Human Leukemia-Lymphoma Associated Antigen Detected by Heteroantisera

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The identification of tumor-specific antigens on leukemia cells would facilitate the diagnosis and possibly the treatment of the disease. To detect such antigens, one widely used approach has been to raise antibodies in animals against leukemia cells or leukemia cell extracts. Although many variations of this approach have been used previously, in most cases the antisera produced have not been unequivocally shown to be leukemia specific. The more recent tests used to determine the specificity of the antisera have been more discriminating and some progress has been made. The tests used must be sensitive, capable of screening a large variety of cells, and able to detect small subfractions of positive cells. In our studies these critera were applied to the two main tests used to determine antiserum specificity. Complement-dependent microcytotoxicity was used to screen large numbers of normal and leukemic cells, and the immunofluorescent staining technique was used to detect small subpopulations of positive cells. The latter technique was essential for locating positive cells in heterogeneous populations such as bone marrow samples or peripheral blood lymphocyte preparations.

The heterogeneity of the antisera produced is dependent on the nature of the immunogen used and the type of animal which is immunized. In the past, a variety of different antigenic sources and animals has been used (1–9). In our studies whole leukemia cells produced very heterogeneous antisera whereas soluble membrane extracts produced more specific reagents. Animals either tolerant to normal cell antigens (1, 2, 8), or nonhuman primates (9) that are phylogenetically closer to man, have been immunized so that the response produced against normal antigens is lowered. However, when these animals were immunized with whole leukemia cells, the antisera that was produced still required absorption with normal tissue to show leukemic specificity.

Our approach has been to immunize rabbits with papain-solubilized extracts of purified cell membranes from human lymphomas (10, 11). The antisera produced were tested without prior absorption by the methods described above. Complement-dependent microcytotoxicity indicated that the antisera had specificity for leukemia cells from about 75 % of patients having all subclasses of leukemia. The

Table I: Cytotoxic titers of rabbit antisera against normal human lymphocytes and leukemia cells

Rabbit Number	Normal Lymphocytes[a]	Leukemia Cells
63	1	128
64	4	1024
66	0	512
68	0	1024
69	8	512
70	8	2048
71	0	10000
74	0	512
75	0	512
77	8	4000
78	0	1500
79	0	400
80	0	256
P413[b]	0	512

[a] Average titer against cells from 100 different donors.
[b] IgG fraction from antisera 68.

more discriminating fluorescein test revealed a small percentage of normal peripheral lymphocytes and normal bone marrow cells that were also positive.

Table 1 shows the complement-dependent cytotoxicity titers of antisera obtained from 13 different rabbits immunized with papain digests of cell membrane from human histiocytic lymphoma. The titers against normal lymphocytes were low (1:8 or less) while titers against leukemia cells were high, ranging from 1:128 to 1:10,000. This strongly indicates that the antigen being detected is present on leukemia cells but is not present on the majority of normal lymphocytes. However, the cytotoxicity test using total lymphocytes as targets is unable to detect a small subfraction of normal lymphocytes such as B cells which might also be positive. The specificity of the antisera for several different types of normal white cells and leukemic cells is presented in Table 2. Peripheral blood leukemia cells from patients with high white cell counts were positive whereas lymphocytes, granulocytes, and bone marrow from normal healthy donors were negative. However, not all leukemia cells were positive; 70–75 % of ALL, AML, and CML and 100 % (7/7) CLL were positive. Thirteen out of fifteen cultured lymphoblastoid cell lines were positive, the lines Molt 4 and 6410/EBV were negative. Normal lymphocytes from remission patients and phytohemagglutinin blast cells were negative.

