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Analysis of Human Leukemic Cells by Use of High-Resolution Two-Dimensional Electrophoresis. I: Results of a Pilot Study

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We analyzed mononuclear leukocytes from patients with various human leukemias by high-resolution two-dimensional electrophoresis. Tumor cells of the granulocytic, monocytic, and lymphoid lineages [obtained from chronic granulocytic leukemia in blast transformation, acute monocytic leukemia, and chronic lymphocytic leukemia (CLL), respectively] can be easily recognized by using a series of cell-type marker proteins identified by comparison of fractionated normal cell populations. B and T cell types of CLL could be distinguished, the results correlating well with those obtained by use of monoclonal-antibody staining methods. In two cases representing almost pure B-cells (classical CLL; 0% T, 85% B) and T-cells (cutaneous T-cell leukemia; 77% T, 0% B), 27 of 29 marker proteins showed quantitative B/T differences comparable to those observed in comparisons of normal B- and T-lymphocytes prepared by cell sorting. These results indicate that cells from relatively well-differentiated leukemias show complex patterns of gene expression very similar to those of the corresponding normal cells and strongly support the use of large marker panels in cell-type determination. Less-well-differentiated acute leukemias [such as acute undifferentiated and acute granulocytic (FAB:M_1)] appear to yield protein patterns corresponding less closely to recognizable mature cell types, and may show expression of novel proteins related to the state of differentiation.

Additional Keyphrases: *patterns of gene expression · cancer · B and T cells · leukocyte proteins*

The major characteristics of a cell, such as morphology and growth rate, are primarily determined by the pattern in which its genes are expressed. Because most expressed genes code for proteins, the most direct method for examining the expression pattern in a given cell type would be one capable of quantitating the synthesis of all (or a large number) of the cellular proteins. Such a method now exists, in the form of high-resolution two-dimensional electrophoresis (*1–3*) combined with computerized data-reduction systems (*4*), thus creating the opportunity to re-explore the categorization of cell types at a new level of sophistication. Using this approach, each of the several thousand proteins expressed in a cell type can be considered a potential marker for some aspect of differentiation or regulatable function.

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The human peripheral blood leukocytes constitute a nearly ideal system for exploring the usefulness of such a protein-cataloging approach. The cells are easily obtained in a viable state, and can be cultured in vitro for extended periods of time. Numerous studies have begun to identify important leukocyte gene-regulation systems [heat shock (*5*), interferon (*6*), mitochondrial inhibitors (*7*), phorbol ester tumor promoters (*6*)], and to determine the physical locations of particular proteins within the cell [e.g., mitochondria (*7*), cytoskeleton (*8*), surface membrane, lysosomes, and nuclei]. In addition, the various major leukocyte classes [neutrophils, monocytes, and lymphocytes (*9*)] and some subclasses [B and T lymphocytes, and suppressor and helper T (K. E. Willard-Gallo and M. Loken, manuscript in preparation)] have been analyzed, yielding sets of "marker" proteins that distinguish one cell type from another. Taken together, these results have begun to establish an interpretive base capable of giving some meaning to the analysis of aberrant cell types such as those occurring in leukemia.

Although the necessity of dissecting and understanding cells at the molecular level is obvious in the context of basic biological research, it is important to ask whether more information, and of what type, would be useful to the hematologist in a clinical context. Examination of leukemic cells by use of monoclonal antibodies (*10*) and enzyme markers (*11*) has helped to define the relationships between leukemic types and normal differentiation stages with much greater precision. However, these techniques also demonstrate clearly the fragmentary nature of present knowledge and argue strongly that even more (and better) markers will be necessary to characterize the variety of cells observed. The motivation for the development of objective markers for all cell types and differentiation stages is not academic; even the classification of acute leukemias into three lymphoblastic and six nonlymphoblastic categories in the French–American–British (FAB) system can, when applied by different pathologists, give rise to differing initial diagnoses in 30 to 40% of patients (*12*). A more exact and objective method of classification is thus highly desirable.

