Immunofluorescence Studies in Human Leukemia

By Henry A. Bates, Rufus O. Bankole and William R. Swaim

LYMPHOID TISSUES of mice injected with Rauscher leukemia virus have been shown, by immunofluorescent technics, to contain antigen(s) reactive with antimurine leukemia antibody prepared against the Rauscher strain.1 Other immunofluorescent studies2,3 have demonstrated that blood and bone marrow cells from patients with leukemia, lymphoma, Hodgkin’s disease, polycythemia, teratoma, mycosis fungoides, carcinomatosis, megaloblastic anemia, anemias of diverse origins and idiopathic thrombocytopenic purpura were also reactive with the antimurine leukemia antibody prepared against the Rauscher strain. Fluorescent anti-human leukemia antibody has been shown to react with bone marrow cells from patients with leukemia, lymphoma, Hodgkin’s disease and lymphosarcoma.2,4,5 Positive reactions have been reported6,7 in patients with Burkitt’s lymphoma using an indirect immunofluorescence test.

The purposes of this paper are to study further the above described immunofluorescent reaction of leukemia blood cells and to determine the variance of this reaction in leukemia patients receiving therapy.

MATERIALS AND METHODS

Preparation of Antisera to Normal and Leukemia Plasma Pellets

All centrifugation, absorption and serum fractionation procedures were carried out at 4°C. Prior to treatment, blood was obtained from a patient with stem cell and from another with subacute myelogenous leukemia. Both patients leukocyte counts were greater than 100,000/mm³. Blood cells were separated from the plasma by repeated centrifugation at 1500 r.p.m. × g. for 15 minutes. Separate 200 ml. pools of the respective plasmas were centrifuged at 35,000 r.p.m. × g. for 4 hours. Each pellet was suspended in phosphate buffer 0.01 M, pH 7.0–7.2 and recentrifuged. The respective pellets were then mixed with an equal volume of Freund’s complete adjuvant (Difco Laboratories, Detroit, Michigan) and each

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handled as follows. Each of three New Zealand white rabbits were pre-bled and inoculated with 10, 0.10 ml. intercutaneous injections of cell-free plasma pellet during the first and third weeks. One ml. intramuscular injections were given during the second, fourth, and fifth weeks. The animals were bled during the sixth week and their sera pooled. Equal volumes of plasma from 20 normal patients were mixed and handled in a similar manner. In this paper, rabbit antiserum to: 1) human stem cell leukemia plasma is designated A.H.S., 2) human myelogenous leukemia plasma, A.H.M., 3) normal pooled human plasma, A.H.N., and 4) pre-immunization pooled rabbit serum, P.R.G.

Fractionation of Antiserum

Absorbance at 276–280 μm was used to monitor collected fractions or determine protein concentration. Purified rabbit gamma globulin fraction II (Nutritional Biochemical Co., Cleveland, Ohio) or human globulin fraction II (Dade Reagents, Inc., Miami, Fla.) were used for preparing reference concentration curves. When dialysis was carried out, the serum to buffer volume ratio was 1:100. Each antiserum, A.H.S., A.H.N., A.H.M. and P.R.G., was equilibrated by dialysis overnight against 0.01 M phosphate buffer pH 7.4 in 0.03 M sodium chloride and then fractionated on a G-100 Sephadex column in equilibrium with the same buffer. Antiwhole rabbit serum goat antiserum (A.W.R.) (Hyland, Los Angeles, Calif.) was used in an agar immunoelectrophoresis test to detect fractions with precipitation bands in the gamma globulin region. These fractions were pervaporated to 1/10 volume and fractionated on a G-200 Sephadex column in equilibrium with 0.1 M phosphate pH 7.8–8.0 in 0.3 M sodium chloride. By agar immunoelectrophoresis, fractions with precipitation bands in the gamma globulin region were pooled and dialyzed against bicarbonate/carbonate buffer pH 8.9–9.0, 0.5 M for 3–4 hours. With constant stirring, 1 mg. of fluorescein isothiocyanate (Baltimore Biological Laboratory, Baltimore, Md.) was added for each 40 mg. of protein. After reacting 12–14 hours, this solution was fractionated on a C-100 Sephadex in equilibrium with 0.01 M phosphate buffer pH 7.4. Agar immunoelectrophoresis was used to determine which fraction contained gamma globulin bound to fluorescein isothiocyanate. This fraction was pervaporated to approximately 1/10 volume and absorbed as follows.

