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Occurrence in Human Bone Marrows of an Antigen Released from Continuous Cell Cultures Derived from Human Leukemia[1] (39460)

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Repeatedly, antigenic particles have been described in human leukemic plasma (1-5). Fluorescent antisera made to those particles primarily stained bone marrow cells from patients with leukemia (1, 3, 4) and occasionally bone marrow cells (2, 3) and lymphoid cells (5) from patients with lymphoma. Antigens of simian sarcoma virus and gibbon ape leukemia virus have been detected on cells from patients with acute leukemia (6) as well as cross-reactive antigens between Rauscher leukemia virus and peripheral leucocytes from such patients (7). There is no defined relation between the cell antigens detected with antisera prepared to the tumor virus and those prepared to plasma particles.

Two cell lines, JIII and Z-597, derived from human leukemias release particles into their culture media (8). These particles are unlike oncornaviruses in biochemical and morphologic properties. Fluorescent antibodies prepared against JIII particles stain JIII cells and Z-597 cells but not a variety of human and non-human cells tested (8). In view of this, and the above phenomena, the antigenic relation of the JIII particle to oncornaviruses was investigated, as well as the distribution of the antigen in bone marrows of a selected human population, primarily patients with leukemia and lymphoma.

Materials and methods. Cell lines. JLSV-9, a Balb/c mouse bone marrow cell line, infected with Rauscher leukemia virus (RLV), was kindly supplied by Dr. John Riggs (California State Dept. of Health, Berkely, Calif.). The uninfected JLSV-9 cell line was obtained from the Office of Program Resources and Logistics Viral Oncology, NIH, Bethesda, Md. D-17 cells, a canine cell line, uninfected and infected with simian sarcoma virus (SSV) were obtained from Dr. John Riggs. JIII cells, derived from a case of monocytic leukemia, were purchased from the American Type Culture Collection Cell Repository, Rockville, Md. All cells were grown in Eagle's minimal essential medium and Hank's balanced salt solution with 10% fetal calf serum and antibiotics. Cells were passaged when they reached confluency.

Antisera. Antiserum against disrupted RLV was obtained from Dr. Paul Levine, NIH, Bethesda, Md. and antiserum against disrupted SSV from Dr. T. G. Kawakami, University of California, Davis, Calif. Preparation of antiserum against JIII particles and conjugation with fluorescein isothiocyanate was described previously (8).

Bone marrow preparations. Two separate series of bone marrow films from patients with a variety of disorders were examined. The first series was obtained from the Collection of the Simpson Memorial Institute, University of Michigan and had been stored at −20° from 1-8 months. These films were fixed in acetone and tested with a 1:8 dilution of JIII antiserum using the direct fluorescent antibody method. A second series was tested to confirm the results of the first. These preparations were acetone-fixed the same day that the specimen was taken from the patient and tested with a 1:8 dilution of JIII antiserum within a week of that time. Only mononuclear leucocytes showing fluorescent intensity greater than 2+ (scale 1+ to 4+) in the cytoplasm and not in an area of dye trapping (at the edge of the slide) were considered positive. Coverslips containing no positive cells must have displayed 100 negative leucocytes to be evaluated as negative. All tests were performed at least

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twice on each sample and were read independently by two persons without knowledge of the patients diagnosis.

Results. Antigenic relationship of JIII particles to oncornaviruses. D-17 cells either uninfected or infected with SSV, and JLSV-9 cells uninfected or infected with RLV were grown on coverslips in Leighton tubes until they reached confluency. The cells were then fixed in acetone prior to testing. With the indirect fluorescent antibody method, a 1:10 dilution of bovine antiserum made against SSV and a 1:20 dilution of conjugated antibovine serum stained D-17 cells infected with SSV but not the uninfected cells. Unconjugated normal bovine serum did not stain these cells. A 1:10 dilution of monkey antiserum made against RLV and a 1:10 dilution of conjugated anti-monkey serum stained JLSV-9 cells infected with RLV but not the uninfected cells. Normal monkey serum did not stain these cells. These results ensure the presence of viral antigens in the virus infected cells and not in the uninfected cells.

