Microfluorometry of Nuclear Acridine Orange Metachromasia in Lymphocytes of Thymus, Spleen, and Blood of AKR and Random-bred Mice

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SUMMARY

Lymphocyte chromatin lability to acid hydrolysis was studied using acridine orange fluorescence metachromasia in a high-lymphocytic-leukemia-susceptibility strain (AKR) and random-bred mice (ICR). Comparisons were made of blood, thymus, and spleen lymphocytes between random-bred, “normal” AKR, and leukemic AKR animals. The leukemic mice were in the stages of the disease characterized by enlarged thymus and spleen but preceding massive elevation of blood lymphocytes. The ranges of the mean chromatin acid lability overlapped and were nearly identical in peripheral blood lymphocytes. However, thymic and splenic lymphocytes showed a marked rise in mean chromatin acid lability in the leukemic animals. The ranges of the mean values of this parameter were also found to be far greater in the lymphopoietic organs of normal AKR than in the random-bred mice. The data indicate that anatomically normal AKR animals of an age in which they are highly susceptible to spontaneous lymphocytic leukemia may contain a greater number of lymphoblasts in both the spleen and the thymus than do comparable random-bred mice. The implications of these findings are discussed in relation to strain differences and the concept of thymic origin of lymphocytic leukemia in mice.

INTRODUCTION

The histopathology of spontaneous murine lymphocytic leukemia has been described by Siegler (12). These studies show that the neoplasm in mice of the AKR strain originates in the thymus followed by dissemination to other organs. The route by which the neoplastic lymphocytes leave the thymus is presumably via the thymic vein rather than through the lymphatics. This conclusion is based on the observation that secondary tumors appear first in the spleen rather than in the thymic capsular lymphatics or internal mammary lymph nodes. The tumor cells proliferate in the splenic lymphoid follicles, thus enlarging the organ and creating a distinctive mottled appearance in section. With continued growth of the secondary splenic tumor, individual tumor cells begin to appear in the splenic venous sinuses and in the liver. It is only at this relatively late stage that neoplastic lymphoblasts begin to increase in number in the blood.

One interesting aspect of murine lymphocytic leukemia is that clinical symptoms are not usually evident until the disease is far advanced. It is only then that the disease is characterized by a terminal phase of massive release of tumor cells into the bloodstream (12). Thus, the blood leukocyte count and differential are not elevated in early stages and are therefore useless for the early detection of the disease. In fact, this condition may exist even in clinical stages in certain domestic species (11).

In view of the frequent use of mice of the AKR strain for the study of lymphocytic leukemia, we decided to investigate the disease with a microfluorometric technique using the metachromatic fluorochrome, AO. Our previous studies of various types of neoplasias in animals using variations of these procedures indicated their potential (1, 2). It was felt that the microfluorometric procedure might prove to be more sensitive in detecting lymphoblasts in the lymphopoietic organs than were the standard staining procedures applied to histological sections. Furthermore, this method might provide a means for quantitating the progress of the disease that could prove valuable in a number of experimental situations, such as in screening of carcinogenic or carcinostatic drugs.

MATERIALS AND METHODS

Two populations of male mice were maintained in standard laboratory cages in an air-conditioned room and provided with food and water ad libitum. One population consisted of retired breeders of the AKR/J strain and the other consisted of comparable random-bred animals (ICR/Dub).

Nine individuals of each strain were randomly chosen for this study. Standard blood smears were prepared from a cut on the tail. Some of the smears were stained with Wright’s stain while others were fixed in ethanol:acetone (1:1, v/v) for 15 min at room temperature. The latter were stored in the fixative in sealed containers at 4°C to be used for AO staining. Differential leukocyte counts were made from the Wright’s stain smears for each animal, differentiating a minimum of 200 cells/slide. Blood from the cut tail was also used at this time for determining the WBC from each animal using a standard hemocytometer method.

The mice were subsequently killed by etherization and

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2 The abbreviation used is: AO, acridine orange.

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autopsied immediately. The thymus and spleen of each animal were removed, and examined grossly and portions were used for preparation of standard histological sections. Other portions of these organs were used to prepare free lymphocytes.