The antisera would also kill leukemia cells when normal human lymphocyte effector cells were used in place of complement. In lymphocyte-dependent antibody lympholysis (LDA) the titer of the rabbit antiserum, 78008, was very high (10^6) against leukemia cells and cultured lymphoblastoid lines 6410 and RAJI

Table II: Cytotoxicity of rabbit antisera against various types of normal and leukemia cells

Cell type	Clinical Status	Cytotoxicity No. positive[1] / No. tested[2]	Average titer
PWBC[3]	AML relapse	30/40	1 = 512
PWBC	CML relapse	9/13	1 = 512
PWBC	ALL relapse	28/41	1 = 512
PWBC	CLL relapse	7/7	1 = 512
Lymphocytes	Normal	0/500	–
Granulocytes	Normal	0/56	–
Bone Marrow	Normal	0/2	–
PHA lymphoblasts	Normal	0/4	–
C L B[4]		13/15	1 = 64
PBWC	Leukemia[5]-remission	0/13	–

[1] More than 80 % cells killed.
[2] Number of different patients tested.
[3] Peripheral white blood cells.
[4] Cultured lymphoblastoid lines.
[5] 8 ALL, 4 AML, 1 CML.

whereas the LDA titer against normal peripheral blood lymphocytes was undetectable (Table 3). The control sera taken from the same rabbit prior to immunization (78000) was negative against leukemia cells. The LDA titers were found to be over 1000-fold higher then the complement-dependent microcytotoxicity titers. This large difference in titer between normal cells and leukemia cells further suggests that the antigen is not found on the majority of normal peripheral blood lymphocytes.

Neither the complement-dependent cytotoxicity test nor the LDA test are, however, capable of detecting minor subpopulations of positive target cells. In order to detect such positive cells in bone marrow or peripheral blood an immunofluorescent staining technique was used. The target cells were first incubated for

Table III: LDA and complement-dependent antibody titers of rabbit antileukemia sera

TARGET	78008 LDA	78008 Compl.	78000 LDA	78000 Compl.
6410	10^6	64	N	N
RAJI	10^6	64	N	N
Leukemia cells	10^6	512	N	N
Normal Human Lymphocytes	N	N	N	N

N = negative

30 min with the rabbit antisera at a dilution of 1:200. After washing, the cells were then treated with fluorescein-conjugated goat antirabbit IgG and examined microscopically for fluorescence. Positive leukemia cells and certain cultured lymphoblastoid lines gave a very bright continuous membrane fluorescence which can be seen in Fig. 1.

