Immunofluorescent Studies of Human Leukemic Cells with Antiserum to a Murine Leukemic Virus (Rauscher Strain)

By Angeliki Katrahoura Ioannides, Fred Rosner, Martin Brenner and Stanley L. Lee

In 1963, Fink and Malmgren, using the technic of immunofluorescence, showed that lymphoid tissue in murine leukemia contains antigen reactive with specific anti-viral antibody. Further studies by Fink, Malmgren, Rauscher, Orr and Karon demonstrated that some human leukemic blood and bone marrow cells also reacted with the murine leukemic virus (Rauscher) antibody. In addition, rabbit antisera prepared against plasma containing virus-like particles from leukemic humans reacted specifically with leukocytes of a significant number of patients with leukemia. The present report describes studies with human leukemic tissue using fluorescein-tagged monkey antiserum against Rauscher murine leukemia.

Materials and Methods

Antiserum

Antimurine leukemia serum prepared against the Rauscher virus was made by immunizing rhesus monkeys against Balb/c mouse plasma containing Rauscher virus. The monkey antiserum was absorbed with Balb/c mouse erythrocytes and plasma until no further reaction was obtained with either of these. Fluorescein isothiocyanate conjugation of the antiserum was effected and then Rhodamine labeled bovine albumin was added. The monkey antiserum was then purified by Sephadex and DEAE cellulose filtration and the final product was lyophilized and stored at 4 C. Prior to use, each ampoule of lyophilized material was restored to solution by the addition of one milliliter of distilled water and four milliliters of phosphate buffered saline (pH 7.1).

Materials Tested

Peripheral blood and bone marrow smears were prepared from all 31 patients with acute and chronic leukemia seen during the study period and from 54 nonleukemic patients whose bone marrows were being examined diagnostically. In some cases, two to four serial bone marrow examinations were available for study. In three patients, imprints of surgical
or autopsy specimens of spleen, liver, kidney and bone marrow were utilized. Blood smears were used only when the peripheral white blood cell count exceeded 50,000/mm$^3$.

Technic

The smears were air dried, fixed immediately in methyl alcohol for 15-20 minutes and stored at 4°C until used. Methyl alcohol was found to be preferable to acetone for storing smears for prolonged periods of time (weeks to months). After being brought to room temperature for 30 minutes, the smears were refixed in acetone for 10 minutes. Then 0.05-0.1 cc. of reconstituted fluorescent Rauscher antiserum was added, the smears were covered with a glass tray to prevent evaporation and allowed to incubate at 20°C for 45 minutes to one hour. The excess antiserum was poured off and the slides were washed thoroughly in phosphate buffered saline (pH 7.1) for 20 minutes.

After air drying for 30-40 minutes, the smears were examined under ultraviolet light in the Leitz fluorescence microscope (HB200 light source, 2 and 4 mm. UCl filters). Permanent mounts of the smears were not made as results were recorded immediately after examination. The intensity of apple-green cytoplasmic (and, when present, nuclear) fluorescence was graded as strong, moderate, weak or none. In most instances the predominant fluorescence was cytoplasmic. Nuclear fluorescence was occasionally noted, but its presence did not follow any consistent pattern. For this reason the cytologic localization of the fluorescent stain has been ignored in the analysis of these data. All smears were identified by code number only and were examined and interpreted independently by two observers. Random duplicate smears were tested with fluorescein labelled anti human globulin and in no instance was more than weak non specific fluorescent detected.

Results

Acute Lymphoblastic Leukemia

Twenty-four sets of smears from eleven cases of acute lymphoblastic leukemia were examined. Multiple bone marrow aspirations were available from seven of these patients. Moderate or strong fluorescence of cells was observed in all instances except for the cells of a single peripheral blood tested on one patient at a time when his white blood count exceeded 1000,000/mm$^3$ and consisted almost entirely of lymphoblasts. Bone marrow smears of four patients were examined during complete remission and during subsequent relapse. No differences in intensity of staining of cells were observed in three of these. Cells from one case stained more intensely during remission than during relapse.

Chronic Lymphocytic Leukemia

Nine sets of smears from five cases of chronic lymphocytic leukemia were examined. In four patients, the cells demonstrated strong fluorescence with Rauscher antiserum. In one of these patients, cells from the buffy coat of peripheral blood failed to fluoresce at a time when cells from the bone marrow fluoresced strongly. The peripheral blood cells of another patient fluoresced strongly on three separate occasions. In one patient the cells from both bone marrow and peripheral blood failed to fluoresce with Rauscher antiserum.