Preparation of Thymic and Splenic Lymphocytes. Portions of each thymus and spleen were minced in 0.25 M sucrose containing 2 mM MgCl2. Aliquots of the cell suspension were transferred to acid-cleaned glass slides, air dried, and fixed in ethanol:acetone as above. The cell densities (cells/unit area) are uniform among slides and low (less than 10 cells/10× field). Furthermore, care was taken to spread the cells uniformly over the slide surfaces. Microscopic examination of samples of these slides showed that the predominant nucleated cell type after this treatment was the lymphocyte and that these were clearly distinguishable from the relatively few other nucleated cell types from these organs.

Chromatin Denaturation. Whole blood smears fixed in ethanol:acetone and slides of fixed, minced thymus and spleen from each animal were used to measure chromatin acid lability by AO microfluorometry. The slides were placed horizontally over glass rods in covered Petri dishes lined with filter paper moistened with water. The slide surfaces were flooded with a solution of RNase (5 mg/50 ml H2O, pH 6.0) and incubated at 37° for 30 min. The RNase preparations were subsequently rinsed in distilled water for 5 min, shaken dry, and placed in 5 N HCl at room temperature for 5 sec. They were immediately quenched in an ice-cold solution of 0.15 M NaCl and 0.015 M sodium citrate dissolved in 10% formalin adjusted to pH 7.0 for 5 min and rinsed in distilled water for 5 min at room temperature. The slides were subsequently stained in AO without storage.

AO Staining. The AO staining procedure was essentially as previously described except that cover slips were mounted over immersion oil (nD58° = 1.5150) (1). In all cases, the times in each solution, the temperatures, and the ratios of the numbers of slides per unit volume of solution were held constant. The final poststaining rinse was limited to 5 min.

Microfluorometry. After RNase treatment there was no cytoplasmic fluorescence, indicating that all RNA was removed. The nuclear fluorescence, under the present conditions, was due to binding of the AO with unmasked, destabilized DNA, and the color was proportional to the degree of denaturation of the chromatin induced by the brief exposure to the acid (see "Discussion"). Fluorescence color was determined by measuring the intensity of nuclear fluorescence at 590 and 530 nm for each nucleus. The numerical value of the metachromatic ratio, fluorescence emission intensity at 590 nm per fluorescence emission intensity at 530 nm (voltage), serves as a measure of the degree of denaturation induced by the acid treatment. Two hundred ten lymphocytes were measured in this manner from each slide. Means and standard deviations were computed for each animal.

The instrument used for microfluorometry was a Lietz Ortholux microscope fitted with an incident light illuminator. The exciting light was generated by a xenon arc. The optical design of the detector, the electronics, and procedure for measurement have been previously described (6). All measurements were done with a 40× objective.

RESULTS

Of the 9 AKR mice used, 2 showed distinctive signs of lymphocytic leukemia upon autopsy. These animals (Animals 1 and 2) exhibited a greatly enlarged, anteriorly displaced thymus, splenomegaly with distinctive mottled appearance, and enlarged mesenteric lymph nodes. Histologically, these organs showed a massive content of lymphocytes and, in the spleen, there was an accentuated follicular pattern caused by infiltration of neoplastic cells in the follicles.

The remaining 7 AKR animals appeared normal upon autopsy. Thus, the 2 animals showing distinctive anatomical evidence of leukemia were judged to be in relatively advanced stages of the disease and are numbered 1 and 2 in all of the tables. The remaining 7 were judged to be normal, or at least in a stage prior to the incipience of the disease, and are numbered 3 through 9 in the tables. Thus, the ranking of the animals is consistent and may be compared among tables for the purpose of establishing correlations.

All of the 9 random-bred mice (ICR/Dub) were found to be normal upon autopsy. This was confirmed histologically. These animals are ranked in all tables in descending order of the mean metachromatic ratios obtained from chromatin acid denaturation.

At the time the AKR mice were killed, the differentials of all were still within the normal range reported for this strain. The differentials obtained from the AKR animals were similar to those of the random-bred animals (Table 1). AKR Animals 1 and 2, which showed definite anatomical and histopathological signs of lymphocytic leukemia, were the highest among this strain and were slightly higher than the highest random bred. However, AKR Animal 3, which showed no anatomical evidence of lymphocytic leukemia, had a slightly higher lymphocytic differential than did Animal 2, which showed clear evidence of the disease. These differences are not statistically significant. Furthermore, the range of lymphocyte differentials among the 9 individuals of the 2 strains were nearly identical, i.e., 7% random bred, 8% AKR.