Undiluted JIII antiserum did not stain either the D-17 cells infected with SSV or JLSV-9 cells infected with RLV using the direct fluorescent antibody method. Neither a 1:4 or 1:8 dilution of unconjugated JIII antiserum with a 1:20 dilution of conjugated antirabbit serum stained either of these virus infected cells using the indirect fluorescent antibody method.

In another series of experiments, acetone-fixed JIII cells were tested with both JIII antiserum and antisera made against either RLV or SSV. A 1:8 dilution of conjugated JIII antiserum stained JIII cells with the direct method, but a 1:5 dilution of either SSV or RLV antisera did not stain these cells with the indirect method.

Presence of antigen of JIII particles on human bone marrow cells. The list of diseases of patients whose preparations were tested in the first series of tests is given in Table I. All preparations were tested with a 1:8 dilution of JIII antiserum using the direct fluorescent antibody method. Certain preparations from patients with Hodgkin's disease, chronic lymphocytic leukemia, chronic granulocytic leukemia, anemia, and abnormal white blood cells were stained by the JIII antiserum. Table II gives the percentage of positive bone marrow preparations according to the general type of disorder. Preparations from patients with lymphoma were stained more frequently (45% positive) than those from patients with leukemia (11%). Bone marrow films from patients with nonmalignant blood disorders were positive with a frequency (25%) between the two.

The list of diseases of patients whose preparations were tested in the second series is listed in Table I. Again certain preparations from patients with Hodgkin's disease were positive as well as preparations from patients with multiple myeloma, atypical lymphocytes, red blood cell aplasia, renal failure, and one patient with undetermined diagnosis. None of the preparations from patients with leukemia stained. Examination of these results grouped according to the general disorder (Table II) revealed results similar to those seen in the first series of tests, except the percentage of positive preparations was slightly lower in the second series. Table II also gives the percentage of positive results of the first and second series of tests combined.

Characteristics and specificity of staining on bone marrow cells. Generally, lymphocytes and monocytes were the types of bone marrow cells which gave positive staining. The percentage of positive cells (only mononuclear cells considered) ranged from 1 to 50%. Figure 1 is a photomicrograph of a positive leucocyte which demonstrates uniform cytoplasmic fluorescence. Conjugated preimmune rabbit serum did not stain the bone marrow cells nor did conjugated antiserum prepared against antigens unrelated to JIII particles. Absorption of JIII antiserum with JIII cell powder reduced the dilution of antiserum which could stain the bone marrow cells from 1:8 to 1:2. Absorption of antiserum with tonsilar lymphocyte powder had minimal effect on the staining capacity of the antiserum when tested on bone marrow cells. This result suggests that the antigen being detected on the bone marrow cells is related or identical to the antigen of JIII particles.

Statistical analysis. There was not a significant difference between the first and sec-

TABLE I. FLUORESCENT ANTIBODY TESTS OF BONE MARROW FILMS FROM PATIENTS WITH VARIOUS DISEASES WITH JIII ANTISERUM[a].

First Series		Second Series	
Disease	No. positive/ total	Disease	No. positive/ total
Acute myelomonocytic leukemia	0/12	Hodgkin's Disease	4/11
Chronic lymphocytic leukemia	2/9	Non Hodgkin's lymphomas	4/15
Chronic granulocytic leukemia	1/4	Reticulum sarcoma	1/2
Acute lymphocytic leukemia	0/1	Histiocytic lymphoma	1/1
Histiomonocytic leukemia	0/1	Poorly differentiated lymphocytic lymphoma	1/5
Hodgkin's Disease	3/7	Well differentiated lymphocytic lymphoma	0/12
Anemia	2/9	Unclassified lymphoma	1/15
Lymphadenapathy	0/2	Chronic granulocytic leukemia	0/2
Abnormal white blood cells	1/1	Prolymphocytic leukemia	0/1
Lymphomas	2/4	Acute lymphocytic leukemia	0/1
Scleroderma	0/2	Acute myelomonocytic leukemia	0/12
		Chronic lymphocytic leukemia	0/3
		Erythroleukemia	0/1
		Cancer metastasis	0/3
		Multiple myeloma	1/3
		Atypical lymphocytes	1/1
		Agranulocytosis	0/1
		Polycythemia	0/1
		Red cell aplasia	1/1
		Anemia	0/13
		Cirrhosis	0/1
		T. B. abscess	0/1
		Renal failure	1/2
		Kidney transplant	0/1
		Seizures syndrome	0/1
		Arthritis	0/1
		Sarcoidosis	0/1
		Undetermined diagnosis	1/1