Acute Myelogenous Leukemia

Twenty-seven sets of smears from fifteen patients with acute myelogenous leukemia were examined. Of the twelve patients studied during life, bone marrow was available from eleven. In five of the patients the cells reacted strongly with the Rauscher antiserum; in the remaining six the cells reacted weakly or not at all. In three cases cells from bone marrow demonstrating complete
Table 1.—Immunofluorescence of Nucleated Cells from Patients with Leukemia and Other Disorders with Antibody Prepared Against Rauscher Murine Leukemia Virus

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of Patients</th>
<th>Number of Specimens</th>
<th>Immunofluorescence Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Positive (Strong or Moderate)</td>
</tr>
<tr>
<td>(Acute ) Relapse</td>
<td>6</td>
<td>13</td>
<td>6</td>
</tr>
<tr>
<td>(Lymphatic) Remission</td>
<td>9</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>(Leukemia ) Total</td>
<td>11</td>
<td>24</td>
<td>11</td>
</tr>
<tr>
<td>(Acute ) Relapse</td>
<td>15</td>
<td>23</td>
<td>7</td>
</tr>
<tr>
<td>(Myelogenous) Remission</td>
<td>4</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>(Leukemia ) Total</td>
<td>15</td>
<td>27</td>
<td>7</td>
</tr>
<tr>
<td>Chronic Lymphatic Leukemia</td>
<td>5</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td>Plasma Cell Myeloma</td>
<td>7</td>
<td>7</td>
<td>2</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>3</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Carcinomatosis</td>
<td>7</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Megaloblastic Anemias</td>
<td>6</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Idiopathic Thrombocytopenic Purpura</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Leukemoid Reaction</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Anemias of Diverse Origins</td>
<td>28</td>
<td>29</td>
<td>4</td>
</tr>
</tbody>
</table>

*One specimen of peripheral blood.
†Includes two samples of peripheral blood.
‡Includes three samples of peripheral blood.

remission reacted no differently than corresponding cells obtained at the time of relapse. However, a strong reaction with Rauscher antiserum during relapse became weak during remission in one patient. In three patients imprints were made from the spleen at the post-mortem table and their cells tested against Rauscher antiserum. In one instance, (Case 9) cells from splenic imprints fluoresced strongly. Cells in lymph node imprints from this patient also stained intensely, but liver and kidney cells did not. On the other hand, cells in splenic imprints from one case did not react while cells in liver imprints from the same patient fluoresced strongly. Cells in imprints from all organs tested from another case failed to fluoresce.

**Chronic Granulocytic Leukemia**

Only one patient was studied. Cells from his bone marrow reacted weakly with Rauscher antiserum.

**Multiple Myeloma**

Seven sets of bone marrow smears from seven patients with multiple myeloma were examined. No reactions occurred in the cells of four patients. Weak fluorescence was observed in the cells of one case, the reaction was weak
to moderate in the cells of another case, and the cells of another case showed moderate intensity of staining. In the latter three cases, weak fluorescence of their cells was noted with nonspecific anti-human globulin.

Miscellaneous Conditions

Bone marrow smears from seven patients with iron deficiency anemia were studied. Weak fluorescence of cells was detected in one instance. Cells in the bone marrows of the other six patients failed to react with Rauscher antiserum.

Bone marrow smears from five patients with megaloblastic anemia were studied. In four instances, moderate to strong reactions were observed in the cells. Cells from bone marrow smears in one case failed to fluoresce.

Five sets of smears of bone marrow aspirates from three patients with lymphoma were studied. Cells in lymph node imprints in two of these cases did not fluoresce. Cells from bone marrow smears in one case reacted with weak to moderate intensity. Cells in one case did not react and cells from another case fluoresced weakly.

Cells from bone marrow smears from two patients with leukemoid reactions secondary to hemorrhage and infection respectively and from two other patients with idiopathic thrombocytopenic purpura fluoresced strongly. Seven patients with metastatic carcinoma were studied. Cells from bone marrow smears from three stained moderately or strongly but in the four other patients the cells reacted weakly or not at all. Cells from bone marrow smears from three out of four patients with cirrhosis and from four patients with uremia failed to fluoresce when treated with Rauscher antiserum. Results obtained on bone marrow smears in additional patients with miscellaneous conditions are indicated in Table 1.

Discussion

Cells from bone marrow aspirates and liver, kidney and spleen imprints from consecutive patients with acute and chronic leukemias were tested for reactivity to murine leukemia (Rauscher) virus antibody employing the technique of immunofluorescence. Patients with various non-hematological conditions in whom bone marrow aspirates were available were similarly studied. Since selection of patients in this latter group was unavoidable (although without bias), statistical comparisons between leukemic and non-leukemic patients were not attempted. The bone marrow cells of all patients with acute lymphoblastic leukemia and of four out of five patients with chronic lymphatic leukemia reacted strongly with Rauscher antiserum. In the bone marrow cells of only half the patients with granulocytic leukemia could such reactions be demonstrated. These findings are interesting in view of the fact that in nearly all the work on transmission of leukemia by filterable agents, the type of leukemia involved has been lymphocytic.