Plasma Absorption Procedures

Plasma was pooled from 20 individuals with normal hemograms and no clinical evidence of leukemia or related disorders. This plasma was pervaporated until the protein concentration was approximately 600 mg./ml. Each ml. of anti-murine leukemia (Rauscher) monkey antiserum (A.M.R.*) and A.H.S., A.H.M., and P.R.G. antiserum was absorbed for 1–2 hours with 30 mg. of this plasma. Each antiserum was centrifuged at 25,000 r.p.m. × g. for 2 hours. The supernatant was removed and its reaction with pooled normal human plasma determined by agar immunoelectrophoresis. The absorption procedure was repeated until no human plasma protein antibodies were detected by this method.

Tissue Absorption Procedures

Livers and spleens were obtained from 2 full-term human stillborns. Gross and microscopic examination of these tissues showed no evidence of leukemia. Equal weights of liver and spleen were titrated and washed with 0.01 M phosphate buffer pH 7.4 until the supernatant fluid was clear. The fluid was decanted and the tissues lyophilized. Bone marrow cells were obtained from normal individuals 41–65 years of age. These cells were pooled and lyophilized without washing. Each ml. of antiserum was absorbed, as described above, with 20 mg. of spleen-liver and 20 mg. of bone marrow powder. After centrifugation at 25,000 × g. for 2 hours, the supernatant was passed through a G-25 Sephadex column in equilibrium with phosphate buffer pH 7.6–7.8, 0.01 M. The fractionated conjugated antisera were reacted with leukocytes from leukemia and normal sources. Tissue

*Antiserum absorbed with normal BALB/c mouse erythrocytes and plasma was obtained through the courtesy of Dr. F. Rauscher, National Cancer Institute, Bethesda, Md.
absorption was repeated until normal cells were 0, +1 and leukemia cells +3 fluorescent intensity.

Collection, Preparation, Staining and Examination of Specimens

From each patient, 10 ml. of blood was obtained by clean venepuncture and added to 12 mg. E.D.T.A. Na₂. Blood samples were centrifuged at 1,000 r.p.m. × g. for 10 minutes and the supernatant plasma removed, leaving a column of plasma equal to the white cell layer. The plasma-white cell layer was removed, placed in a centrifuge tube and allowed to sediment at room temperature for 1 hour. The white cell layer in plasma was then removed and added to 0.85 per cent sodium chloride in a 1:9 ratio. This was gently mixed and centrifuged at 1,000 r.p.m. × g. for 5 minutes. The supernatant was removed and a sample of the white cells spread in a thin film onto microscope slides, air dried and fixed 10 minutes in cold (4°C.) acetone. Fixed slides were stored at −20 C. until stained.

Prior to use, conjugated antiserum was serially diluted 1:2 to 1:64 in phosphate buffer pH 7.6, 0.01 M and then reacted with leukemia blood cells. The highest dilution giving a +3 fluorescent reaction with at least 25 per cent of the observed cells was noted. Prior to use, each conjugate was diluted 1:2 with bovine albumin-conjugated Rhodamine (Baltimore Biological Laboratory, Baltimore, Md.). Under moist conditions, the specimen was reacted for 30–45 minutes at 20 C. It was then washed 20 minutes in 0.02 M phosphate buffer pH 7.4, air-dried, mounted in buffered glycerol (9 parts analytical grade glycerol, 1 part 0.02 M phosphate buffer pH 7.8) and coverslipped. The slides were stored (not more than 48 hours) at 4 C. until examined. A Leitz microscope equipped with an ultraviolet source (HBO 200) BG-12 or UV-2 selective filters (4 mm. thick) and an ultraviolet barrier filter was used to examine these smears. All smears, identified only by code number, were observed and interpreted independently by two individuals. Cytoplasmic fluorescence predominated with only a rare cell showing intranuclear fluorescence. While observing cells with a 54 × oil immersion objective lens, intensity of fluorescence was graded as 0 (non-reactive), +1 (weakly reactive), +2 (reactive), or +3 (strongly reactive). Results in this paper express the intensity shown by the majority of fluorescent cells in a smear. In this study, an arrow pointing downward in the figures indicated that less than 25 per cent of the total cells observed were of the indicated intensity of fluorescence, and an upward pointing arrow indicated that more than 25 per cent of the cells were of the indicated fluorescence.