The range of the total WBC was found to be greater among the AKR animals (5,420 to 29,000 cells/cu mm) than among the random bred animals (11,000 to 20,700 cells/cu mm) and the high extreme was above the reported mean for the AKR strain. Also (Table 1) the range of lymphocytes per cu mm of blood was greater in the AKR animals.
Table 1
Leukocyte counts and differentials on AKR and ICR mice

<table>
<thead>
<tr>
<th>Animal</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
<th>Granulocytes</th>
<th>WBC (cells/cu mm)</th>
<th>Lymphocytes/cu mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AKR</td>
<td>ICR</td>
<td>AKR</td>
<td>ICR</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>70 67</td>
<td>2 6</td>
<td>28 27</td>
<td>29,000 16,000</td>
<td>14,000 4,050</td>
</tr>
<tr>
<td>2</td>
<td>68 56</td>
<td>4 5</td>
<td>27 40</td>
<td>7,300 11,000</td>
<td>4,964 6,160</td>
</tr>
<tr>
<td>3</td>
<td>69 62</td>
<td>4 8</td>
<td>27 30</td>
<td>11,400 13,000</td>
<td>7,886 8,060</td>
</tr>
<tr>
<td>4</td>
<td>63 61</td>
<td>3 6</td>
<td>34 33</td>
<td>5,450 13,000</td>
<td>3,433 7,930</td>
</tr>
<tr>
<td>5</td>
<td>65 60</td>
<td>5 6</td>
<td>30 34</td>
<td>9,500 11,150</td>
<td>6,175 6,890</td>
</tr>
<tr>
<td>6</td>
<td>62 60</td>
<td>3 5</td>
<td>35 35</td>
<td>17,300 20,700</td>
<td>10,726 12,627</td>
</tr>
<tr>
<td>7</td>
<td>62 56</td>
<td>4 4</td>
<td>34 40</td>
<td>7,250 16,400</td>
<td>4,493 9,184</td>
</tr>
<tr>
<td>8</td>
<td>61 58</td>
<td>6 7</td>
<td>35 35</td>
<td>16,000 19,400</td>
<td>10,004 11,252</td>
</tr>
<tr>
<td>9</td>
<td>62 60</td>
<td>6 12</td>
<td>32 28</td>
<td>10,050 12,700</td>
<td>6,231 7,620</td>
</tr>
</tbody>
</table>

both the WBC and number of lymphocytes per unit volume of blood showed no obvious relation to the autopsy data. Note, for example, the extreme difference in WBC between AKR Animals 1 and 2 (29,000 versus 7,300), both of which were clearly leukemic. Thus, no definite conclusion as to the presence or absence of lymphocytic leukemia among these animals could have been reached using only these standard hematological parameters as a spot check.

Mean chromatin acid lability in peripheral circulating blood lymphocytes is shown in Table 2. It will be seen that the mean values for this parameter are similar in blood lymphocytes of AKR and random-bred mice. Note the nearly identical values obtained from the leukemic AKR mice and the highest of the ICR. Chart 1 shows nearly identical, overlapping ranges of the means of this parameter in this tissue. These data indicate that, at the stages of lymphocytic leukemia exhibited by AKR Animals 1 and 2, the number of abnormal circulating lymphocytes is too low to be detected by this method as well as by cell counts.

Chromatin acid lability of thymic and splenic lymphocytes is also compared in Table 2. Note that the chromatin acid lability shows marked differences between AKR and ICR animals. This difference is significant at \( p = 0.01 \) when the means are tested for significance as pairs (for example, AKR1 versus ICR1, etc.), or if the values for the 9 individuals of each strain are pooled and the pooled value means are tested. In addition, when the means are ranked in descending order, they correlate well with the autopsy-histopathological evidence, i.e., those mice of the AKR strain that were definitely leukemic show the greatest lymphocyte chromatin acid lability.

Chart 1 shows the ranges of the means of chromatin acid lability in the thymus and spleen as measured by AO metachromasia. In these organs the difference in range between strains is quite marked. The dashed lines in this chart are drawn through the highest mean values for AKR individuals that did not show anatomical evidence of lymphocytic leukemia. Although the means of chromatin acid lability overlap in range, the upper (and lower) extremes are higher among the "normal" AKR animals than among the random bred. Thus, although the highest metachromatic ratios are found in the animals with confirmed disease, the mean values from some of the AKR animals that appeared normal exceed the extremes from the random-bred group. This latter observation indicates that the microfluorometric measurements of chromatin acid lability may be capable of detecting abnormal cells among the lymphocytes populating these 2 lymphopoietic organs prior to their detection by ordinary histological stains. This is particularly striking in the spleens of AKR Animals 3 through 9, which did not show enlarged thymuses.

