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Immunology

Distribution of Ia-like molecules on the surface of normal and leukemic human cells

(leukemia Ia antigens/T cells/B cells/Null cells)

STUART F. SCHLOSSMAN*, LEONARD CHESS*, ROBERT E. HUMPHREYS†‡, AND JACK L. STROMINGER*†

* Sidney Farber Cancer Center, Harvard Medical School, Boston, Massachusetts 02115; and † The Biological Laboratories, Harvard University, Cambridge, Massachusetts 02138

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ABSTRACT **Antiserum to a glycoprotein antigen complex of 23,000 and 30,000 dalton subunits (p23,30), isolated and purified from a human lymphoblastoid B cell line, was shown to be highly specific for human bursal-equivalent-processed (B) cells, reactive with 15–20% of human Null cells, but completely unreactive with human thymus-processed (T) cells. The p23,30 antigen is widely distributed on chronic lymphatic leukemic cells, 85% of acute lymphatic leukemic cells, all acute myelogenous leukemic cells, but not on chronic myelogenous leukemic cells. A rabbit antiserum specific for normal human thymocytes has also been prepared; it is reactive only with precisely that subset of acute lymphatic leukemic cells (15%) whose members do not have p23,30 on their surfaces.**

Insight into the cellular origin and differentiation pathways for a variety of lymphoid neoplasms has resulted from recent studies identifying normal lymphocyte differentiation antigens and receptors on the surface of these neoplastic cells (1–5). For example, it is well recognized now that the majority of cases of chronic lymphatic leukemia (CLL) and most of the lymphoblastic lymphomas bear surface immunoglobulin, Fc, and complement receptors, and thus point to a bursal-equivalent-processed (B) cell origin for these neoplasms. Moreover, recent studies published while the present paper was in preparation have indicated that a human B-cell-specific alloantigen is expressed on cells of approximately 80% of cases of acute lymphoblastic leukemia (ALL) (6). In contrast, thymus-processed (T) lymphocyte receptor for sheep erythrocytes is present on blast cells from a minority of patients with ALL, and on the neoplastic cell associated with the Szezary syndrome (5, 7, 8). However, since the B-cell-specific antigens are expressed on certain T lymphocytes and stimulated T cells, their presence on a subset of ALL cases does not clearly associate this subgroup with a B cell origin (9).

Recently, a glycoprotein antigen complex of 23,000 and 30,000 dalton subunits (p23,30) was isolated and purified from a human lymphoblastoid B cell line and was used to prepare an antiserum (anti-p23,30) which has been shown to be highly specific for human B cells, but to be completely unreactive with both T cells and human T cell lymphoblastoid lines (10, 11). Moreover, this antiserum does not recognize known HLA components, B_2 microglobulin, or other surface components that are associated with human B cells, including the Fc receptor, complement receptor, or surface immunoglobulin. This antibody (anti-p23,30) apparently recognizes a unique antigen on B lymphocytes that is probably the human counterpart of murine Ia antigens (12, 13). In the present, study, the distribution of this antigen on a variety of other normal and leukemic human cells of both lymphoid and myeloid origin has been explored.

Evidence will be presented showing that p23,30 antigen is widely distributed on CLL cells, 85% of ALL cells and, unexpectedly, on all acute myelogenous leukemic cells (AML) but not on chronic myelogenous leukemic cells (CML). A heterologous antiserum specific for normal human thymocytes has also been prepared; it is reactive only with precisely that subset of ALL cells (15%) whose members do not have p23,30 on their surfaces.

MATERIALS AND METHODS

Patient Material. Peripheral blood and bone marrow were obtained from 46 patients with the hematologic diagnosis of leukemia seen at the Sidney Farber Cancer Center, the Children's Hospital Medical Center, or the Peter Bent Brigham Hospital in Boston. Blood was obtained prior to introduction of therapy and all patients were diagnosed on the basis of general clinical features and cytologic evaluation of both peripheral blood and bone marrow specimens.