Specificity of the Staining Reaction

Leukocytes from 10 individuals with no clinical evidence of leukemia or related disorders were reacted with unabsorbed and absorbed, conjugated A.M.R., A.H.S., A.H.N., and P.R.G. Leukocytes from patients with acute stem cell, lymphatic and myelogenous leukemia were reacted with A.H.N., anti-human globulin rabbit antiserum (Sylvana, Milburn, N.J.), anti-Herpesvirus hominis rabbit antiserum (Sylvana), anti-adenovirus type 7 rabbit antiserum (Sylvana) and P.R.G. Viral controls consisted of Hep-2 (human epithelial carcinoma) cells infected with either adenovirus type 7 or Herpesvirus hominis and uninoculated Hep-2 cells. All controls were stained with the same antisera. Blocking reactions were studied with A.H.N., A.H.M., A.H.S., A.M.R., and P.R.G. absorbed, unconjugated antisera. These were reacted with leukocytes from patients with: 1) stem cell, 2) lymphatic, and 3) myelogenous leukemia. After this reaction, each type of specimen was individually stained with absorbed, conjugated A.H.S., A.H.M., A.M.R., P.R.G. and unabsorbed A.H.N.

RESULTS

Prior to absorption, leukocytes obtained from non-leukemia patients had a +3 or +2 intensity of fluorescence when stained with A.H.S., A.H.M., and A.M.R. conjugated antisera. The nature of this preabsorption fluorescence is not known; however, it was reduced in intensity to +1 or 0 after two absorptions with human liver-spleen, marrow tissue powders. A.H.N. and P.R.G. antisera
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did not show reactivity with normal cells. Cells obtained from leukemia patients in acute or relapse state usually fluoresced with a +3 or +2 intensity after staining with either absorbed or unabsorbed A.H.S., A.H.M., or A.M.R. antisera.

Leukocytes from patients with acute stem cell, lymphatic and myelogenous leukemias when stained with A.H.N., anti-human globulin rabbit antiserum, anti-Herpesvirus hominis rabbit antiserum, anti-adenovirus type 7 rabbit antiserum and P.R.G. showed a +1 or 0 fluorescence. Control studies with Hep-2 cells infected with Herpesvirus hominis or adenovirus type 7 had a +2 fluorescence when stained with the respective specific antiviral antisera. Uninoculated Hep-2 cells did not fluoresce when stained with the various antisera. When peripheral blood cells from stem cell, lymphatic or myelogenous leukemias were each stained separately with absorbed, conjugated A.H.S., A.H.M., or A.M.R., the intensity of fluorescence was +3 in the acute phase. Individual pretreatment of each type of specimen with absorbed, unconjugated A.H.S., A.H.M., or A.M.R. reduced this fluorescent intensity to +1 or 0. A.H.N. and P.R.G. failed to show a similar blocking reaction. Table 1 lists the results when blood smears obtained from normal individuals, patients with leukemia and other hematopoietic disorders were stained with absorbed conjugated A.M.R. None of the normal individuals studied showed fluorescent staining. Twenty-four of the 45 patients studied had significant fluorescence. Those cases which were in relapse showed the greatest degree of fluorescent staining.

Case Studies

Since the majority of the patients with chronic myelogenous and lymphatic leukemia listed in Table 1 had or were receiving some form of therapy, the question arose whether the number of cells and intensity of fluorescence was related in any way to clinical relapse or remission following therapy. The fluorescent antibody data (Figs. 1-5) was compiled without knowledge of the clinical state or therapy by one investigator (H.A.B.) and independently correlated by another (W.R.S.)

Case # 1 (Fig. 1, chronic myelogenous leukemia) was started on busulfan therapy on 5/26/67. A partial remission was achieved with a gradual reduction in total white cell count and disappearance of blasts. A reduction in the number and intensity of fluorescing cells correlated with these hematologic improvements; however, a few cells continued to show significant fluorescence with A.M.R. There was no fluorescence detected with the two antibodies prepared from human sources by 10/4/67. The persistence of thrombocytosis indicated that a full remission was not achieved during this period of investigation.

Case #2 (Fig. 2, chronic myelogenous leukemia) received busulfan therapy from 8/4/66 to 7/20/67. On 3/30/67, when the fluorescent antibody studies were begun, the patient was in clinical remission. During the next few weeks there was a progressive rise in total white count and platelet count. The appearance of many fluorescing cells on 5/1/67 correlated with a partial relapse.
Table 1.—Summary of Immunofluorescence Data Obtained in a Variety of Hematologic Diseases and Normal Persons with Absorbed Anti-murine Leukemia Antibody Prepared Against the Rauscher Strain (A.M.R.)

