IMMUNOLOGICAL STUDIES IN ACUTE LEUKEMIA

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INTRODUCTION

There is increasing evidence that leukemia specific or associated antigens exist in man and that these antigens are capable of stimulating specific antitumor responses.[1] The purpose of this study was to investigate three questions: 1. Do tumor specific or tumor associated antigens exist in adult acute leukemias?, 2. Do patients in hematologic remission, family members and unrelated individuals show immunological reactivity to these antigens?, and 3. If reactivity is shown, what is its significance?

MATERIALS AND METHODS

Population Studied. Over 50 patients with acute leukemia (primarily acute myelogenous leukemia), their families and a group of 40 nonrelated adults were studied. In general, all patients were studied during the initial florid phase of acute leukemia (80–90% blasts in the peripheral blood) before receiving anti-leukemic therapy. All patients were adults over 17 years of age, and all but six of the siblings studies were adults.

Histocompatibility Typing and Testing for Cytotoxic Antibody. HL-A typing and testing for cytotoxic antibody activity were performed in triplicate cultures in Falcon plastic microtitre trays. One μl (10^6 cells per ml) of cell suspension (nylon column-purified lymphocytes) was incubated with 2 μl of each typing serum or each unknown serum for 30 min at room temperature. The wash step, described by Amos et al.[2] was included to remove excess antibody. The cells were then incubated for 1 hr at room temperature with 4 μl of rabbit complement. Trypan blue dye was added and reactivity determined by dye exclusion. A cytotoxic reaction was considered positive if at least 20% of cells were killed.

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Cell Freezing. Cells frozen for later, repeated HL-A typing, or for use as stimulating or target cells in the assays, were collected in heparin (Upjohn Co., Kalamazoo, Mich.) by leukophoresis, washed, and suspended in RPMI 1640 medium (Grand Island Biological Co., Grand Island, N.Y.) with 15% fetal calf serum (FCS) (Grand Island Biological Co.) Dimethyl sulfoxide was added to a final concentration of 7.5% and the cells were distributed in aliquots into 2 ml polyethylene vials. Cells were cooled to −80 C in liquid nitrogen at a rate of 1 to 2 C per min, and then stored in the vapor phase of liquid nitrogen. When frozen cells were subsequently needed, they were thawed rapidly in 100 volumes of medium at 37 C and washed three times in medium before use. Viability (trypan blue exclusion) of thawed leukemic blasts was usually over 80% and no significant difference between frozen and fresh cells was discernible in our assays, when they were used as stimulating or target cells.

Mixed Leukocyte Culture (MLC) Assays. Leukemic blasts and normal lymphocytes were tested when freshly derived. Leukemic blasts were often frozen and thawed for later use as stimulating cells; only fresh (never frozen) cells were used as responding cells. All tests and the appropriate controls on a particular patient and normal family members or unrelated adults were done on the same day. One-way MLC assays with appropriate controls were performed as a micro-technique as described by Thurman *et al.*[3]

In the one-way cultures employed, the stimulating cells were treated with mitomycin C. Responding cells were designated A, B, C, etc. and corresponding mitomycin-treated stimulating cells were designated Am, Bm, etc. The following designations for lymphocytes of different origin are used in this report: P = patients in remission (presumably normal lymphocytes); A, B = siblings, HL-A identical with their respective sibling patient and with each other; K= unrelated normal control subjects; T = leukemic blasts, autologous to P, and HL-A identical to A and B.