Cell Preparation. Heparinized blood and bone marrow samples were collected and the lymphocytes and blast cells were purified on Ficoll–Hypaque density sedimentation (14). Normal lymphocytes, normal bone marrow cells, and leukemic cells were stored frozen at −196° at a controlled rate of freezing in 10% dimethylsulfoxide and 20% serum. In addition, whenever possible fresh unfrozen Ficoll–Hypaque-sedimented normal and leukemic cells were examined. Normal bone marrow samples were obtained from patients in the course of their evaluation for nonhematologic malignancies.

Granulocytes, red cells, and phytohemagglutinin-induced T cell blasts were prepared by previously described techniques (15). Single cell suspensions from normal human thymuses and fetal liver were prepared, Ficoll–Hypaque purified, and washed as above.

Preparation of Antisera. p23,30 antigen and rabbit antiserum to this antigen were described in a previous paper (10, 11).

Antisera to a single population of acute lymphoblastic cells were raised by injecting two rabbits intravenously with 3×10^6 lymphoblasts from B.K., a patient with ALL. The injections were repeated 14 days later and the animals were bled on day 21. Anti-lymphocyte serum (ALS) was raised in rabbits injected with 3×10^6 Ficoll–Hypaque-purified normal human mononuclear cells. Each rabbit received two injections spaced 14 days apart and was bled on day 21.

Abbreviations: CLL and ALL, chronic and acute lymphatic leukemia, respectively; CML and AML, chronic and acute myelogenous leukemia; B cell, cell processed by a bursal equivalent; T cell, cell processed by the thymus; ALS, anti-lymphocyte serum; NRS, normal rabbit serum.

‡ Present address: Department of Pharmacology, University of Massachusetts Medical Center, 55 Lake Ave. North, Worcester, Mass.

All antisera were heated at 56° for 30 min. Antibodies to B.K. cells and normal lymphocytes were absorbed with AB erythrocytes, fetal liver cells, and CLL cells. The absorptions were carried out at a 3:1 serum:packed cell ratio and were performed at 4° on a rotary shaker for 60 min at 4°. Antibody to p23,30 required no further absorption except where specifically indicated in the *text*.

Immunofluorescence Analysis. Binding of anti-B.K., ALS, and p23,30 was studied primarily by indirect immunofluorescence using fluorescein-conjugated goat anti-rabbit Fc which had been previously digested with pepsin and fractionated on Sephadex G-150 to remove undigested material. The goat Fab_2 fluoresceinated anti-rabbit Fc was passed through a human IgG-Sepharose column to remove cross reactive antibody to human IgG. Immunofluorescent staining was carried out at 4° on cell populations and analysis of fluorescence-staining cells was performed with a Becton-Dickenson fluorescence-activated cell sorter (FACS-1), the output of which can provide a histogram of the number of positive fluorescent cells stained against the intensity of fluorescence. Detailed methodology, analysis, and cell separation capabilities of the FACS-1 were described previously (16). In brief, 3×10^6 cells were reacted with appropriate antibodies or normal rabbit serum controls for 30 min at 4°, washed three times with minimal essential medium containing 5% fetal calf serum, stained with fluoresceinated Fab_2 goat anti-rabbit Fc, washed three times again, and analyzed at room temperature. All sera were centrifuged at 100,000 × *g* for 20 min prior to use. The instrument was gated to exclude red cells and the intensity of fluorescence was recorded for each cell. Background fluorescence was determined by analyzing appropriate negative controls, which, as described above, included normal rabbit serum plus fluoresceinated goat anti-rabbit serum or fluoresceinated goat anti-rabbit serum alone. The percentage of specifically labeled cells as well as a fluorescence profile of the labeled cells was obtained by comparing the number of cells giving fluorescence signals above background. In addition, a histogram plotting cell number versus intensity of fluorescence was obtained.

Separation of B, T, and Null Lymphocytes from Peripheral Blood. Highly purified populations of human T, B, and Null cells were isolated by methods which have been described previously in detail (15, 17, 18).

