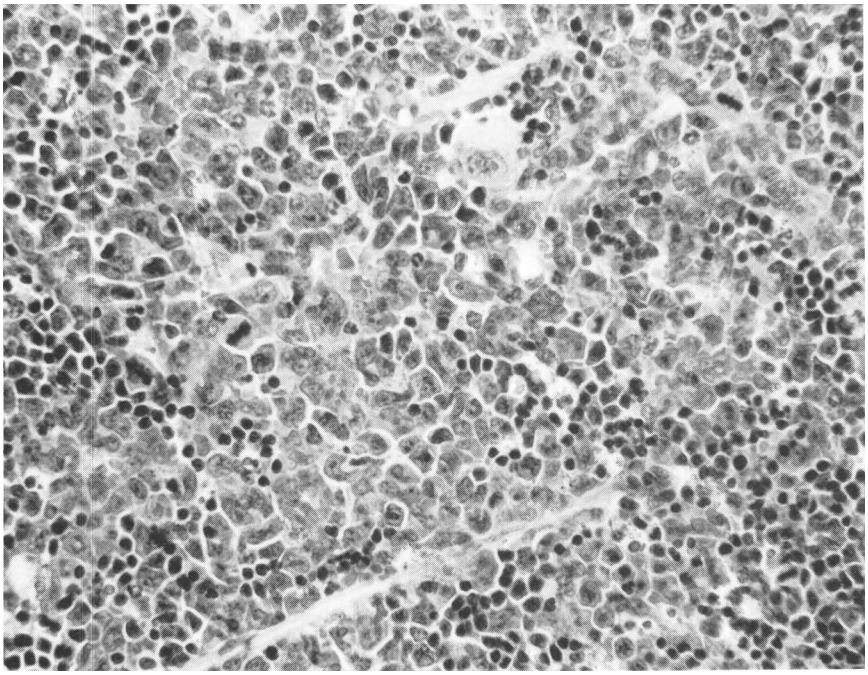

FIG. 4. Spleen from a mouse moribund from the IP subline. The red pulp is largely replaced by pleomorphic monocytoid cells that contrast with the smaller darker lymphocytes (× 410).

lations in the three cell lines. In an attempt to obtain more objective data we compared the plating efficiency of the SQ, SP, and IP sublines. Freshly harvested cells of each tumor line were diluted serially, plated in semisolid medium, and incubated at 37° for up to 7 weeks. Cell growth was scored by examining plates at approximately 4 days intervals. Clusters of 50 or more cells were scored as colonies. "Compact" colonies were those that grew rapidly (were detectable macroscopically in 10–15 days and were 1 to 2 mm in diameter), had very dense centers, and were surrounded by a narrow border of cells growing into the agar. "Diffuse" colonies usually grew more slowly, did not have compact centers, and cells were diffusely distributed in the semisolid agar. No attempt was made to identify the cell type in colonies as was done by Metcalf, *et al.* (8). Even though the data in Table II are somewhat of a preliminary nature they reveal rather clear cut differences in the growth capabilities of cells of the SQ, SP, and IP lines. Colonies failed to grow out from the SQ and SP lines if the inoculum contained less than 10^5 cells per ml. At the 10^5 inoculum growth was so dense that colonies overlapped and accurate counts could not be made. A rather wide range of colony types was observed with the SQ line. With the SQ line colonies could not be detected macroscopically until cultures were 4–5 weeks old. The SP line showed the same threshold effect as the SQ line, although colonies were usually of the compact or diffuse type. Colonies were detectable macroscopically after 10–15 days of incubation. When a cell inoculum of 3×10^2 or 3×10^1 of the IP line was used, plating efficiency was of the order of 5%, colonies became visible macroscopically in 10–15 days, growth was essentially the same in medium containing a final concentration of either 10 or 20% fetal calf serum, colonies were either of the diffuse or compact type, and the two colony types occurred in equal proportions.

Discussion. To develop a practical model of transplantable AMML in mice three sublines of the WEHI-3B tumor were adapted by serial passage in syngeneic BALB/c mice. The SQ subline was employed as a model to simulate solid tumor growth, the IP subline to represent the ascitic growth form, and the SP subline as a model for splenic transmission. For each subline the time in days until death was a linear function of the $\log_{10}$ cell dose inoculated (Fig. 2). The slopes of the regression curves of the three sublines were essentially the same and remained stable after each subline was fully adapted by serial passage. The stability of the cell lines demonstrated in this report was important because Warner, Moore and Metcalf (4) described marked changes among subpassages of WEHI cells on serial transplantation. Variability in the properties of transplantable lines of rat AMML has been a problem in the development of some of these models (10).

The main value of the dose-response data is that they permit precise quantification of AMML tumor cell populations. Thus, it becomes possible by use of techniques described previously (11) to quantify how immunologic reactions or drugs affect the course of the experimental disease. However, the morphologic diversity of AMML cell populations was

TABLE II. PLATING EFFICIENCY OF THE SQ, SP, AND IP SUBLINES OF WEHI-3B.

Cell line	Passage numbers	% plating efficiency with inoculum concentrations:[a] 10^5	10^4	10^3	10^2	10^1	Time for colonies to become visible macroscopically	Colony types[c]
SQ	12	TNC[b]	0	0	0	0	4 to 5 weeks	Diverse
SP	37	TNC	0	0	0	0	10 to 15 days	Diffuse or compact
IP	103, 107	TNC	TNC	TNC	4.97	4.95	10 to 15 days	Diffuse or compact

[a] Percentage of viable cells inoculated into plates that developed into discrete colonies containing 50 or more cells each. Cells at the indicated densities were inoculated into Costar Cluster[6] plates using triplicate wells/cell dilution. Cells were incubated at 37° for up to 7 weeks in a CO_2 incubator. Cells in plates were fed at approximately 4 day intervals by adding 1–2 ml of fresh semisolid growth medium. Plating efficiencies were calculated by dividing the number of colonies/plate by the number of viable cells in the inoculum × 100. Results are from two representative experiments.