In all cultures, the proportion of stimulating (mitomycin-treated) to responding cells was always 1:1. In all cultures, except where otherwise stated, the medium was supplemented with 15% FCS. FCS was inactivated at 56 C for 30 min before use. FCS was always stored at 4 C for at least two weeks before use to reduce any possible mycoplasma contamination.[4]

Mean count/min were determined with a Packard liquid scintillation beta counter. The stimulation index (SI) is the ratio of count/min in cultures stimulated by autologous or allogeneic leukemic cells or allogeneic normal cells, divided by the count/min in cultures stimulated by autologous mitomycin-treated normal lymphocytes, i.e., SI = (A − Tm)/(A − Am). All mean count/min, SE and SD were calculated and ana-

lyzed by Student's "t" test. It was found that all culture combinations studied, for which SI $\geq$ 2.0, had statistical significance, with $P < 0.05$. In each experiment, the criterion of validity of the results was that each cell type used as a stimulating population had to be capable of stimulating at least one normal responding lymphocyte population, and each responding population had to be capable of reacting significantly towards at least one other allogeneic cell.

^{51}Cr Cytotoxicity Assay of Cell-mediated Immunity (CMI). The techniques used for measurement of CMI were essentially those described by Canty *et al.*[5] A Ficoll-Hypaque gradient was used to purify lymphocytes from heparinized fresh venous blood; the cells were washed in Hank's balanced salt solution with 6% heat-inactivated FCS and then suspended in Eagle's minimal essential medium containing 10% FCS, at a concentration of 1.5×10^7 cells/ml. Leukemic blasts (either fresh or thawed after storage in liquid nitrogen) were used as target cells. The target cells were similarly prepared, to a concentration of 2.0×10^7 cells/ml. To 1.0 ml target cell suspension was added 250 μc ^{51}Cr (0.25 ml $Na_2{}^{51}CrO_4$, sp act 200 to 500 $\mu c/\mu g$ sodium chromate). The target cells were incubated in a shaking water bath for 45 min at 37 C, excess chromium washed off and the cells suspended at a concentration of 2.0×10 cells/ml in Eagle's minimal essential medium with 10% FCS. Attacking cells (from siblings or controls, or autologous remission cells) and labeled target cells were combined in a 1:150 target:attacking cell ratio. Cultures were set up in triplicate, in a total volume of 1.05 ml, in plastic petri dishes (13 $\times$ 110 mm). The cultures were incubated at 37 C in a humidified 5% CO_2 atmosphere for 4 hr, on a rocking platform. After incubation, the cultures were centrifuged and the supernatants assayed for ^{51}Cr release, using a Packard gamma scintillation counter. Cytotoxicity in experimental cultures was expressed as the percent of the total release of ^{51}Cr from labeled, freeze-thawed, disrupted target cells.

Labeled target cells (1.0×10^5) were added to unlabeled target cells (1.5×10^7) and subjected simultaneously to the same treatment as the experimental cultures, in order to provide control information on spontaneous leakage; this was found to range from 5 to 35% of the total release from freeze-thawed cells. This autologous test system served as the standard control for all ^{51}Cr assays.

Calculations used for cytotoxicity were performed as follows:

$$\text{Percent release} = \left(\frac{\text{count/min } ^{51}\text{Cr-release, experimental}}{\text{count/min } ^{51}\text{Cr-release, freeze-thaw}} - \frac{\text{count/min } ^{51}\text{Cr-release, control}}{\text{count/min } ^{51}\text{Cr-release, freeze-thaw}}\right) \times 100.$$

Student's t test was used to compare the percent lysis of experimental and control cultures. Findings were considered significant when $P < .025$. In many instances, triplicate culture values were very close, giving statistically significant results, even with low absolute percentages of ^{51}Cr release.