RESULTS

Distribution of p23,30 antigen on normal human cells

The binding of rabbit anti-p23,30 antibody to the surface of various cell populations was investigated by indirect immunofluorescence on a the fluorescence-activated cell sorter utilizing fluoresceinated goat Fab_2 absorbed with human Ig and specific for rabbit Fc as the developing reagent. Binding curves plotting fluorescence intensity versus cell number (40,000 cells counted) are shown with human peripheral lymphocytes in Fig. 1A. Approximately 20% of human peripheral lymphocytes bind p23,30 antibody. In 25 patients studied, the binding varied between 15 and 25% and was consistent with the complement-mediated lysis data (10).

Indirect fluorescent binding studies were then carried out with separated T, B, and Null cell populations (Table 1). All human B cells stained with p23,30 antibody strongly in dilutions of the antisera as great as 1/1000 (Fig. 1B). In contrast, T cells did not stain with any of the dilutions of p23,30 antibody tested (Fig. 1C). The Ig-negative sheep erythrocyte rosette negative Null cell population, on the other hand, was

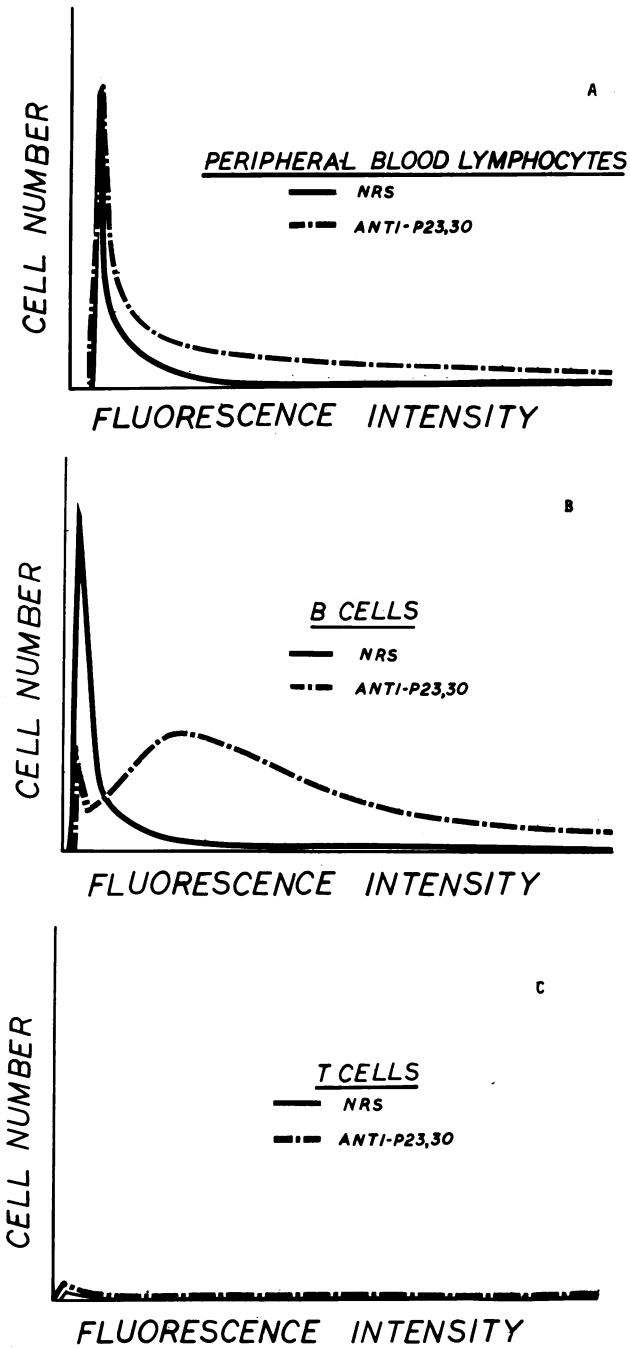

FIG. 1. Fluorescence-activated cell sorter analysis of unseparated peripheral blood lymphocytes (A), B lymphocytes (B), or T lymphocytes (C) reacted with anti-p23,30 or normal rabbit serum (NRS) and developed with fluoresceinated goat Fab_2 anti-rabbit Fc. The abscissa represents the relative intensity of fluorescence and the ordinate represents the number of cells counted per channel. For each analysis 40,000 cells were counted.

heterogeneous, since approximately 15–20% of this cell population stained.