<table>
<thead>
<tr>
<th>Diagnosis and/or Clinical State of Disease</th>
<th>Number of Cases</th>
<th>A.M.R.* Results</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>+3</td>
<td>+2</td>
</tr>
<tr>
<td>Myelogenous Leukemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute/Subacute relapse</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Chronic remission</td>
<td>7</td>
<td>—</td>
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<tr>
<td>relapse</td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td>Myelofibrosis</td>
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<td>—</td>
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<td>Lymphatic Leukemia</td>
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</tr>
<tr>
<td>Acute/Subacute relapse</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Chronic remission</td>
<td>1</td>
<td>—</td>
</tr>
<tr>
<td>partial remission</td>
<td>4</td>
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<tr>
<td>relapse</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Macroglobulinemia</td>
<td></td>
<td></td>
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<tr>
<td>partial remission</td>
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<td>—</td>
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<tr>
<td>Stem Cell Leukemia</td>
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</tr>
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<td>1</td>
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<tr>
<td>Multiple Myeloma</td>
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<td>1</td>
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<tr>
<td>Normal Persons</td>
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* Degree of fluorescence, see text for details.

Busulfan therapy was discontinued on 7/20/67 because of thrombocytopenia. By 8/31/67 there was a reduction in the number of fluorescing cells; however, the few cells remaining were more intensely fluorescent. A bone marrow examination on 10/24/67 was interpreted as chronic myelogenous leukemia in partial remission. On 11/9/67 the total white count was 8,000/mm³ and the patient seemed to be in remission. However, a small number of intensely fluorescent staining cells continued to be present.

Case #3 (Fig. 3, chronic myelogenous leukemia) received busulfan from 6/28/67 to 7/24/67. A majority of the white cells showed intense fluorescence with A.M.R. The patient's spleen was treated with 600 roentgens in air over an 8-day period. Even though the total white count decreased from 659,000/mm³ to 5,000/mm³ following splenic irradiation, the majority of the cells continued to show a significant, although reduced (8/29/67) intensity of fluorescence. The results with busulfan therapy were more striking. With this type of therapy, continued for several months, there was progressive decrease in both number and intensity of fluorescence until no fluorescent cells could be detected by 1/16/68.

Case #4 (Fig. 4) was found to have a mass in the mediastinum during August 1967. A complete blood count and blood smear were within normal limits. A scalene node biopsy revealed malignant lymphoma. Radiation therapy was started on 8/29/67 and 3,350 roentgens in air administered to the mediastinum. Toward the end of the four week period of radiation therapy a few immature lymphoid cells were noted on the blood smear. On 10/2/67, 20
Fig. 1.—Chronic myelogenous leukemia. The open circle and solid line indicate the anti-murine leukemia antibody (Rauscher strain), the solid circle and dashes indicate the anti-human stem cell antibody and the X's and dashes the anti-human myeloid antibody. The downward pointing arrow indicates that less than 25 percent of the total cells observed were of the indicated intensity of fluorescence, and an upward pointing arrow indicates that more than 25 percent of the cells were of the indicated fluorescence. These same symbols are used for Figures 1–5.

<table>
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<tr>
<th>Date</th>
<th>WBC x10^3/mm^3</th>
<th>Platelets x10^3/mm^3</th>
<th>Blasts %</th>
<th>Fluorescence</th>
<th>Therapy</th>
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<td>10/67</td>
<td></td>
<td></td>
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<td>5-25</td>
<td>42</td>
<td>475</td>
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<td>6-22</td>
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<td>626</td>
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<td>820</td>
<td>1.7</td>
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<td>12</td>
<td>657</td>
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Busulfan 5/26

Fig. 2.—Chronic myelogenous leukemia.