In this paper we report some results obtained in a pilot study of leukocytes obtained from leukemia patients. Our major objectives were to identify technical difficulties and, given some technical success, to begin to assess the usefulness of the high-resolution two-dimensional electrophoretic mapping approach in the study of various forms of leukemia. The results indicate that a systematic examination of the leukemias is feasible and justified.

Materials and Methods

Leukemic leukocyte specimens. Blood specimens from patients with leukocyte counts exceeding 15 000 per microliter or with known hematologic diseases were obtained from the Mayo Clinic central processing or hematology laboratories within 1 to 5 h of venipuncture. Patients were identified by

a serial number within the study, prefixed by the letter Z. A volume of 1 to 4 mL, collected with EDTA (or occasionally heparin) as anticoagulant, was centrifuged upon receipt to separate the "buffy coat." We observed no systematic differences between lymphocytes from specimens containing heparin or EDTA. Control specimens, obtained daily from normal, healthy individuals, were processed in the same way as the leukemic specimens. Differential counts were made on blood smears as a guide to selecting specimens from which mononuclear cells were isolated. Of the 31 patients in the study, 14 had chronic lymphocytic leukemia.

Isolation of mononuclear cells, labeling, and sample preparation. Leukocyte buffy coats were collected with a Pasteur pipette and diluted into 3 mL of complete RPMI 1640 medium (GIBCO, Grand Island, NY 14672). Each sample was layered over 3 mL of "Ficol-Paque" (Pharmacia Fine Chemicals, Piscataway, NJ 08854) in a fresh siliconized tube and centrifuged for 30 min. Bands of mononuclear cells were collected, washed once in RPMI 1640 medium minus methionine (RPMI-met; GIBCO Selectamine kit plus fetal bovine serum, 100 mL/L, and antibiotics) and finally diluted in RPMI-met to a concentration of approximately 4×10^6 cells per milliliter. Cell suspensions were dispensed into the wells of a standard 24-well tissue culture plate (400 μL per well). Twenty-five microcuries of [^{35}S]methionine (1200 Ci/mmol; Amersham, Arlington Heights, IL 60005) was added to each well and the cells were incubated at 37 °C for about 18 h. After this labeling, the cells were quickly pelleted by centrifuging the cell suspension for 1 s in capillary-bottom Microfuge tubes. The supernate was removed, and the cells were immediately lysed in 60 μL of a pH 9.5 NP-40/urea solution as described previously (*4, 5*). After this solubilization, samples were centrifuged for 1 min in the Microfuge to pellet nuclear DNA, frozen at −80 °C, and transported to Argonne on solid CO_2.

Two-dimensional electrophoresis and computer analysis. Samples were analyzed by using the 18 × 18 cm ISO-DALT system as previously described (*2, 3, 13*). In general, a 20-μL sample containing labeled proteins from ~600 000 leukocytes (~40 μg of protein) was applied to each gel. Isoelectric focusing was performed in 40-place tube gel devices, and SDS-electrophoresis by using slab gels cast in batches of 20 and run 10 gels per DALT tank. Gels were stained with Coomassie Blue, destained, dried, and autoradiographed on Kodak XAR-2 film. Selected films were scanned and processed in the TYCHO system as previously described (*4*).

Quantitation of T- and B-cells in CLL samples. Blood smears were made from the blood specimens to be processed for study. T and B lymphocytes on the smears were identified by use of an indirect immuno-alkaline phosphatase "stain" involving monoclonal antibodies specific for T cell-surface antigen and HLA-DR (Ia-like antigen). For each smear, 200 lymphocytes were counted to establish the percentage of lymphocytes positive for T-cell surface antigen (T cells) and HLA-DR (B cells).