The distribution of p23,30 antigen on phytohemagglutinin-induced T cell blasts, thymocytes, normal bone marrow granulocytes, and fetal liver cells was investigated. In all cases tested no significant staining was noted.

Distribution of p23,30 antigen on leukemic cells

In seven patients tested, all CLL cells bound anti-p23,30 antibody and the intensity of fluorescence was either equal

Table 1. Reactivity* of anti-p23,30 and anti-B.K. with normal, leukemic, and related tissues

Cell source	Antisera	
	Anti-p23,30	Anti-B.K.
B cells (normal)	25/25	0/25
T cells (normal)	0/25	0/25
Null cells (normal)	12/12	—
Thymocytes	0/3	3/3
Normal marrow	0/5	0/5
ALL	21/24	3/24
AML	10/10	0/10
CLL	7/7	0/7
CML	0/2	0/2

* Data are presented as number of samples positive/number of samples tested.

to or greater than that obtained with normal B cells (Fig. 2).

The reactivity of p23,30 antibody on acute lymphoblastic leukemic cells is illustrated in Fig. 3. Of the 24 ALL patients analyzed, strong staining was found in 21 with p23,30 antibody in dilutions of 1/150 or greater. Three patients with cytologically indistinguishable acute lymphoblastic cells were totally nonreactive with p23,30 antibody. On the other hand, both anti-p23,30-positive and -negative ALL cells stained strongly and were indistinguishable when tested with ALS, a polyvalent anti-lymphocyte serum. More important, precisely those three patients who were p23,30 negative, including B.K., reacted with anti-B.K. antibody (Fig. 4) (Table 1). Anti-B.K. antibody was unreactive with p23,30-positive cells and, of all the normal cells tested, only the thymocyte population tested were specifically stained (Table 1).

Ten cases of acute myelogenous leukemia were analyzed with p23,30 antibody and all stained strongly (Table 1). The staining pattern, as shown in Fig. 5, was similar to that obtained with normal B cells and p23,30-positive ALL and CLL cells. In these instances, as with other leukemic cells, no discernible differences in staining patterns were obtained using either peripheral blood blasts or bone marrow blasts. It should be emphasized that normal bone marrow had maximally 1–2% cells which stained and that the specificity of this staining was not certain, since similar staining profiles

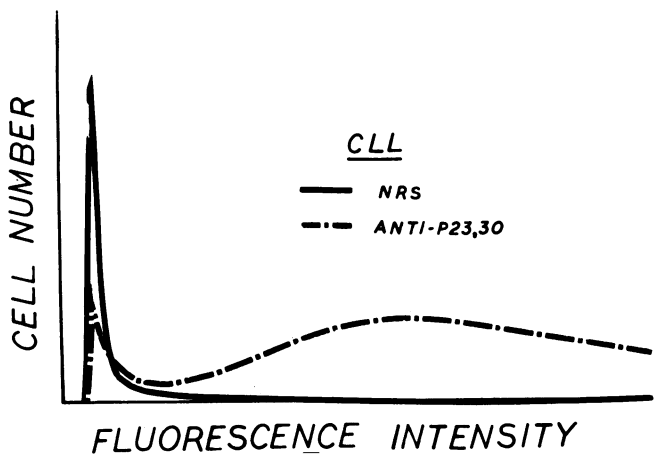

FIG. 2. Fluorescence-activated cell sorter analysis of chronic lymphatic leukemic (CLL) cells reacted with anti-p23,30 or normal rabbit serum and developed with fluoresceinated goat Fab_2 anti-rabbit Fc.

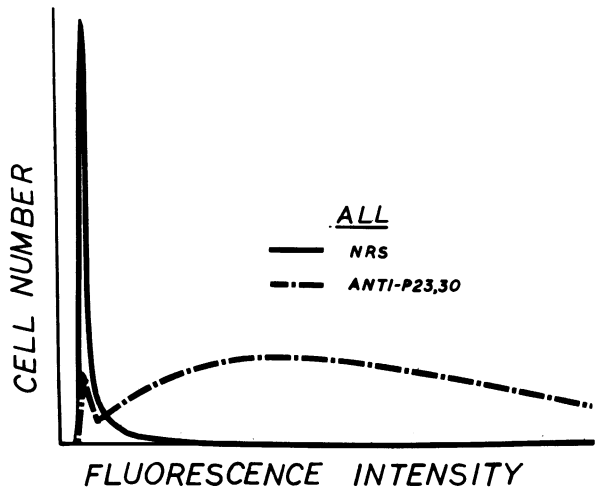

FIG. 3. Fluorescence-activated cell sorter analysis of acute lymphatic leukemic (ALL) cells reacted with anti-p23,30 serum or normal rabbit serum and developed with fluoresceinated goat Fab_2 anti-rabbit Fc.