Macrophage Migration-inhibition Factor (MIF) Assay. Tests for the demonstration of MIF production by cells of siblings or patients in response to culture with leukemic blasts were performed using modifications of the techniques described by Rocklin and David[6] and Thor.[7] Cells from HL-A identical siblings and from siblings not HL-A identical were assayed for MIF production in response to culture with leukemic blasts from patients. Freshly drawn heparinized venous blood was allowed to settle in 50-ml syringes for 90 min. Leukocyte-rich plasma was extruded, and the cells were centrifuged. Erythrocytes were lysed with Tris-NH_4Cl. The leukocyte pellet was washed with RPMI 1640 medium, containing penicillin, streptomycin, L-glutamine and bicarbonate buffer, but no FCS. Lymphocytes from siblings (responding cells) and leukemic blasts (stimulating cells) were suspended in RPMI 1640 medium with 1% FCS. The ratio of responding cells to stimulating cells was 5:1, 5.0×10^6 responding cells were mixed with 1.0×10^6 stimulating cells in a total volume of 4 ml. Responding and stimulating cells were also cultured independently, at 5.0×10^6 cells/ml. The cultures were incubated at 37 C in a 5% CO_2 humidified atmosphere for 72 hr with no change of medium. The supernatants were harvested and filtered through a 0.45 μm Swinnex 25 filter. Supernatants from unmixed cultures were used as controls in the different experimental combinations. FCS, 9%, was added to each supernatant. Each such supernatant was tested directly in Sykes-Moore chambers for ability to inhibit migration of guinea pig peritoneal exudate (mineral oil induced) cells from capillary tubes placed in the chambers. After 24 hr of migration at 37 C, the areas of migration were projected onto paper and measured with a compensating planimeter.

The experimental mixtures were those supernatants from cultures in which leukemic blasts and lymphocytes from normal individuals were brought into contact. The controls were supernatants of responding cells alone, and the combined supernatants from independently cultured stimulating and responding cells. Other controls included responding cells from normal unrelated controls plus leukemic blasts, and lymphocytes plus antigens to which the control had shown positive skin tests. The means of the areas of migration seen with the experimental mixture were compared with those obtained with supernatants of the responding cells alone and with supernatants combined from independently cultured stimulating and responding cells. The means were compared

using Student's t test, and the results considered significant when $P < 0.05$.

Results

Cytotoxic Antibody. We observed an 18 year old male who presented to our patient service with a diagnosis of acute lymphocytic leukemia. He had a WBC of 100,000 per μl with 90% lymphoblasts. Definitive treatment for his disease was delayed for 3 days while we performed certain immunological studies. He was given 3 units of blood, however, to correct a rather severe anemia. At the end of the 3 day period his WBC had dropped to 1,000 per μl still showing a differential of 90% lymphoblasts. The 3 blood donors were identified and one of them was noted to have a cytotoxic antibody to this individual's lymphoblasts. On the basis of this observation each patient and each family member as well as pertinent blood donors were assayed for serological evidence of cytotoxic antibody to leukemic cells.

Over the past 4–5 years we have discovered 12 sera from putative normal, non-transfused individuals without histories of pregnancy that demonstrated complement dependent cytotoxicity to cells from patients with acute lymphocytic leukemia. Most of the sera were identified in blood donors after we had observed fall in peripheral counts following blood transfusions. Repeated testing of these sera against a large panel of normal lymphocytes, cells from patients with acute lymphocytic leukemia, acute myelocytic leukemia, infectious mononucleosis, etc. have confirmed our initial observations[8] that these sera show a consistent though less than 100% reactivity with peripheral blood cells of patients with acute leukemia particularly with cells of patients with acute lymphocytic leukemia.

Patients in Remission. Fourteen patients in remission were tested 34 times versus autologous tumor in the MLC assay. Six of these patients (43%) responded at least one time to their tumor. Table I gives examples of such reactions. Essentially all of the remission patients responded within the normal range to allogeneic normal cells. No remission patients responded to HL-A identical siblings, and no HL-A identical normal siblings responded in MLC to their sibling's remission lymphocytes.

Studies of approximately 25 sera collected from 8 patients at various times during their course have been tested for possible "blocking" effects[9] on MLC reactions to tumor cells. We have not, however, been able to demonstrate any consistent blocking or enhancement of these responses.