Human tissues other than bone marrow aspirates produced erratic results. Cells from the peripheral blood from a patient with acute lymphoblastic leukemia failed to fluoresce with Rauscher antiserum at a time when
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the cells of the bone marrow of the same patient fluoresced strongly. Similar findings occurred in one patient with chronic lymphocytic leukemia. Imprints obtained post-mortem from various organs from three patients with acute myelogenous leukemia (spleen and liver in one case; kidney, bone marrow, lymph node and spleen in another case; liver, kidney, bone marrow and spleen in another case) did not show consistent fluorescence with Rauscher antiserum.

Since bone marrow cells from approximately one third of nonleukemic individuals tested reacted with Rauscher antiserum, the immunofluorescent technique certainly does not represent a diagnostic test for leukemia. That fluorescence occurs in bone marrow cells of nonleukemic patients requires explanation. Recent investigations of viral leukemias in animals indicate that at least in some cases two or more viruses must be simultaneously present for clinical disease to develop. If this situation should hold for human leukemia, it would not be surprising to find persons harboring one of the viruses and yet free of leukemia. The present experimental results would fit this model if Rauscher virus shares an antigen with one of the causative agents of human leukemia.

Bone marrow cells from four out of five patients with megaloblastic anemias fluoresced strongly with Rauscher antiserum. It has been suggested that this finding may be due to the marked erythroid activity in the bone marrows of such individuals—that Rauscher antiserum may contain an antibody to an antigen specific to erythropoietic cells but not necessarily viral. In support of this suggestion is the fact that Rauscher virus produces in mice a clinical and hematological picture very similar to human erythroleukemia. Furthermore, the strongest immunofluorescent reactions reported by Fink, Malmgren, Rauscher, Orr and Karo occurred in bone marrow cells of patients with erythroleukemia.

The present studies do not confirm this thesis, however. Differential bone marrow counts failed to show any correlation between the amount of erythroid activity and the degree of immunofluorescence, not only with regard to the bone marrow cells of patients with megaloblastic anemias but in all the patients studied.

A viral etiology for human leukemia has been postulated by many investigators ever since the earliest description of the viral etiology of avian leukemia by Ellerman and Bang in 1908. A murine leukemia virus was first demonstrated by Gross in 1951 and this work was subsequently confirmed. Certain forms of dog, cat and cattle leukemias may also be caused by viruses. There is as yet no direct proof that human leukemia is etiologically related to a virus although a recent report describes the transmission of Burkitt's lymphoma, probably a variant of human lymphoblastic leukemia, into monkeys.

By immunofluorescence, Fink and Malmgren detected viral antigen in leukemic tissues of mice and rats infected with Rauscher virus. These studies were extended by the same investigators and, subsequently, cross reactivity was shown between human leukemic tissue from some patients and antiserum prepared against murine (Rauscher) leukemia virus. These authors postulate a viral etiology of human leukemia and suggest that antigenic similarities
occur among strains of leukemia virus infecting various species.

The present studies confirm the work of others\(^3\) in the search for a possible viral etiology of human leukemia. The present findings seem to indicate that equally strong reactions in bone marrow cells of patients with acute lymphoblastic leukemia occur whether such patients are in remission or in relapse as tested by serial bone marrow aspirations. Fink, Karon, Rauscher, Malmgren and Orr\(^3\) also state that “preliminary findings in other leukemias (other than erythroleukemia) indicate that the reactivity with the anti-Rauscher virus antibody does not correlate with the stage of the disease.” These preliminary observations are in agreement with our own findings. On the other hand, Fink, Karon, Rauscher, Malmgren and Orr\(^3\) found that 79 percent of cases of acute leukemia (type unspecified) in relapse fluoresced strongly with antihuman “leukemia virus” antibody whereas this occurred in only 25 percent of patients in remission.

**SUMMARY**

Cells from bone marrow aspirates and liver, kidney and spleen imprints from patients with leukemia and other nonleukemic hematological and nonhematological disorders were tested for reactivity with murine leukemia (Rauscher) virus antibody using the technique of immunofluorescence. The cells of all eleven patients with acute lymphoblastic leukemia and four out of five patients with chronic lymphatic leukemia reacted strongly. In only six of the 15 patients with myelogenous leukemia and approximately one-third of the 47 patients with miscellaneous disorders (Table 1) was strong immunofluorescence demonstrated.

**SUMMARIO IN INTERLINGUA**

Cellulas ab aspiratos de medulla ossee e imprimitas de hepate, ren, e splen ab patientes con leucemia e con altere non-leucemic disordines hematologic e non-hematologic esseva testate pro reactivitate con anticorpore anti virus de leucemia murin (Rauscher) con le utilisation del technicia immunofluorescentic. Le cellulas de omne le dece-un patientes con acute leucemia lymphoblastic e de quatro del cinque patientes con chronic leucemia lymphatic reageva fortemente. In solo sex del 15 patientes con leucemia myelogene e in circa un tertio de 47 patientes con miscellaneous disordines un forte immunofluorescentia poteva esser demonstrate.

**ACKNOWLEDGMENTS**

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