were obtained with normal rabbit serum. In addition, two patients with chronic myelogenous leukemia and one patient with plasma cell myeloma were analyzed and their cells were unreactive with either anti-p23,30 or anti-B.K. antibodies.

Absorption of p23,30 antibody with leukemic cells

The reactivity of anti-p23,30 antibody with all CLL, AML, normal B cells, and 85% of ALL cells suggested the possibility that either a single antigen on all cells or several different antigens were recognized by anti-p23,30. To resolve these possibilities, differential absorptions with CLL, AML, and ALL cells were undertaken. A panel of ALL, AML, and B cells was used as test cells. Separate absorptions of 1 ml of a 1/20 dilution of anti-p23,30 with 5×10^8 ALL cells (twice), 5×10^8 AML cells (twice), or 5×10^8 CLL cells (twice) markedly diminished staining with the test panel of ALL, AML, or normal B cells. Fig. 6 illustrates the staining pattern obtained with anti-p23,30 on ALL cells prior to and following absorption with AML, CML, CLL, or ALL cells.

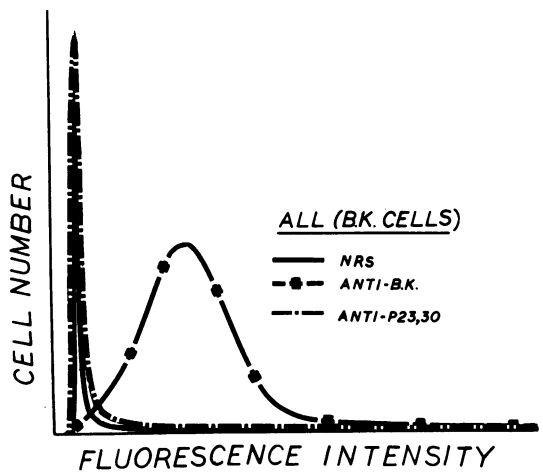

FIG. 4. Fluorescence-activated cell sorter analysis of acute lymphatic leukemic (ALL) cells from case B.K. with anti-p23,30 serum, anti-B.K. serum (thymocyte specific), or normal rabbit serum and developed with fluoresceinated goat Fab_2 anti-rabbit Fc.

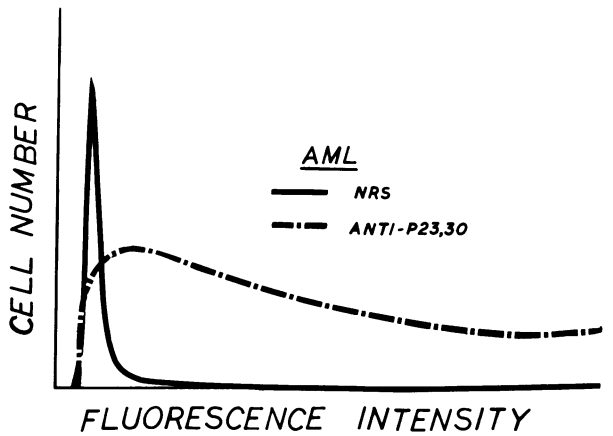

FIG. 5. Fluorescence-activated cell sorter analysis of acute myelogenous leukemic (AML) cells with anti-p23,30 or normal rabbit serum and developed with fluoresceinated goat Fab_2 anti-rabbit Fc.

Similar studies were performed with AML, CLL, and B cells as the test cells. No unique leukemia-specific antibody could be demonstrated, since extensive absorption with ALL, CLL, and AML cells removed reactivity with all other leukemic cells as well as normal B cells. In contrast, absorption with 2×10^8 (twice) fetal liver cells (not shown) or CML (Fig. 6) did not reduce the staining appreciably.