Since it is known that local differences in cell density cause variations in the metachromatic ratios of denatured, AO-stained cells by inducing differences in the diffusion rate of the dye out of the cell (2), care was taken to spread the cells evenly over the slide surface. Also, low densities were used coupled with short diffusion time and the cover-
slips were mounted over immersion oil. These precautions resulted in low intra- and interslide variation (Table 3). The mean metachromatic ratios reported here are based on small samples of 50 lymphocytes/slide. The means in Table 2, on the other hand, are derived from 200 cells and thus show a lower S.D.

**DISCUSSION**

The microfluorometric procedure used in the present study is still used primarily in experimental settings and is only now being tested for applicability as a serious diagnostic tool. Therefore, while it is not the purpose of this paper to elaborate on the physicochemical basis of the procedure, the principles upon which it is based are summarized in order to aid the interpretation of the data.

AO, after suitable blockade of other anions, is thought to bind to native DNA by intercalation between base pairs in the interior of the helix. The DNA:AO complex, when excited, fluoresces with maximum emission in the green region of the spectrum (530 nm). If, however, the chromatin is denatured, i.e., loses its highly ordered structure, the dye binds to available sites on the DNA by aggregation between anionic phosphates as a result of dye:dye interaction. This type of nucleic acid:AO complex, when excited, fluoresces with maximum emission in the red region of the spectrum (590 nm). Thus, if denatured chromatin in a fixed cell devoid of RNA is stained with AO under appropriate conditions (8, 9), the color of the fluorescent nucleus provides an index of the degree of denaturation effected, i.e., the greater the extent of denaturation, the redder the nucleus.

Although the precise consequence of exposure of the cell to acid is not perfectly understood at the molecular level, it is clear that it probably results in the extraction of nuclear proteins. Removal of such components of chromatin very probably results in the destabilization of DNA, thus reducing its highly ordered structure. Furthermore, it appears possible that cells in different functional states might lose these proteins through the action of the acid at different rates or to different degrees. Whatever the molecular mechanism, it appears that acid hydrolysis, coupled with measurement of AO metachromasia, may provide a method for distinguishing cells in different functional states. Although the present data cannot be interpreted in relation to the molecular structure of DNA, they do provide a sensitive empirical

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**Table 2**

Mean lymphocyte metachromatic ratios \( \left( \frac{F_{590}}{F_{530}} \right) \) resulting from acid treatment

<table>
<thead>
<tr>
<th>Animal</th>
<th>Thymus</th>
<th>Spleen</th>
<th>Blood</th>
</tr>
</thead>
<tbody>
<tr>
<td>AKR</td>
<td>ICR</td>
<td>AKR</td>
<td>ICR</td>
</tr>
<tr>
<td>1</td>
<td>0.63 ± 0.16</td>
<td>0.50 ± 0.08</td>
<td>0.62 ± 0.10</td>
</tr>
<tr>
<td>2</td>
<td>0.62 ± 0.16</td>
<td>0.48 ± 0.09</td>
<td>0.62 ± 0.09</td>
</tr>
<tr>
<td>3</td>
<td>0.61 ± 0.08</td>
<td>0.45 ± 0.07</td>
<td>0.59 ± 0.11</td>
</tr>
<tr>
<td>4</td>
<td>0.57 ± 0.07</td>
<td>0.42 ± 0.07</td>
<td>0.57 ± 0.07</td>
</tr>
<tr>
<td>5</td>
<td>0.53 ± 0.07</td>
<td>0.42 ± 0.06</td>
<td>0.49 ± 0.15</td>
</tr>
<tr>
<td>6</td>
<td>0.51 ± 0.07</td>
<td>0.39 ± 0.06</td>
<td>0.49 ± 0.08</td>
</tr>
<tr>
<td>7</td>
<td>0.47 ± 0.04</td>
<td>0.38 ± 0.07</td>
<td>0.48 ± 0.07</td>
</tr>
<tr>
<td>8</td>
<td>0.46 ± 0.04</td>
<td>0.37 ± 0.10</td>
<td>0.45 ± 0.07</td>
</tr>
<tr>
<td>9</td>
<td>0.41 ± 0.17</td>
<td>0.33 ± 0.20</td>
<td>0.39 ± 0.17</td>
</tr>
</tbody>
</table>

* \( F_{590}/F_{530} \), fluorescence emission intensity at 590 nm per fluorescence emission intensity at 530 nm (voltage).
* Mean ± S.E.