Seven patients in remission were tested a total of 17 times to autologous leukemic blasts for MIF production. Three of these patients did not

TABLE I

Examples of Mixed Leukocyte Culture Responses of Remission Lymphocytes to Autologous Leukemic Blasts

Patient	Diagnosis*	Culture†	Mean Count/Min ± S.E.	S.I.‡	*P* Value
J.B.	AML	P – Pm	1,501 ± 118	1.0	
		P – Tm	5,035 ± 371	3.4	<0.0005
		P – Km	10,190 ± 914	6.8	<0.0005
C.P.	AML	P – Pm	443 ± 57	1.0	
		P – Tm	8,149 ± 785	18.4	<0.0005
		P – Km	10,128 ± 385	22.9	<0.0005
P.S.	AML	P – Pm	293 ± 72	1.0	
		P – Tm	236 ± 51	0.8	NSD
		P – Km	28,981 ± 1679	98.9	<0.0005
L.B.	ALL	P – Pm	2,041 ± 89	1.0	
		P – Tm	1,878 ± 166	0.9	NSD
		P – Km	5,994 ± 436	2.9	<0.005

* AML = acute myelogenous leukemia, ALL = acute lymphocytic leukemia

† P = remission lymphocytes, T = autologous frozen leukemic blasts, K = lymphocytes from unrelated controls.

‡ S.I. = stimulation index.

TABLE II

Migration Inhibition Factor Responses of Remission Lymphocytes to Autologous Leukemic Blasts

Patient	Diagnosis*	Assay†	Area‡ ± S.E.	Percent Inhibition	*P* Value
P.S.	AML	(P) (T)	1.03 ± 0.06		
		P + T	0.86 ± 0.04	17%	<0.05
H.B.	AML	(P) (T)	1.76 ± 0.08		
		P + T	1.40 ± 0.08	26%	<0.0125
R.B.	ALL	(P) (T)	2.34 ± 0.10		
		P + T	2.30 ± 0.08	0%	NSD

* AML = acute myelogeneous leukemia, ALL = acute lymphocytic leukemia.

† P = remission lymphocytes, T = autologous frozen tumor, (P) (T) – P and T cultured separately, supernatants combined for assay; P + T = P and T cultured together, supernatant assayed.

‡ Arbitrary planimeter units.

respond but 4 (57%) did react repeatedly to autologous tumor cells. Table II illustrates examples of such tests.

Fifteen patients in remission were evaluated 34 times for reactivity to autologous tumor by the CMI test. Twenty-five of 34 (74%) were positive, and the lymphocytes of 13 of 15 patients were able to lyse ^{51}Cr labelled autologous tumor (87%). Table III shows examples of CMI reactions of patients in remission to their autologous tumor cells.

Family Studies. Thirty-eight normal individuals were tested for reactivity against the leukemic blasts of their HL-A identical siblings in the MLC assay. Positive reactions were demonstrated in 32 of 38 instances (84%). In 6 instances these tests were repeated using lymphocytes from the afflicted siblings in remission as stimulating cells. In every case no reactivity was demonstrated. Examples of these tests are displayed in Table IV.

In tests for anti-leukemic reactivity employing the MIF test we noted that 15 of 37 (40%) of family members showed positive tests. Ten of 17 (58%) of normal family members reacted in the MIF test to unrelated

TABLE III

Responses of Patients in Remission to Autologous Leukemic Blasts in the ^{51}Cr-release Cell-mediated Immunity Assay

Patient	Diagnoses*	Percent Lysis† (exptl. above control)	*P* Value
D.C.	AML	17.5%	<0.001
J.D.	AML	5.2%	<0.001
M.M.	AML	4.1%	<0.025
H.R.	AML	4.3%	<0.001
J.B.	ALL	6.6%	<0.001
M.T.	AML	1.9%	<0.01
H.E.	AML	−2.0%	NSD

* AML = acute myelogeneous leukemia, ALL = acute lymphocytic leukemia.

† See text for method of calculation.

