Brief Definitive Report

THE OCCURRENCE OF THE HL-B ALLOANTIGENS ON THE CELLS OF UNCLASSIFIED ACUTE LYMPHOBLASTIC LEUKEMIAS*

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Surface markers of human lymphocytes have been useful in the identification of cellular origins of neoplastic lymphocytes. Surface IgM, IgD, and Fc and complement receptors are usual B-cell markers and they indicate that the majority of cases of chronic lymphocytic leukemia, macroglobulinemia, hairy cell leukemia, and lymphocytic lymphoma are due to the monoclonal proliferation of B cells (1–4). Human T lymphocytes are known to form rosettes with sheep erythrocytes. The presence of this marker on the majority of the abnormal cells indicates that the Sezary syndrome is a T-cell disorder (4).

The situation with respect to acute lymphoblastic leukemia (ALL) is not as clear. Extensive studies (5–7) with these B- and T-cell markers have demonstrated two major types of ALL. Approximately 20% of the cases are clearly T-cell origin and show the characteristic reactions of normal T cells. However, the majority of ALL cases show a proliferation of cells that lack all the usual T- and B-cell markers. Their origin and relationship to known lymphocyte subtypes remains unknown.

Recently, a series of non HL-A human alloantigens recognized by pregnancy sera and selectively expressed on B lymphocytes have been described (8,9). These antigens appear closely related to the Ia system of the mouse and have been termed HL-B (8). The present studies describe the application of these new B-cell markers to ALL lymphocytes with special emphasis on the major unclassified type.

Materials and Methods

Cell Preparation. Peripheral blood samples were obtained from patients with known diagnosis of acute lymphoblastic leukemia. Their age ranges from 10 m to 30 y. Mononuclear cells were isolated by Ficoll-Hypaque discontinuous gradients. They were washed three times with phosphate-buffered saline (PBS) and were suspended in PBS with 2% bovine plasma albumin and 0.02% sodium azide (PBS-BPA) at a concentration of 2×10^7 cells/ml.

Immunological Studies. Rhodamine-conjugated $F(ab')_2$ fragments specified for μ, δ, γ, κ, λ, and rhodamine-conjugated IgG specified for α were prepared and immunofluorescent staining of lymphocytes for surface Ig was performed as described previously (10). In addition, a fluorescent reagent specific for both δ and μ was obtained by mixing equal volumes of the anti-δ and anti-μ antisera. Rosette formation with sheep erythrocyte and the detection of the Fc receptor by aggregated Ig binding were performed as described previously (2, 8).

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Pregnancy sera were obtained at the time of delivery. The sera were used after heat inactivation. Indirect immunofluorescent staining was performed as described previously (8). Briefly, 5×10^5 lymphocytes were incubated for 30 min with 0.025 ml of either a pregnancy serum or a normal serum in 10×75 mm plastic tubes precoated with BPA. After four washings with PBS-BSA, the lymphocytes were stained for 30 min with the $F(ab')_2$ fragments specified for IgG. After three washings with PBS-BPA, the cells were processed for examination by fluorescent microscopy.

Results

Six cases of ALL were studied by various T- and B-cell membrane markers (Table I). They were selected because of a high percentage of peripheral lymphoblasts. In cases A and B, the vast majority of the lymphoblasts formed rosettes with sheep erythrocytes and these lymphoblasts had neither surface Ig or the Fc receptors. In the remaining four cases (C–F), the lymphoblasts had no detectable surface Ig of classes δ, μ, γ, α, κ, and λ. In Table I, the percentages of cells stained for either μ or δ or both were included for surface Ig (11). They did not form rosettes with sheep erythrocytes. In cases E and F, only 60% and 41% of the mononucleated cells were blasts. These blasts could be readily distinguished from the small lymphocytes under phase-contrast microscopy. These blasts did not form E rosettes whereas substantial percentage of the small lymphocytes formed E rosettes.

Indirect fluorescent studies with selected HL-B-specific pregnancy sera were carried out in these six cases of ALL (Table I). In cases A and B, no significant numbers of the lymphoblasts stained with any of the sera. In case C, two of the four sera used stained practically all the lymphoblasts while one stained only 20% and the other stained less than 2%. A similar staining pattern was shown in case D. In cases E and F, the 57% and 36% staining cells by serum 111 represented the staining of the majority of lymphoblasts in the peripheral blood of these cases. No significant numbers of small lymphocytes stained positively with the sera. In all cases, no significant numbers of blasts stained with normal sera. The staining patterns of the lymphoblasts were shown in Fig. 1. In Fig. 1 *a* the phase micrograph shows prominent nucleoli in the blasts in case D. In Fig. 1 *b*, a granular circumferential staining pattern by serum 58 is shown. The panel of pregnancy sera utilized, including several not shown in Table I, gave positive results with the B cells of almost all normal individuals.

TABLE I

Immunological Studies of Acute Lymphoblastic Leukemia Indicates Non-T Non-B Leukemic Cells Stained with HL-B Alloantisera

Patient	Leukocytes, no./mm³ (% blasts)	Surface Ig*	Sheep erythrocyte rosette	Fc receptor	HL-B by indirect fluorescence				
					Pregnancy sera				
					007	58	111	227	265
		%	%	%					
A	70,000 (99)	<1	98	2	2	5	3	2	—
B	60,000 (98)	<1	99	<1	<2	<2	<2	<2	—
C	65,000 (97)	<1	<1	<1	—	94	<2	20	96
D	23,000 (98)	<1	<1	<1	98	97	<2	—	—
E	10,500 (60)	<1	23	3	62	61	57	68	—
F	3,400 (41)	3	52	5	21	—	36	—	—

* Surface Ig refers to the cells bearing either IgM or IgD or both.