**Table 3**

Mean lymphocyte metachromatic ratios \( \left( \frac{F_{590}}{F_{530}} \right) \) on individual slides

<table>
<thead>
<tr>
<th>Slide</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>1.77 ± 0.30</td>
<td>1.54 ± 0.37</td>
<td>1.46 ± 0.25</td>
<td>1.28 ± 0.47</td>
<td>1.10 ± 0.26</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.23 ± 0.29</td>
<td>1.07 ± 0.34</td>
<td>0.86 ± 0.16</td>
<td>0.92 ± 0.25</td>
<td>0.91 ± 0.13</td>
</tr>
</tbody>
</table>

Duncan's new multiple range test

Duncan's new multiple range test

<table>
<thead>
<tr>
<th>Slide</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thymus</td>
<td>1.10</td>
<td>1.28</td>
<td>1.46</td>
<td>1.54</td>
<td>1.77</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.86</td>
<td>0.91</td>
<td>0.92</td>
<td>1.07</td>
<td>1.23</td>
</tr>
</tbody>
</table>

* \( F_{590}/F_{530} \), fluorescence emission intensity at 590 nm per fluorescence emission intensity at 530 nm (voltage).
* All slides prepared from a single normal animal (n = 50).
* Mean ± S.D.
* Slides twice the cell density of others and of those used in Table 2.
* Means over a common rule not significantly different at 5% level.
method of cell recognition. This is in the same sense as the use of Giemsa or quinacrine banding of metaphase chromosomes. While the molecular basis of these procedures is still imperfectly understood, they are nonetheless valuable as a means of recognizing individual homologous chromosomes.

It has been demonstrated in a number of systems that the chromatin of cells active in the synthesis of RNA is more labile to thermal denaturation than is the chromatin of their quiescent counterparts (3-7, 10). These systems include a number of neoplasias of diverse etiology, tissue origin, and species (1, 2). Thus, enhanced chromatin lability, whether due to the rupture of hydrogen bonds (as in thermal denaturation) or to the probable extraction of nuclear proteins, may be characteristic of "blast" cells in general. Furthermore, the thermal and acid lability of chromatin may be related since nuclear proteins, and in particular the histones, confer structural stability to the DNA to which they are bound.

One of the problems encountered with the use of AO metachromasia as a means of monitoring chromatin lability is the fact that local variations in cell density on the slide surface induce variations in metachromasia. This appears to result from differences in the rate of diffusion of the dye out of the cells because of interaction between neighboring cells (2). We have found, however, that this effect can be minimized, and perhaps even eliminated, if the following conditions are met. The cells must be spread uniformly over the slide surface this is aided by using low cell densities in the suspension applied to the slide. Thus, in this manner, cell:cell dye diffusion interactions are greatly reduced both because the cells are far apart and because they are not clumped around the edges of the smear. We have also found that intraslide variation is significantly reduced by mounting the stained cells under immersion oil rather than buffer as in the original Rigler procedure (9). We believe that this prevents further diffusion of the dye bound in the aggregate form. Thus, the cells retain most of the dye bound while in equilibrium with the dye solution. These data are presented fully in a subsequent paper in preparation.

Measurement of the metachromatic ratio of 50 cells along a transect across the spread on a slide, including the edges, showed that the metachromatic ratio of a given cell is not related to its position on the slide if the above conditions are met. A relationship between metachromatic ratio and the position of the cell on the slide surface would be expected if the value of the ratio were affected by local cell density. That such was not the case leads us to conclude that low cell densities and oil mounting effectively eliminate this technical problem. Indeed, when high cell densities are used together with buffer mounting, large position-related variations are seen. Therefore, such individual variations that do exist with low-density, oil-mounted preparations are probably due to a different response to acid treatment by individual cells and, in fact, AO metachromasia is probably a highly sensitive means of detecting such biological differences within a cell population.