TABLE IV

Responses of HL-A Identical Siblings to Leukemic Blasts in Mixed Leukocyte Culture

Patient	Diagnosis*	Culture†	Mean Count/Min ± S.E.	S.I.‡	*P* Value
V.L.	AML	A − Am	608 ± 46	1.0	
		A − Tm	2,436 ± 13	4.0	<0.0005
		A − Km	2,703 ± 351	4.4	<0.0005
H.B.	AML	A − Am	667 ± 258	1.0	
		A − Tm	5,847 ± 325	8.8	<0.0005
		A − Km	2,192 ± 292	3.3	<0.0005
J.B.	ALL	A − Am	506 ± 16	1.0	
		A − Tm	1,138 ± 69	2.2	<0.0005
		A − Km	3,039 ± 232	6.0	<0.0005
E.C.	AML	A − Am	2,812 ± 335	1.0	
		A − Tm	1,214 ± 48	0.4	NSD
		A − Km	7,660 ± 453	2.7	<0.0005

* AML = acute myelogeneous leukemia, ALL = acute lymphocytic leukemia.

† A = normal HL-A identical lymphocytes, T = frozen leukemic blasts, K = lymphocytes from unrelated controls.

‡ S.I. = stimulation index.

leukemic blasts. Examples of family member reactions to leukemic blasts are shown in Table V.

The CMI assay was used to study reactivity of family members to leukemic blasts. Of 83 family members tested against the leukemic blasts of the affected individual, 43 (73%) responded by significant release of ^{51}Cr label. When tested against unrelated leukemic blasts, 27 of 37 responded (73%). Examples of CMI responses are noted in Table VI.

Normal Unrelated Control Reactions to Leukemic Blasts: The CMI and MIF tests presumably measure the sensitized state. These tests were used to measure anti-leukemic reactions in order to describe the extent and prevalence of antileukemic immune reactivity in a normal population.

Using the CMI test we noted that 37 of 46 (80%) of normal unrelated

TABLE V

Migration Inhibition Factor Responses of Siblings to HL-A Identical Leukemic Blasts

Patient	Diagnosis*	Assay†	Area‡ ± S.E.	*P* Value
P.S.	AML	(A) (T)	1.40 ± 0.05	
		A + T	1.17 ± 0.07	<0.05
H.B.	AML	(A) (T)	0.63 ± 0.06	
		A + T	0.46 ± 0.04	<0.05
C.P.	AML	(A) (T)	1.20 ± 0.10	
		A + T	0.71 ± 0.05	<0.01
J.B.	ALL	(A) (T)	1.99 ± 0.05	
		A + T	1.47 ± 0.09	<0.01

* AML = acute myelogeneous leukemia, ALL = acute lymphocytic leukemia.

† A = normal HL-A identical sibling lymphocytes, T = frozen leukemic blasts (A) (T) cultured separately, supernatants combined for assay; A + T cultured together, supernatant assayed.

‡ Arbitrary planimeter units.

TABLE VI

Responses of HL-A Identical Siblings to Leukemic Blasts in the ^{51}Cr-release Cell-mediated Immunity Assay

Patient	Diagnosis*	Sibling†	Count/20 min			% Release Experimental Above Control‡	*P* Value
			Control (mean ± S.E.)	Experimental (mean ± S.E.)	Freeze-Thaw (mean ± S.E.)		
C.B.	ALL	A	22,701 ± 1,024	27,017 ± 426	81,848 ± 1,444	5.3	<0.025
D.B.	AML	A	14,459 ± 201	15,642 ± 150	23,304 ± 1,051	5.5	<0.0025
P.S.	AML	A	3,115 ± 34	3,396 ± 18	50,278 ± 1,532	0.7	<0.01
J.W.	AML	A	3,571 ± 63	4,196 ± 123	45,043 ± 809	1.5	<0.0125
J.B.	ALL	A	9,912 ± 245	12,147 ± 109	23,779 ± 946	8.9	<0.0025
		B	9,912 ± 245	12,167 ± 167	23,779 < 946	9.5	<0.0025

* ALL = acute lymphocytic leukemia.

† A and B = normal HL-A identical Siblings.

‡ See text for calculation.

controls reacted to leukemic blasts. Twenty-nine of 37 (78%) persons responded by MIF production to leukemic blasts.