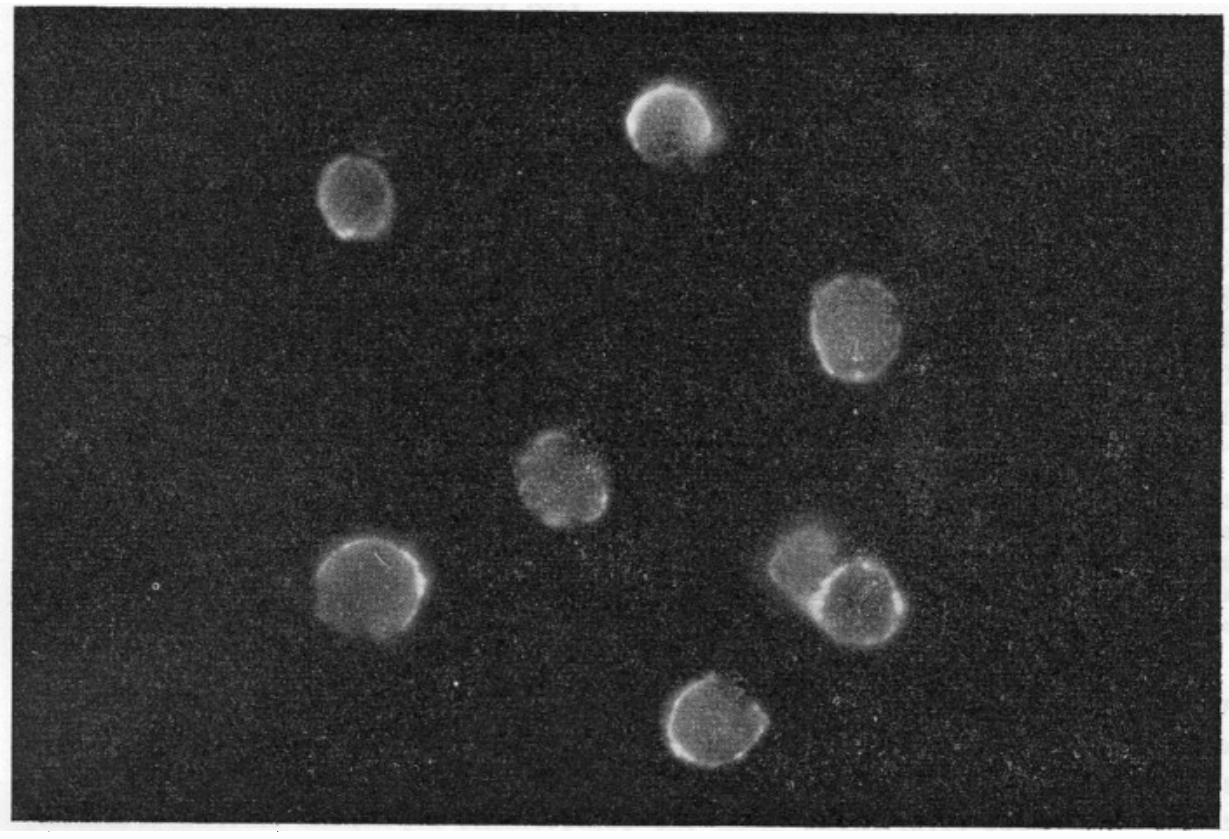

Fig. 1: Immunofluorescence staining of leukemia cells.

Whereas in the microcytotoxicity and LDA tests the rabbit antisera were negative against normal peripheral lymphocytes, the fluorescence test showed the presence of 6–15 % positive cells among peripheral lymphocytes from 40 healthy donors. Bone marrow cells from normal healthy donors had 0–2 % fluorescein-positive cells. This positivity of normal lymphocytes did not appear to be due to non-specific binding to B cells through the Fc receptors because F $(ab)_2$ fragments of the rabbit antibody gave similar results. Therefore, the leukemia-associated (LA) antigen appears also to be found on a subpopulation of normal peripheral lymphocytes. Preliminary results indicate that the positive peripheral blood lymphocyte is a B cell.

Bone marrow samples from children with leukemia were examined before and after chemotherapy. The numbers of fluorescein-positive cells were found to correspond to the stage of the disease as determined by morphological examination (Table 4). In newly diagnosed cases, the number of fluorescein-positive cells in the bone marrow was high (over 80 %). After chemotherapy the numbers of positive cells decreased to 0–2 % which was in general agreement with the number of blast cells found at this time by morphological examination. In relapse cases the increase

Table IV: Bone marrow samples tested for LA by fluorescein technique

Patient	Treatment	Diagnosis	% Blasts (Morphology)	% LA Positive (Fluorescen)
G. E.	Untreated	ALL New Case	87	90
	Chemotherapy	ALL Remission	1	Neg.
P. R.	Untreated	ALL New Case	93	70
	Chemotherapy	ALL Remission	0	2
S. J.	Untreated	ALL New Case	97	80
	Chemotherapy	ALL Remission	0	1
A. E.	Chemotherapy	ALL Remission	0	10
S. J.	Chemotherapy	ALL Partial Relapse	8	30
R. M.	Chemotherapy	ALL Relapse	72	60

of fluorescein-positive cells was again commensurate with the increased numbers of blast cells.

Although the immunofluorescence test was able to detect small numbers of positive leukemia cells its potential for early diagnosis of relapse remains to be determined. Before this can be achieved, the 0–2 % positive cells present in normal bone marrow must be eliminated. At the present time a more practical use of the test might be to distinguish between different clinical forms of leukemia. A preliminary study in children indicated that negative cases which constitute 25 % of the ALL cases studied appear to have a more virulent form of the disease which is characteristic of T cell leukemia. If this result can be substantiated, then the presence or absence of this cell marker may aid diagnosis and therapy of not only acute lymphocytic leukemia but also acute and chronic myeloid leukemia.

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