We believe that microscopic AO fluorometry as well as flow fluorometry should be pursued. Although the former is not as rapid as the latter, it retains several major advantages. Microscopic AO fluorometry not only enables one to measure the level of metachromasia, but it also allows the observer to discriminate between individual cells. Thus, mixtures of cell types may be studied together without the need to separate the types by buoyant density. Indeed, separation by density may be virtually impossible for cells of the same type that differ only in their functional state. Furthermore, separation of cell types by buoyant density could itself eff ect physicochemical differences in the structural state of the chromatin. Finally, the simplicity of the measuring apparatus, with its attendant, relatively low cost and the ease with which the cell preparations can be made, make microscopic fluorometry attractive as a cytodiagnostic tool.

The present data show that lymphocyte chromatin acid lability exhibits a relationship with anatomical evidence of lymphocytic leukemia. Furthermore, this parameter shows a distinct difference between a high-incidence strain and random-bred mice. However, these differences appear in thymic and splenic lymphocytes only. Blood lymphocytes fail to reveal any differences between strains or between confirmed and potential leukemic animals.

Our findings are in some ways consistent with the currently held concept of the sequence of histopathological events occurring during development of lymphocytic leukemia in AKR mice. This concept holds that the neoplasm originates in the thymus, disseminates via the blood to the spleen where it forms secondary tumors, and ultimately metastasizes to the liver and peripheral lymph nodes (12). It is only in the late stages that the level of blood lymphoblasts increases markedly. Thus, our failure to find significant changes in chromatin acid lability in blood lymphocytes in animals that were clearly leukemic suggests that the number of blast cells in circulating peripheral blood is too low to be detected in the sample sizes taken and that AKR Animals 1 and 2 had not yet progressed to the point of massive flow of neoplastic cells into the circulation. This conclusion is, of course, sustained by the lymphocyte differentials, which show, at best, only an insignificant elevation of blood lymphocytes in the animals in which the leukemia was anatomically confirmed. It will be recalled that 210 blood lymphocytes from each animal were measured for chromatin lability. Thus, in the 2 AKR mice showing distinct anatomical signs of leukemia, a total of 420 cells were measured. If the measurements of this parameter are pooled for the 9 AKR animals, a total of 1890 blood lymphocytes were examined with no evidence of abnormal cells. Thus, again, we conclude that the number of neoplastic lymphoblasts circulating in the peripheral blood in stages prior to massive dissemination is too low to be detected by the present method and cannot be used for early detection of lymphocytic leukemia.

The discrepancy between the present data and previous histopathological studies lies in the fact that lymphocytes with blast cell-staining characteristics with AO were detected simultaneously in the thymus and spleen of animals that showed no other evidence of leukemia. In other words, if the tumor indeed originates in the thymus and then secondarily disseminates to the spleen, one would not expect to find an increased number of lymphoblasts in the spleen of an animal in which neither the thymus nor the spleen was
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enlarged. However, if one excludes the mean chromatin acid lability of the confirmed leukemic animals, the range of the mean ratios is greater for AKR than for random-bred animals in both lymphopoietic organs. The AKR Animals 3 through 9 showed no evidence of lymphocytic leukemia, i.e., the thymus and spleen were both of normal size. Thus, if elevated chromatin acid lability is indeed indicative of lymphoblasts, the data suggest the presence of a greater number of these cells in the normal AKR animals than in the random bred. Since the occurrence of this phenomenon was established by sampling a large population of lymphocytes, it is improbable that it could have been detected histologically. Many factors, such as glucocorticoid levels, age, sex, and immune response, could affect the levels of lymphopoiesis (11). However, the present experiments were designed to minimize such factors. For example, care was taken to use animals free of infection, of the same age and sex, and to handle them all in a consistent manner to minimize stress. Thus, it appears unlikely that the observed differences in chromatin lability in splenic and thymic lymphocytes between strains resulted from such factors.

A more probable explanation would be that the observed differences are genetic. Such differences are well known in the circulating blood. Standardized differential counts show widely divergent means and ranges of leukocytes that are characteristic of specific strains (11). However, our differential counts revealed no significant differences between the AKR and ICR mice. There were, however, rather marked differences in the WBC with the AKR animals showing a much wider range, even if the 2 confirmed leukemic individuals are excluded.

These facts lead us to question the concept that lymphocytic leukemia in AKR mice always originates in the thymus and raises the possibility that the neoplasm may, in some cases, arise simultaneously in both lymphopoietic organs. However, the present data do not permit a definite conclusion and the question needs further investigation.

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