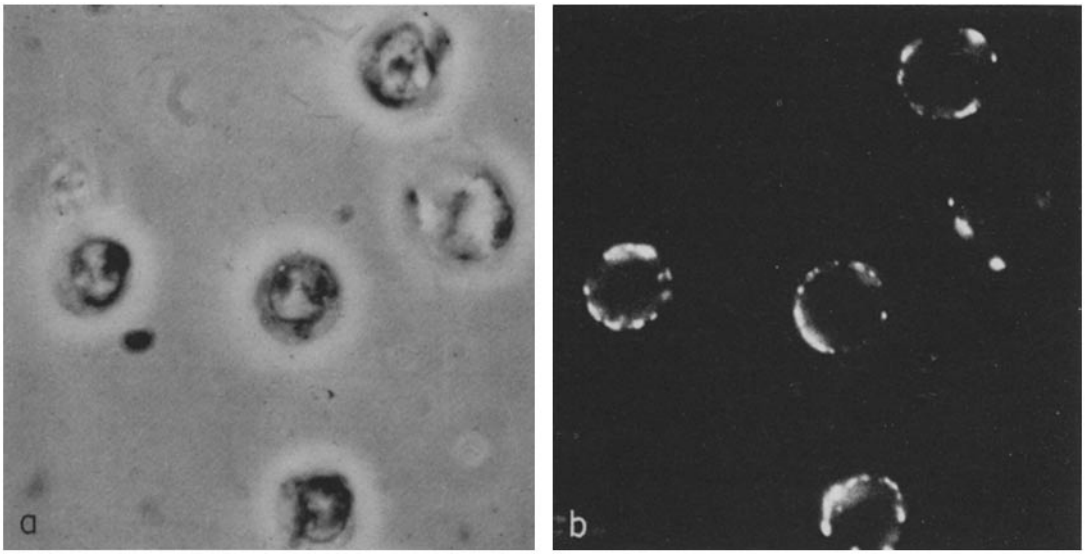

FIG. 1. HL-B antigens detected by indirect fluorescence on ALL lymphoblasts in case D. (*a*) phase micrograph and (*b*) fluorescent micrograph of the same field showing staining with serum 58.

Discussion

In the six cases of acute lymphoblastic leukemia studied by various membrane markers, two appeared to be T-cell leukemias since the vast majority of the lymphoblasts formed sheep erythrocyte rosettes and lacked surface Ig and the Fc receptor. The four other cases lacking all the usual markers would have to be classified as "non-T non-B" leukemias (5). Thus in this small series, one third of the cases can be classified as E rosette-forming T-cell ALL. This percentage is higher than that reported previously (5–7) but the difference is probably due to the selection of cases with high numbers of lymphoblasts in the peripheral blood.

The HL-B alloantigens detectable by pregnancy sera appear to represent the human counterpart of the Ia antigens of the mouse (8,9). These antigens are easily detectable on lymphocytes from patients with chronic lymphocytic leukemia and lymphoblastoid lines of B-cell origin. They are undetectable on human thymocytes and the two T-cell lymphoblastoid lines studied. Thus, they are clearly preferentially expressed on B cells although some evidence for their presence on certain T cells, particularly stimulated T cells, has been obtained (8). In the two cases of E rosette-forming ALL, HL-B antigens were not detectable on the blasts whereas in the four non-T non-B ALL, the vast majority of the lymphoblasts had readily detectable HL-B alloantigens. This finding would suggest that the latter might represent B-cell leukemias. By this criterion, the majority of the acute lymphoblastic leukemias could be of B-cell origin and these leukemia cells might represent clonal proliferation of early B cells, possibly B stem cells. Thus, ALL might be placed with chronic lymphocytic leukemia, macroglobulinemia, multiple myeloma, and lymphocytic lymphoma to be in the

category of B-cell malignant lymphoproliferative disorders. If this is indeed the case, the majority of the neoplastic lymphocytes are of B-cell origin. It is also known, however, that the HL-B antigens are present on monocytes and possibly some cells of the myeloid series. Thus, the possibility that the HL-B-bearing ALL lymphocytes represent even earlier bone marrow stem cells requires consideration.

Recently, Greaves and his colleagues described a set of acute lymphoblastic leukemia-associated antigens detectable by heterologous antisera against acute lymphoblastic leukemia cells (6). These antigens were not present on normal peripheral lymphocytes and were present on the lymphoblasts of the majority of their non-E rosette-forming ALL cases. It is clear that these leukemia-associated antigens are distinct from the HL-B antigens described above although apparently associated with the same group of ALL cases. The possibility of some indirect relationship or the common reflection of the stem cell nature of these cells remains to be determined.

Summary

Six cases of acute lymphoblastic leukemia were studied by a variety of T- and B-lymphocyte surface markers. Two appeared to represent T-cell leukemias with the lymphoblasts forming sheep erythrocyte rosettes. The other four lacked all the usual membrane markers. However, indirect immunofluorescence with alloantisera detected the presence of the Ia-related HL-B antigens on the cells of the latter four cases; these antigens were absent in the first two cases. The primary association of the HL-B antigens with B cells raises the possibility that the positive group of cases are of B-cell lineage.

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