Discussion

The present studies confirm previous reports that leukemia associated or specific antigens exist and that patients in remission as well as normal individuals show a high degree of reactivity to these tumor cells.

Antibody to human leukemia associated antigens have been reported to occur only infrequently in autologous sera.[10, 11] Mitchell *et al.*,[12] however, have demonstrated a high frequency of cytophilic antileukemic antibodies in patients with leukemia. In these studies antibodies of leukemic patients are able to "arm" naive mouse macrophages and demonstrate specific rosette formation by such "armed" macrophages against autochthonous and allogeneic leukemia cells of the same histological type. Yoshida and Imai, using immune adherence, demonstrated that most leukemia patients have antibody against their tumor cells, but since the sera were not tested against normal or remission cells, the significance of these results is difficult to determine.[13] Gutterman *et al.*[9] have demonstrated, by immunoflorescence, that eight of twenty-four patients with acute myelogenous leukemia had immunoglobulin (IgG) present on the surface of leukemia cells, though he could not clearly determine if this immunoglobulin was a tumor cell product or a serum antibody. That a serum antibody reactive with tumor cells was present in this study is suggested by the fact that 7/8 of the immunofluorescence positive cell preparations had an autologus *serum* inhibition of a blastogenic response of autologous remission lymphocytes to tumor cells.

Studies on the reactivity of leukemic cells with human sera have been reported in the context of anomolous reactivity of such cells with HL-A typing sera.[14, 15] Such *extra reactions* suggesting no clear "extra" HL-A antigen pattern, were common in patients with chronic lymphocytic leukemia.[15] Walford *et al.*,[14] using absorptions, further characterized these "extra" reactions in CLL as falling within clear cut patterns, possibly defining six specificities not directly related to HL-A antigens. The blast cells from patients with acute myelogenous leukemia were found to develop patterns of reactivity consistent with changes in antigens – usually the assumption of an additional antigen. Several cases of acute myelogenous leukemia reported by Evans and Pegrum[15] showed a total of more than the four antigens inconsistent with the then current 2 loci hypothesis for HL-A. While it is possible that either or both the patterns seen (isolated extra reactions or "extra antigen") may be related directly to a change in the manner of expression of HL-A antigens, it also remains possible that new leukemia specific antigens are detected by these sera.

A number of workers have reported cell-mediated immune responses to acute leukemia. Oren and Herberman,[16] for instance, noted that patients with acute leukemia could demonstrate delayed hypersensitivity to autologous leukemic cells and to antigens extracted from them by classic skin testing.

Stimulation of remission cells by autologous leukemic blasts in the MLC assay was first reported by Fridman and Kourilsky[17] and confirmed by others.[18–21] Anti-leukemic reactivity demonstrable in HL-A identical siblings using MLC assays was first described by Bach *et al.*[22] Halterman *et al.*[23] or Schweitzer *et al.*[24] were unable to confirm these observations. The present report, however, represents the largest series studied and strongly confirms the reactivity of HL-A identical siblings.

Rosenberg *et al.*[25] reported that 3 of 10 normal monozygotic twins had lymphocytes capable of cytotoxic reactions to ^{51}Cr labelled leukemic blasts. Several other family members and normal controls also demonstrated positive CMI tests against leukemic blasts. Similar observations have been noted by others.[26–28] Our studies would suggest that this reactivity is more widespread than previously realized.

Hilberg *et al.*[29] have reported MIF production in response to leukemic blasts, by patients in remission. Our studies provide confirmation and extend these observations.

On the basis of reports of others reviewed above as well as our own studies it appears that there is widespread immunological reactivity to leukemic blasts.

Although alternative hypotheses are possible the data are consistent with the following hypothesis: Acute leukemia is caused by very ubiquitous viruses that cause a high infectivity rate in the existing population but result in low morbidity (i.e., acute leukemia). Such a hypothesis would explain the high frequency of immunological reactivity (i.e., exposure to the agent) but the rarity of the disease in the general population.