[b] Colonies were Too Numerous to Count.

[c] See text for detailed description.

a complicating factor, i.e., the slopes of the regression curves may have represented the sum effect of interacting cell populations. Thus, it was important to determine if the lethality of a single malignant myelomonocytic leukemic cell was altered by other cells counted in the same test population. The results of the statistical analysis with the IP subline provided evidence consistent with single cell kill, i.e., the presence of nonmalignant cells in preparations did not affect the malignancy of single IP cells under these test conditions.

Although the dose-response and histopathologic data indicated that all three sublines were similar, they could be distinguished from each other by the use of more sensitive assay techniques. For example, the data in Table II shows that the plating efficiency of the IP line was approximately 5%, colonies 1–2 mm in size grew out in 10–15 days, and were either compact or diffuse. In contrast, the SP and SQ lines both showed a marked "threshold effect", i.e., colonies failed to grow out when an inoculum of $<10^5$ cells was used. At the 10^5 concentration colonies grew at such high densities that they overlapped each other. For the SP line colonies grew out to macroscopically visible size in 10–15 days and were either "compact" or "diffuse." For the SQ line colonies were not detectable macroscopically until 4–5 weeks of incubation and a rather diverse array of colony types were observed. While the data in Table II are admittedly of a preliminary nature, they nevertheless provide rather convincing evidence for qualitative differences in the cell populations that comprise the three transplantable lines. Diversity within a given line also has been documented. For example, 13 cloned lines of cells of been established in culture from the IP line. They differ in their morphologic properties, growth characteristics, and relative malignancy for BALB mice. For example, the LD_{100} dose of a line designated A3 is 10^6 viable cells with a mean survival time of mice of 50.3 days. For a line designated MW-7, the LD_{100} dose is $<10^3$ and mean survival time is 32.2 days. Detailed studies of the type described by Metcalf *et al.* (8) are being carried out to more adequately characterize the cell populations that comprise the SQ, SP, and IP lines.

The histopathologic changes caused by the IP subline were consistent with the initial multiplication of the malignant cells in various abdominal organs and tissues. After progressive cell growth and infiltration of tissues, cell populations reached proportions sufficient to appear in the peripheral blood. Such results are typical of transplanted leukemias. In the original report by Warner, Moore and Metcalf (4) detailed hematologic data were not provided for the WEHI-3B line. Because of the recognized difficulties in the precise identification of the cell types that are found in AMML (12) we classified cells into major categories that could be identified with reasonable accuracy. The most impressive hematologic change was the increase in the cell populations in the granulocyte compartment (neutrophils). An increase in mature granulocytes occurred as early as 12 days after inoculation and became approximately tenfold before death. A similar pattern occurred with the cells classified as "immature" granulocytes (principally metamyelocytes and bands). Blast cells were detected in the peripheral blood as early as 8 days after inoculation and increased in number until death. Mature and immature monocytes increased in the peripheral blood by 18 days after inoculation and remained elevated until death. These hematologic changes, and the accompanying pathologic changes in tissue, were consistent with characterization of the disease as myelomonocytic leukemia as reported originally (4). We also confirmed that invasion of bone marrow was minimal in the transplanted disease. This does not necessarily detract from the experimental model since it will be of interest to determine whether the failure of WEHI-3B cells to infiltrate the bone marrow in large numbers is immunologically mediated or has some other basis. For example, resistance to allografts of bone marrow in mice appears to be mediated (13) by a ^{89}Sr-sensitive bone marrow effector cell (M cells) and is relatively independent of classical B and T cell immunologic mechanisms.

The proliferative changes observed in tissues, as well as some of the hematologic alterations in the peripheral blood, are similar to those found in other transplanted myeloid leukemias. For example, in their studies of RMF transplanted leukemia Husseini *et al.*

(14) also found a stimulatory effect of transplanted myeloid leukemia cells on normal hematopoiesis. Studies in progress have shown that a cloned line of WEHI-3B cells (HUCL-1) produces colony stimulating factor (CSF) *in vitro*. Perhaps the production of CSF *in vivo* by malignant WEHI cells accounts for some of the hematopoietic or histopathologic changes seen in this disease. It also may account for the "threshold effect" observed with the SQ and SP lines in the plating efficiency tests. Thus, it will be of interest to compare various cloned lines of WEHI-3B cells for their relative malignancy *in vivo*, their capacity to produce CSF, and accompanying histopathologic changes. The current study was done to carefully define the disease so that the results of such experiments could be evaluated accurately.

Summary. Three transplantable sublines of WEHI-3B myelomonocytic leukemia in BALB/c mice were developed by splenic (SP), intraperitoneal (IP) and subcutaneous (SQ) passage. All three sublines then were compared for stability on passage, lethality for mice, and major hematologic and histopathologic effects. The time in days until death was a linear function of the $\log_{10}$ dose of viable cells injected for each cell line. Evidence was provided that a single cell of the IP line was lethal to mice and that death from all three cell lines approximated single cell kill. All three sublines were stable after adaptation by serial passage. Detailed hematologic and histopathologic studies were done on mice that were inoculated iv and ip with the SP and IP sublines. Changes typical of myelomonocytic leukemia were observed except that bone marrow invasion was minimal.

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