mg. of nitrogen mustard were injected into the left pleural space to control an effusion. Other lymph node bearing areas of the patient began to increase in size and chlorambucil was started on 10/4/67. During the next 10 days, the total white count increased with the majority of the cells being immature lymphoid forms. On 10/5/67, the majority of the cells stained intensely with A.H.S. and only a few cells stained significantly with A.M.R. As the white count increased, the number of cells showing significant fluorescence with the anti-murine leukemia antibody also increased. On 10/4/67, treatment with 6-mercaptopurine and prednisone was begun. Within 5 days the number of cells staining significantly had decreased and by 10/25/67, no fluorescent staining was detectable. 6 MP was discontinued because of leukopenia and prednisone was continued. Fluorescent staining cells reappeared within 24 hours after discontinuance of 6 MP and by 11/6/67, a majority of the cells showed intense fluorescence. The reason for the reduction in the number of fluorescent cells on 11/9/67 is not clear. A five-day course of 6 MP beginning 11/16/67 was without apparent effect on fluorescence and was discontinued because of leukopenia. There were only a few cells showing +1 or +2 fluorescence from 11/9/67 to 12/21/67. On 12/28/67, the white count had increased to 77,000/mm^3 and there were many fluorescing cells on the blood smear. The patient was hos-
pitalized on 1/13/68 and expired on 1/14/68. At the time of death, without specific therapy, the white cell count was 1,100/mm³ with 75 per cent immature lymphoid forms. Only a few cells per slide revealed significant fluorescence.

Case #5 (Fig. 5, chronic lymphatic leukemia) initially had many fluorescing cells in the blood smear. With therapy, the partial reduction in white count correlated with a reduction in the number of fluorescent cells and intensity of fluorescence. The incompleteness of the remission through 12/30/67 was characterized by the continued presence of fluorescing cells and an elevated white count.

**DISCUSSION**

The etiology of human leukemia is unknown. The fact that a viral etiology for murine leukemia has been established has lead to extensive attempts to demonstrate a similar etiology for human leukemia. Studies using the electron microscope and/or immunofluorescent technics have resulted in variable and inconclusive results. When immunologic technics are used, attempts are made to define one component of the reaction, generally the prepared antibody, and thereby indirectly characterize the antigenic component of the reaction. Such antigenic characterization requires antibodies with a high degree of specificity. In this study an attempt was made to prepare such antibodies. As in previous reports it was necessary to absorb our antisera with normal human tissue antigens before immunofluorescent differences between normal and leukemia leukocytes could be observed. The absorption procedures employed removed antibodies against antigens for normal human spleen, liver, bone marrow, plasma, erythrocytes, platelets and leukocytes.
These antibodies are common components of hyperimmunization procedures using plasma pellets as an antigen(s).

Many laboratories have prepared antibodies to study immunologic reactions of blood cells obtained from patients with leukemia and a variety of related disorders. Comparison and interpretation of results between laboratories are difficult. Differences in preparation of materials and source of absorbants may account for some of these differences. In our study when absorbed conjugated antisera (A.H.S., A.H.M., A.M.R.) were reacted with cells from acute or relapse stages of leukemia intensity of fluorescence was similar for all three antibodies. However, when serial studies on leukemia patients were done, variations in intensity of fluorescence were observed. Since titration and standardization of antibody were done with cells obtained from the acute phase of leukemia, quantitative antibody differences toward other cellular antigen(s) could have accounted for these differences. Such antigens being detected when the acute phase antigen was absent or reduced in concentration. This variation in acute phase antigen in cells from leukemia and related disorders (Table 1) may indicate the presence of a replicating agent or its genome capable of altering the cellular processes of blood cells during their morphogenesis, with the subsequent production of a specific antigen. It is possible that the antigenic variation observed, reflects a quantitative difference in an antigen(s) common to
normal and neoplastic cells. It has been shown, using a cytotoxicity test, that chronic lymphatic leukemia blood cells contain normal leukocyte isoantigens with a normal or above distribution frequency. The antigenic pattern of these cells usually remained constant in all phases of the disease and during therapy. Cells from granulocytic leukemia patients were also reported to contain similar isoantigens. However, a considerable variation of patterns was reported. The hypothesis that leukemia cells contain specific antigens is supported by immunodiffusion studies. These studies showed that myelogenous leukemia cells contain an antigen(s) not found in normal or chronic lymphatic leukemia cells. Positive immunofluorescent results obtained in our study with both myelogenous and lymphatic leukemia cells are not wholly consistent with these findings. Whether different antigens were detected by the two methods is not known.

When our antisera were repeatedly absorbed with normal bone marrow, fluorescence was abolished. Since A.M.R. reactivity was also eliminated by this method, it was assumed that this procedure was non-specific, rather than directed toward specific human isoantigens. Whereas, the cytotoxicity reactions were concerned with variations in known normal isoantigens. Whereas, variable antigen(s) detected by immunofluorescence has not been characterized. Therefore, it remains to be determined whether this antigen(s) is specific for leukemia cells or is some unknown normal isoantigen(s).

