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Detection of an Antigen Associated with Acute Leukemia

Abstract. *Antiserums to a purified cell membrane component from a Burkitt's lymphoma tissue culture cell line were produced in rabbits. These antiserums were cytotoxic to peripheral white blood cells from 8 of 15 patients with acute leukemia and 5 of 41 relatives, but not to peripheral white blood cells from leukemia patients in clinical remission or from normal individuals. These antiserums appear to be detecting an acute leukemia associated antigen or antigens.*

Serums obtained from rabbits immunized with purified cell membrane components (*1*) from several tissue culture cell lines were cytotoxic to all of a large panel of peripheral blood lymphocytes (*2*). However, when a cell membrane fraction from the tissue culture cell line Raji (derived from the patient with Burkitt lymphoma) was used to immunize rabbits, a more limited pattern of cytotoxic reactivity was observed. Serums from two of three animals immunized with the same cell membrane component were found to be cytotoxic only to the peripheral white blood cells from some patients who had acute lymphocyte or acute myelocytic leukemia (*3*). These serums were not absorbed before they were tested.

The cytotoxic reactivity of these antiserums to the peripheral white blood cells of 15 patients with acute leukemia, 41 of their relatives, and 527 random donors was determined by a microdroplet cytotoxicity test (*4*).

Table 1 summarizes the cytotoxic reactivity (as determined with two rabbit antiserums) of the peripheral white blood cells from the leukemia patients, together with the clinical status of the disease at the time of the testing. These two antiserums showed positive cytotoxic reactivity with the peripheral white blood cells of 8 of 15 leukemic patients tested. The percentage of blast cells in the bone marrow is an indication of active (acute) disease or remission. Six patients had from 14 to 90 percent blast cells in their bone marrow and were considered to be in an acute phase of the disease. Reactive peripheral white blood cells were found in two patients who had 5 percent blast cells in the bone marrow. In contrast, peripheral white blood cells from two of the seven patients showing no cytotoxic reaction with the two antiserums had 10 to 20 percent blast cells in their bone marrow. The remainder of the patients could be considered in clinical remission, and no cytotoxic reaction of their peripheral white blood cells was observed. A total of 41 relatives was tested; more than 60 percent cytotoxicity was observed with the peripheral white blood cells from four relatives, either siblings or one or the other parent. The two rabbit antiserums showed no cytotoxicity to peripheral white blood cells from 527 random donors.

The HL-A alloantigenic profiles of cells from the leukemia patients, their relatives, and the normal donors were simultaneously determined with 78 alloantiserums capable of detecting 15 HL-A alloantigens. The cytotoxic reactivity of the two rabbit antiserums did not correspond to the presence of a particular HL-A alloantigen or a combination of different HL-A alloantigenic determinants.

An attempt was made to determine whether the antigen present on leukemic cells might also be present on normal cells but in concentrations or density that did not allow direct cytotoxic reactions. These studies were done with a cytotoxic inhibition assay.

Two tissue culture cell lines—Raji and R-4265—were labeled with ^{51}Cr. The two rabbit antiserums were tested against these cells in the presence of complement at varying dilutions, and a dilution was selected which lysed 70 to 80 percent of 10^4 target cells. The

Table 1. Diagnosis, clinical status (percent blast cells in bone marrow) and cytotoxic reactions of peripheral white blood cells from patients with acute lymphocytic (ALL) or acute myelocytic leukemia (AML). Positive reaction (+) indicates more than 60 percent cell death with two rabbit antiserums. Negative reaction (−) indicates less than 10 percent cytolysis.

Patient	Diagnosis	Blast cells in bone marrow (%)	Cytotoxic reaction
Hm	AML	90	+
Gd	AML	71	+
Rt	ALL	68	+
Hr	ALL	15	+
Sm	ALL	15	+
Hs	ALL	14	+
Dl	AML	5	+
Ad	AML	5	+
Dh	AML	20	−
Mt	ALL	10	−
Dt	ALL	3	−
El	ALL	<5	−
Ba	ALL	<5	−
Cl	AML	<5	−
Ob	ALL	<5	−

Table 2. Inhibition of antibody activity (AA) to the acute leukemia associated antigen with peripheral white blood cells (PWBC), bone marrow (BM), PHA stimulated white blood cells (PHA-WBC), and long-term cultured lymphoblast (CLB). N.I., no inhibition of cytotoxicity.

Cell type	Number tested	Clinical status	Cell numbers needed to inhibit AA by 50 percent
PWBC	9	ALL,AML (acute)	$1,2,3,4,5 \times 10^4$; $1,2,5 \times 10^5$; 1×10^6
PWBC	3	ALL,AML (remission)	N.I.* 10^6
PWBC	17	Normal	N.I. 10^6
BM	2	Normal	N.I. 10^6
BM	2	ALL,AML	10^4; 10^4
PHA-WBC	2	Normal	N.I. 10^6
CLB	9		$2,3,5,7 \times 10^3$; $1,2,3,5 \times 10^4$
Hela, Hep.	2		N.I. 10^6

* The cytotoxicity of the antiserums detecting the leukemia associated antigen to 10^4 target cells was not inhibited by concentrations of 10^6 cells.

cells used for inhibition were added in numbers varying from 10^3 to 10^6 cells. The number of cells that inhibited the lysis of the target cells by 50 percent (35 to 40 percent release of the ^{51}Cr) was determined and used as a criterion for the presence of an antigen associated with acute leukemia. Cell numbers greater than 10^6 were anticomplementary and therefore could not be reliably tested. The inhibition data are summarized in Table 2.