[a] The direct fluorescent antibody test was used with a 1:8 dilution of JIII antiserum.

TABLE II. RESULTS OF FLUORESCENT ANTIBODY TEST ON BONE MARROW FILMS GROUPED ACCORDING TO THE GENERAL DISORDER.

	First series		Second series		First and Second series	
Disease	No. positive/total	Positive (%)	No. positive/total	Positive (%)	No. positive/ total	Positive (%)
All lymphomas	5/11	45	9/26	34	14/37	38
All leukemias	3/27	11	0/20	0	3/47	6
Other cancers	–	–	1/6	14	1/6	16
Nonmalignant blood disorders	3/12	25	2/7	28	5/19	26
Nonmalignant disorders	0/2	0	1/8	12	1/10	10

ond series of tests when the fraction of positive or negative preparations from lymphoma patients were compared by chi square tests ($\chi^2 = 0.38$, p 70.50). There were not enough positive preparations from patients with leukemia to do chi square tests to compare series 1 and 2, but those results and results of preparations from patients with nonmalignant blood disorders were very similar. (Table II). Using the combined groups, chi square tests were also used to determine whether the fraction of positives in preparations from patients with leukemia and those with lymphoma are significantly different. A chi square value of 13.42 ($p < 0.001$) was calculated. A chi square value of 1.00 ($p > 0.30$) was calculated when results from tests of preparations from patients

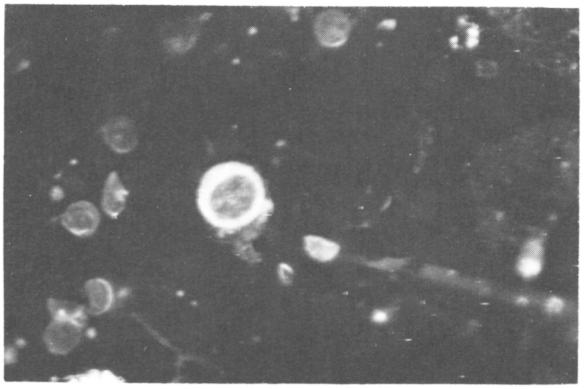

FIG. 1. Bone marrow leucocyte with cytoplasmic fluorescence. This preparation was stained with a 1:8 dilution of the JIII antiserum using the direct fluorescent antibody test. 400 ×.

with lymphoma and nonmalignant blood disorders were compared. There appears to be a significant difference in results to the test of preparations from patients with leukemia and lymphoma but not of results of preparations from patients with lymphoma and this selection of nonmalignant blood disorders at a 5% level.

Another series of statistical tests were performed which dealt with comparing five different characteristics of the patients with lymphoma to the fluorescent antibody test results. Chi square tests were done analyzing the variables sex and stage of disease, the Fisher exact test was done on the variables, treatment of patients and bone marrow involvement with malignant cells, and the *t* test was done on the variable, age of patients at time of diagnosis. There was a significant relationship between age of the patients and the fluorescent antibody test result $t = 2.82$ ($p < 0.01$).