DISCUSSION

Previous studies have indicated that normal tissue antigens can be expressed on human leukemic cells and under certain circumstances these antigens help define the cellular origin of the leukemic cell. In the present studies we have demonstrated with the use of several antisera that antigens present on normal thymocytes and B cells are distributed on distinct subclasses of acute lymphatic leukemic cells and that normal B cell antigens can be found on a wide variety of leukemic cells of both lymphocytic and myeloid origin.

It was found that antibody p23,30 prepared against a non-HLA, nonimmunoglobulin cell surface antigen complex derived from a human B cell lymphoid line reacted strongly with approximately 20% of normal human peripheral lymphocytes. The reactivity of unseparated cell populations

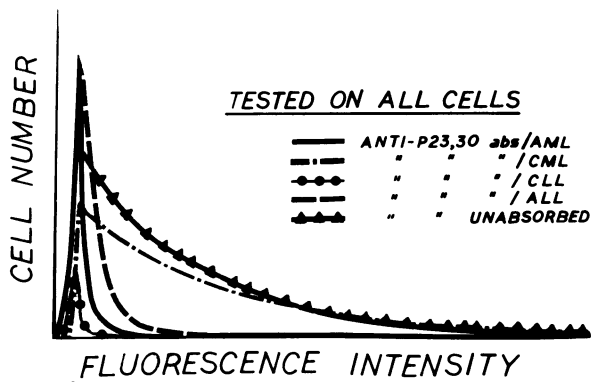

FIG. 6. Fluorescence-activated cell sorter analysis of acute lymphatic leukemic (ALL) cells with anti-p23,30 serum (unabsorbed), anti-p23,30 serum absorbed (abs), twice in all cases, with 5×10^8 AML cells, anti-p23,30 serum absorbed with 5×10^8 CML cells, anti-p23,30 serum absorbed with 5×10^8 CLL cells, and anti-p23,30 serum absorbed with 5×10^8 ALL cells, all developed with fluoresceinated goat Fab_2 anti-rabbit Fc.

could be totally accounted for by the presence of the p23,30 antigen on all of peripheral B cells and approximately 15% of the Null cell subset. No specific staining of the T cell subset could be demonstrated. That only 15% of the Null cell population reacted with this antisera is of interest, since earlier studies from our laboratory demonstrated a functional similarity between the Null and B cell subsets and, further, it was shown that a subset of Null cells were capable of differentiating into B cells as measured by the development of both surface immunoglobulins and immunoglobulin production (19). Of the other normal cells tested, it was not possible to demonstrate the presence of p23,30 antigen on normal thymocytes, fetal liver cells, mature granulocytes, or red cells. In addition, several T cell lines and nonlymphoid malignant cell lines were also unreactive. In contrast, all long term B cell lines tested were positive (10).

In view of the strong reactivity of anti-p23,30 with both circulating B cells and a subset of Null cells destined to differentiate into B cells (unpublished), it was not surprising that chronic lymphatic leukemic cells were strongly reactive with anti-p23,30. Studies from several laboratories have shown that CLL cells maintain many of the characteristics of B cells—that is, the presence of variable quantities of surface immunoglobulins, Fc, and complement receptors. The p23,30 antigen appears to be an important constituent of the plasma cell membrane of the B cell which is distinct from β_2-microglobulins, Fc, and complement receptors (10, 11). Thus, the presence of this antigen on CLL cells defines yet another surface antigen relating this class of leukemic cells to normal B cells.