Peripheral white blood cells in numbers from 5×10^4 to 10^6 from each of nine patients with acute lymphocytic or acute myelocytic leukemia with active disease inhibited the cytotoxic activity from the serums and were considered positive. The peripheral white blood cells from three individuals (other than those tested in direct cytotoxicity studies) who were diagnosed as having acute leukemia and who were clinically in remission showed no positive reaction, as judged by the inhibition of cytolysis. Peripheral white blood cells from 17 normal individuals were used, and no inhibition of the cytotoxic reactivity of the antiserums was detected. Bone marrow from two normal individuals was also tested and no inhibition of the antibody activity was found; however, bone marrow from two individuals who had acute lymphocytic or acute myelocytic leukemia inhibited the cytotoxicity of antiserums at cell numbers of 10^4.

To ascertain whether this antigen was an antigen associated with blast cells, peripheral white blood cells from two individuals were exposed to phytohemagglutinin (PHA) for 72 hours. At the end of this time at least 60 percent of the cells were blast cells as determined by morphologic criteria. These PHA-transformed cells did not inhibit the cytotoxic activity of the rabbit serums at 10^6 cells.

It was found that all long-term lymphoid tissue culture cell lines did inhibit this antibody activity. The concentrations of cells needed for inhibition ranged from 2×10^3 to 5×10^4. Two of these cell lines were derived from individuals who were reputedly normal, and seven of the cell lines had been derived from patients with lymphoid malignancies. Two epithelial cell lines, Hela and Hep-2, at concentration of 10^6 did not react with these antiserums. It would appear therefore that tissue culture conditions are probably not responsible for these antigens. The lymphoid tissue culture cells appeared morphologically similar to the blast cells seen in acute lymphocytic or acute myelocytic leukemia. A common antigen on the acute leukemia cells and lymphoid tissue culture cells may reflect similar genetic mechanisms allowing uncontrolled cellular differentiation and proliferation.

It is indeed difficult to establish whether or not differences found between normal and acute leukemic cells represent a quantitative phenomenon. It is technically difficult to obtain the large numbers of cells from patients with leukemia who are in remission and from normal donors needed to do the absorption analysis to establish this point definitively. Our results of the inhibition of cytotoxicity suggest that this antigenic determinant either is present in small quantity or is not exposed on the peripheral white blood cells from the acute leukemic patients in remission, normal peripheral white blood cells, bone marrow, PHA-transformed lymphocytes, and the cells of two epithelial lines.

Of particular interest is the observation that cells from several relatives of the leukemic patients reacted with the antiserums detecting the leukemia-associated antigen or antigens. A similar observation has been made by Morton and Malmgren in studies of cell surface antigens from patients with sarcoma and from their relatives (*5*). This suggests that there may be a common environmental agent inducing the expression of this antigen on the white blood cell surface. The fact that most, if not all, lymphoid tissue culture cell lines contain a demonstrable virus or viral genome may suggest that this antigen is associated with some virus that induces transformation of normal cells into lymphoid or myeloid malignancies. There have been several reports of the presence of viral particles in acute leukemia cells. Whether the antigen detected with these antiserums becomes demonstrable when the cells are infected with this virus remains to be determined.

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Autoimmune Murine Thyroiditis
Relation to Histocompatibility (H-2) Type

Abstract. *Immunization of 33 inbred strains of mice with thyroid extract emulsified in complete Freund's adjuvant showed differences in both thyroid autoantibody response and autoimmune thyroid damage, related to the histocompatibility (H-2) type of the strain. Congenic mice of the same H-2 type exhibited the same pattern of antibody response and thyroiditis, regardless of the strain's genetic background, thus showing a close relation between histocompatibility determinants and autoimmunity.*

Significant differences in the immune responsiveness of different mouse strains to various antigens have been reported (*1*). Lately it has been shown that, in the case of mice immunized with synthetic amino acid polymers, the ability to mount an immune response is under the control of a single autosomal gene, closely linked to the major histocompatibility locus, H-2 (*2*). Gasser described a linkage between the agouti locus and the ability of inbred mice to form antibodies against mouse erythrocyte antigens (Ea-$1^{a,b}$) present only in red cells from wild-type *Mus musculus*. The linkage with the agouti locus implies a linkage with histocompatibility loci H-3 and H-6 (*3*). Susceptibility to various types of murine leukemia was also related to the H-2 locus (*4*).

We know of no correlation thus far reported between histocompatibility type and susceptibility to an autoimmune disease. We report here the existence of a relation between autoimmune thyroiditis of the mouse and the major histocompatibility (H-2) locus. Murine autoimmune thyroiditis is a