Positive and negative fluorescent antibody results were grouped into age deciles in order to further examine the relationship between age of patients and the fluorescent antibody results. Table III shows the number of positive and negative preparations in each age decile. The difference was small between the number of positive and negative preparations in the age deciles up to age 50, since 23 preparations were examined, and 12 were positive while 11 were negative. In contrast, the results of preparations from patients over the age of 50 showed that of 14 tested, only 2 were positive and 12 were negative. It appears that patients with lymphomas over age 50 are less likely to have the antigen of JIII particles on their cells as those under 50.

TABLE III. AGE DISTRIBUTION (AGE AT TIME OF DIAGNOSIS) OF PATIENTS AND FLUORESCENT ANTIBODY TEST RESULTS.

	Numbers of patients		
Age decile	Positive	Negative	Total
11-20	3	0	3
21-30	2	3	5
31-40	2	5	7
41-50	5	3	8
51-60	2	8	10
61-70	0	4	4

Discussion. The antigen displayed on bone marrow cells with fluorescent antibody is similar or identical with the JIII particulate antigen(s) since the antibody is adsorbed by JIII cell powder but not by tonsilar lymphocyte powder. It is unlikely the antigen is a histocompatibility antigen because of the age distribution of its frequency. It is clearly unrelated to representative oncornaviruses. The JIII antigen is present only in the bone marrow of some leukemia and lymphoma patients. It is not known whether the bone marrow samples which did react positively to the JIII antibody would have reacted positively with antibodies prepared to plasma particles or to oncornaviruses. These malignancies may have multiple etiologies. The bimodal age distribution of Hodgkin's disease suggests this, as well as a different pathology, course and prognosis in younger patients compared to those over 50 years of age (9). If the JIII

antigen is etiologically significant, this may account for the high frequency of the JIII antigen in younger people with lymphoma. From animal models of infectious etiologic agents, acute productive infections of cells are to be anticipated in addition to malignant transformation, ie., the presence of the agent is likely not restricted to tumor cells. This may be the meaning of the positive preparations from patients with nonmalignant blood disorders.

The significance of the JIII antigen might be clarified by determining its presence in pathologic specimens, in conjunction with simultaneous tests for other etiologic candidates and from a study of lymphomas arising in epidemiologic clusters.

Summary. Fluorescent antibodies prepared against extracellular particles from a continuous culture of cells derived from a monocytic leukemia stained JIII cells but not cells infected with Rauscher leukemia virus or simian sarcoma virus. These antibodies reacted with 38% of bone marrow preparations from patients with lymphoma, 26% of preparations from patients with nonmalignant blood disorders and 6% of preparations from patients with leukemia. Bone marrow films from patients with lymphoma over the age of 50 stained less frequently than those from patients under 50. These particles released from JIII cells are not antigenically related to two of the commonly studied oncornaviruses, but may be indicative of the etiology or disease process of lymphoma in young patients.

1. Fink, M., Malmgren, R., Rauscher, F., Orr, H., and Karon, M., J. Natl. Cancer Inst. **33,** 581 (1964).
2. Fink, M. A., Karon, M., Rauscher, F. J., Malmgren, R. A., and Orr, H. C., Cancer **18,** 1317 (1965).
3. Dabich, L., Leopold, B. A., and Zarafonetis, C. J. D., Univ. of Mich. Med. Cen. J. **35,** 15 (1969).
4. Yohn, D. S., Horoszewicz, J. S., Rose, R. E., Mittelman, A., Chai, L. S., and Grace, J. T., Cancer Research **28,** 1692 (1968).
5. Hirshaut, Y., Reagan, R., Perry, S., Veta, V., and Barile, M., Cancer **34,** 1080 (1974).
6. Sherr, C. J., and Todaro, G. J., Science **187,** 855 (1975).
7. Mann, D. L., Halterman, R., and Leventhal, B. G., Proc. Nat. Acad. Sci. USA **70,** 495 (1973).
8. Smith, C. C., Ackermann, W. W., and Maverakis, N. H., Proc. Soc. Exp. Biol. Med., **152,** 645 (1976).
9. MacMahon, B., Cancer Research **26,** 1189.

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