In rodent systems the polyoma virus is widespread and most rodents show reactivity to this virus as well as to polyoma induced tumors. The widespread evidence of serological reactivity to EBV virus but the relatively low incidence of Burkitt's lymphoma is also of interest. These observations lend some credence to the hypothesis of ubiquitous highly infective viruses that only rarely produce morbidity. Analogous observations have been made with the non-oncogenic virus of poliomyelitis, namely, that for every case of paralytic polio there are many infected individuals with minimal or no evidence of clinical disease.

The reports of recurrence of acute lymphocytic leukemia in donor cells following marrow transplantation for this disease are compatible with a viral etiology for some forms of acute leukemia.[30, 31] More pertinent

perhaps are the reports of finding high molecular weight virus-related RNA in human leukemic blasts[32, 33] as well as RNA-directed DNA polymerase (reverse transcriptase)[32-34] which appears to be specifically related to the reverse transcriptase isolated from two primate Type C viruses (Woolly monkey simian sarcoma virus, SiSV; and the gibbon ape leukemia virus, GALV.[35]). Most recently the report of the isolation of a Type C RNA tumor virus from a cultured line of human acute myelogenous leukemia[32] provides further support for the viral etiology of acute leukemia.

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DISCUSSION

DR. FRANK H. GARDNER (Galveston): I was interested in the one patient you described. Those anecdotal stories are often the beginning of clinical studies. There have been some efforts in CLL by the hematology group of Duke to infuse plasma to control the white count. Have you persued that technique with this boy who had the blood transfusion?

DR. SANTOS: In this one case we actually did look at the effect of administration of plasma obtained from one of the individuals who had demonstrated cytotoxic antibody. The patient in question enjoyed a 3 month remission from chemotherapy induction therapy. He was followed very closely and observed to relapse with increasing blasts in the marrow but not in the peripheral blood. At that time he was given 6 units of the appropriate plasma over a 3 day period prior to induction therapy. The plasma treatment did not change the percentage of blasts in the marrow. Perhaps this wasn't a good test. You will remember the initial observation was made when this patient had a WBC of about 100,000 and when there was about 90% blasts in the peripheral blood.

Other investigators have noted that the administration of fresh blood can have a therapeutic effect and we have heard of a few cases where this was traced to the presence of a cytotoxic antibody.

Our major question, however, was this: Why should so many individuals show this type of immunological reactivity to acute leukemic blasts? One could simply conclude that the tests are meaningless or that on the other hand they indicated that a large population of adults were exposed to antigens identical to or cross reactive with antigens on the surface of acute leukemic blasts. This latter interpretation was more attractive to us since the observations with polyoma virus fits this pattern. In addition, there are analogies with poliomyelitis in that many people were infected with that virus for every case of actual paralytic polio.

DR. K. J. R. WIGHTMAN (Toronto): Dr. E. A. McCulloch and associates have been doing work similar to yours in Toronto, and also finds that portions of the DNA in the nucleus of leukaemic cells appears to be of viral origin. However, that seems to fit in with the concept which is so exciting, which is that part of the equipment of normal people and normal cells may also consist of bits of viral material in the genome, which can have a useful function in the development of the species by producing mutations which may then survive as the result of natural selection. We're beginning to have an entirely new idea of what viruses are, what they are doing and what they are good for, as well as the harm they may do. In the report we have just heard, it seems likely that viral material in the genome is being expressed at the cell surface in some way, leading to the production of antigenic material. This is a most interesting finding, and one which may well have implications in the treatment of leukaemia at some later date.

DR. SANTOS: Huebner proposes that we all have this in the genome. I'm not proposing that. I'm proposing that it is an exogeneous agent (i.e., virus), that we all get infected with. But that is the unusual situation where one develops acute leukemia. Incidentally, a similar story is being brought up for EBV virus. Many people have titers without any clinical history. There are situations where with the very ubiquitous agents with high infectivity it's only a few who get the disease.