Like others, we found that the number of cells with fluorescence was greatest during the acute or relapse stages of leukemia and least during remission. Furthermore, chemotherapy effective in maintaining a remission state was characterized by a decrease in the number and intensity of fluorescent blood cells; whereas, a relapse, while under treatment, was associated with an increase.

Our immunofluorescent study of leukemia leukocytes included antisera prepared against *Herpesvirus hominis* and adenovirus type 7. No detectable
reaction to these sera was observed. This suggested that the antigens of *Herpesvirus hominis* and adenovirus type 7 were not key antigens in the cells of the leukemia patients studied.

**Summary**

Plasma obtained from patients with leukemia, was concentrated by high speed centrifugation and used to prepare antisera in rabbits. Unabsorbed, anti-human leukemia plasma and anti-murine leukemia virus (Rauscher) antisera showed similar immunofluorescence with normal and leukemia leukocytes. After absorption of these antisera with normal human antigens, normal cells were non-reactive and leukemia cells still reactive. Each antisera was shown to contain antibodies capable of cross reacting with and/or blocking the immunofluorescent reaction of cells from acute myelogenous, stem cell, or lymphatic leukemia cases. Pre-immunization rabbit serum and anti-human normal plasma antisera failed to show these reactions. These and antisera to other antigens (human globulin, Herpesvirus, adenovirus) were also non-reactive with acute phase leukemia cells. Such findings indicated a specificity for human leukemia cells by the described antisera and that human leukemia cells and Rauscher murine leukemia virus may have common antigens.

Serial studies on leukemia patients showed that the number and intensity of fluorescing cells varied with the clinical state of the patient. The three antisera gave similar reactions in acute leukemia. Differences were noted in remission. It was postulated that these differences were due to other cellular antigens, masked during the acute phase reaction. Leukocytes from patients with Hodgkin's disease, lymphoma(s) and multiple myeloma also showed fluorescence when reacted with anti-murine leukemia (Rauscher) virus antiserum. Whether this common antigenicity with leukemia cells indicates similar altered cell growth and/or related etiology remains unresolved.

**SUMMARIO IN INTERLINGUA**

Plasma obtenite ab patientes con leucemia esseva concentrate per centrifugation a alte velocitate e useate in le preparation de antiseros in conilios. In forma nonabsorbite, antiseros anti human plasma leucemic e anti virus de leucemia murin (Rauscher) manifestava simile immunofluorescentias con leucocytos normal e con leucocytos leucemic. Post le absorption de iste antiseros con normal antigenos human, cellulas normal esseva non-reactive, durante que cellulas leucemic continuava esser reactive. Esseva monstrate que cata-un del antiseros contineva anticorpore capace de entrar in reaction cruciate con c/o de bloccar le reaction immunofluororescentic de cellulas ab casos de leucemia myelogene, a cellulas primordial, o lymphatic. Sero de conilio obtenite ante le immunisation e antiseros anti normal plasma human non monstrava iste reactiones. Iste seros e etiam antiseros anti altre antigenos (globulina human, virus de Herpes, adenovirus) esseva etiam nonreactive con cellulas ab leucemia in phase acute. Tal constatationes indicava un specificitate pro cellulas ab leucemia human per le antiseros descritibe e le possibilite que cellulas ab leucemia human e virus de leucemia murin Rauscher ha antigenos commun.

Studios serial in patientes con leucemia monstrava que le numero de cellulas fluorescente e le intensitate de lor fluorescence variava con le stato clinic del paciente. Le tres antiseros produceva simile reactiones in leucemia acute. Differentias esseva notate in le remission. Esseva postulate que iste differentias esseva causate per altre antigenos celular, mascate durante le reaction de phase acute. Leucocytos ab patientes con morbo de Hodgkin, con lymphoma o lymphomas, e con myeloma multiple etiam monstrava fluoro-
resentia in reaction con antiserо anti virus de leukemia murin (Rauscher). Si non iste antigenicitate commun con cellulas de leukemia indica similaritate del alteration in le crescencia cellular e/o etiologias affin remane a resolver.

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REFERENCES

7. Klein, G., Clifford, P., Klein, E., and Stjernsward, J.: Search for tumor specific immune reactions in Burkitt lymphoma patients by membrane immunofluorescence re-
Immunofluorescence Studies in Human Leukemia

HENRY A. BATES, RUFUS O. BANKOLE and WILLIAM R. SWAIM