The finding that p23,30 antibody reacted strongly with cells from 21 of 24 patients with acute lymphoblastic leukemia was unanticipated in view of the widely held assumption that most childhood leukemias were either T cell or undifferentiated cell disorders. The intensity of staining with p23,30 antibody with acute lymphoblastic leukemic cells was equal to or greater than that found with the normal B cells. The three p23,30-negative cases, however, were totally unreactive even though they were cytologically indistinguishable from cells obtained from p23,30-positive patients. Moreover, both p23,30-positive and -negative cells were equally reactive with anti-ALS. Of greater importance was the observation that the p23,30-negative ALL patients were reactive with both anti-B.K. antisera (thymocyte specific) as well as with an anti-T cell serum (unpublished). In contrast, the p23,30-positive patients were completely unreactive with either of these sera. Thus, all acute lymphoblastic leukemic cells could be accounted for as either having B or T cell surface markers. Similar results were obtained by Fu *et al.* with still another set of reagents capable of reacting with B cells. Their results showed that the majority of CLL cells and acute lymphoblastic cells stained with these B cell alloantisera. On the other hand, T cells and sheep erythrocyte rosette-positive ALL cells were unreactive (6). Their antibodies were thought to be directed at a human Ia antigen, and were capable of inhibiting mixed lymphocyte cultures (MLC). Similarly, anti-p23,30 antiserum is B-cell-specific, inhibits mixed lymphocyte cultures, and, perhaps of greater importance, p23,30 is structurally related to murine Ia antigens (10–13). In contrast, Greaves *et al.* showed that most ALL patients reacted with an antibody which was neither T- nor B-cell-specific (20). How their leukemia-specific antigens are related to p23,30 or other B cell alloantisera remains to be defined. However, it is clear that as the heterogeneity of the lymphoid system as defined by cellular probes

and functional assays becomes more fully appreciated, further distinctions of subsets of ALL cells may be defined.

Perhaps the most important and least anticipated observation was the demonstration that anti-p23,30 reacted with all of the acute myelocytic leukemic cells tested, despite the fact that it was unreactive with normal bone marrow, mature granulocytes, CML cells, or a single instance of plasma cells. One could argue that the finding of the p23,30 antigen on 85% of ALL and all AML cells reflects a B cell derivation of these leukemias, as the retention of B cell surface immunoglobulins, Fc, and complement receptors on CLL cells indicates a B cell origin of these tumors.

Alternatively, one could view the p23,30 antigen as an early differentiation antigen. Under these circumstances the presence of this antigen on most childhood and adult leukemia cells may reflect merely an early stage of cell surface differentiation. The entire hematopoietic system develops from stem cells which appear first in the yolk sac and then, in most species, in fetal liver and in bone marrow. These cells can, in an appropriate microenvironment, differentiate into more mature myeloid, erythroid, or lymphoid cells. Cells migrating from the bone marrow into the thymus mature along the T cell line, develop T cell antigens, and, perhaps in man, lose p23,30 antigen, whereas those migrating into a bursal equivalent differentiate into B cells. The B cell can further differentiate in an appropriate cellular environment into mature plasma cells. The presence of p23,30 on human peripheral blood B cells may suggest that these cells also are at an early stage of cell surface differentiation. In support of this view is the evidence that peripheral B cells have both IgD and IgM as their major classes of surface immunoglobulins (21, 22), surface markers characteristically found during earlier stages of B cell differentiation. The failure to detect p23,30 antigen on normal bone marrow cells would be consistent with this suggestion, since the majority of the cells analyzed are more fully differentiated. This latter point is emphasized by the absence of this antigen on cells from two patients with chronic myelogenous leukemia and the intriguing finding that the single myeloma cell population analyzed was also negative.

Another interpretation of the above findings would be that anti-p23,30 is recognizing different antigens on myeloid and lymphoid cells. However, absorption studies make this interpretation unlikely, since absorption of anti-p23,30 antisera with ALL, AML, and CLL cells removed antibody not only for the homologous absorbing cells but for other leukemic cells and B cells as well. Thus, it would appear that the anti-p23,30 serum reacts with similar, if not identical, antigens distributed on the populations tested. Thus, one must assume that AML represents a disorder of B cells or may express derepressed B cell antigens, or one may take the more likely view that p23,30 antigen is retained on undifferentiated stem cells destined to differentiate into both myeloid and lymphoid cells and that such antigens are retained by B cells.

Finally, the fact that the functional and structural behavior of p23,30 is analogous to that of murine Ia antigens raises the possibility that Ia antigens may play a role in cell differentiation of a wide variety of cells which are not necessarily limited to the lymphoid system.

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