Results

Cell-Type-Specific Marker Proteins in Leukemia

We examined peripheral leukocytes from patients with chronic granulocytic leukemia (CGL), acute monocytic leukemia, and chronic lymphocytic leukemia (CLL) for the presence of characteristic marker proteins that had been identified in studies of corresponding normal cells (*9*). The principal granulocyte markers (G:1–4) are strongly expressed in mononuclear cells from a CGL patient (Figure 1*A*), but not in normals or in the other leukemias examined. These cells also show several major uncommon proteins (Q:4 for example, perhaps associated with the numerous eosinophils or basophils). The major monocytic marker proteins (M:1, 2, 8–11 and Mitcon:5) are likewise strongly expressed in the peripheral blood cells (mostly blasts) obtained in a case of acute monocytic leukemia (Figure 1*C*), but not in normal or non-monocytic leukemic leukocytes. Several other proteins are apparent (typified by Q:5 and Q:6 in panel *C*, and the Q:7 complex identified in Figure 3) that were not previously seen in either normal monocytes or macrophages. Both the CGL and the acute monocytic leukemia show strong expression of Calgon:1, a marker shared by normal granulocytes and monocytes.

Peripheral blood cells from CLL patients (Figure 1*E* and 2) yield patterns rather similar to normal lymphocytes if the lymphocyte subtype differences are taken into account (cells usually show an abundance of B-cell markers B:1, -2, and -5, owing to the more common occurrence of B-CLL).

T- and B-Lymphocyte Markers in CLL

By making use of enzyme-labeled monoclonal-antibody lymphocyte typing techniques, we were able to select two CLL cases in one of which the peripheral blood lymphocytes comprised 0% T-cells, 85% B-cells (case Z72, leukocytes $60 \times 10^3/\mu$L) and in the other 77% T-cells, 0% B-cells (Z15, leukocytes $149.6 \times 10^3/\mu$L). Careful analysis of these samples (Figure 2, Table 1) showed quantitative differences between the T and B leukemias in 29 proteins, which also differ significantly between normal T and B cells isolated by use of monoclonal antibodies and fluorescence-activated cell sorting (K. E. Willard-Gallo and M. Loken, manuscript in preparation). For 27 of these 29 proteins, the differences between leukemic T and B cells were in the same sense (i.e., increase or decrease) as those between normal T vs B cells (Table 1). The exceptions were Mitcon:4 (same as T:12 in Table 1), a mitochondrially encoded protein whose expression is affected by various influences, including intracellular calcium and heat shock, and a minor phosphoprotein about which little is known (B:9, square in Figure 2). One of the principal markers showing consistently large T–B differences in both normal and leukemic cells is the LDH-B chain (circle numbered 1 in Figure 2*B*); B-cells appear to produce less of this polypeptide than do T-cells. The T-CLL has the unusual feature (for a lymphocyte) of producing large amounts of the polypeptides labeled Q:2 (Figure 2*B*), which appear to be involved in phagocytosis and are usually characteristic of monocytes, granulocytes, or fibroblasts.

Less Well-Differentiated Leukemias

Analysis in terms of cell-type specific markers is more difficult for leukemias that appear to involve proliferation of immature leukocyte precursors. An acute granulocytic leukemia (FAB classification M_1; Z77, Figure 1*B*) showed only very low amounts of most granulocyte or monocyte markers. However, a greatly increased amount of a novel protein (Q:3) appears; this protein may be characteristic of an early myeloid precursor, because it is also expressed by the promyelocytic leukemic cell line HL-60 under some conditions. The monocyte/granulocyte specific mitochondrial protein Mitcon:5 (labeled in Figure 1*C*) is also more greatly expressed than would be expected in the absence of other monocyte or granulocyte markers.

A case of acute undifferentiated leukemia (Z74; Figure 1*D*) also shows a lack of mature monocyte or granulocyte markers (this time including a lack of Mitcon:5), but shows large amounts of several new proteins exemplified by Q:8.

Two cases showing additional interesting markers are presented in Figure 3. One (Z90; an acute monocytic leukemia classified FAB M_5) shows abundant monocyte markers

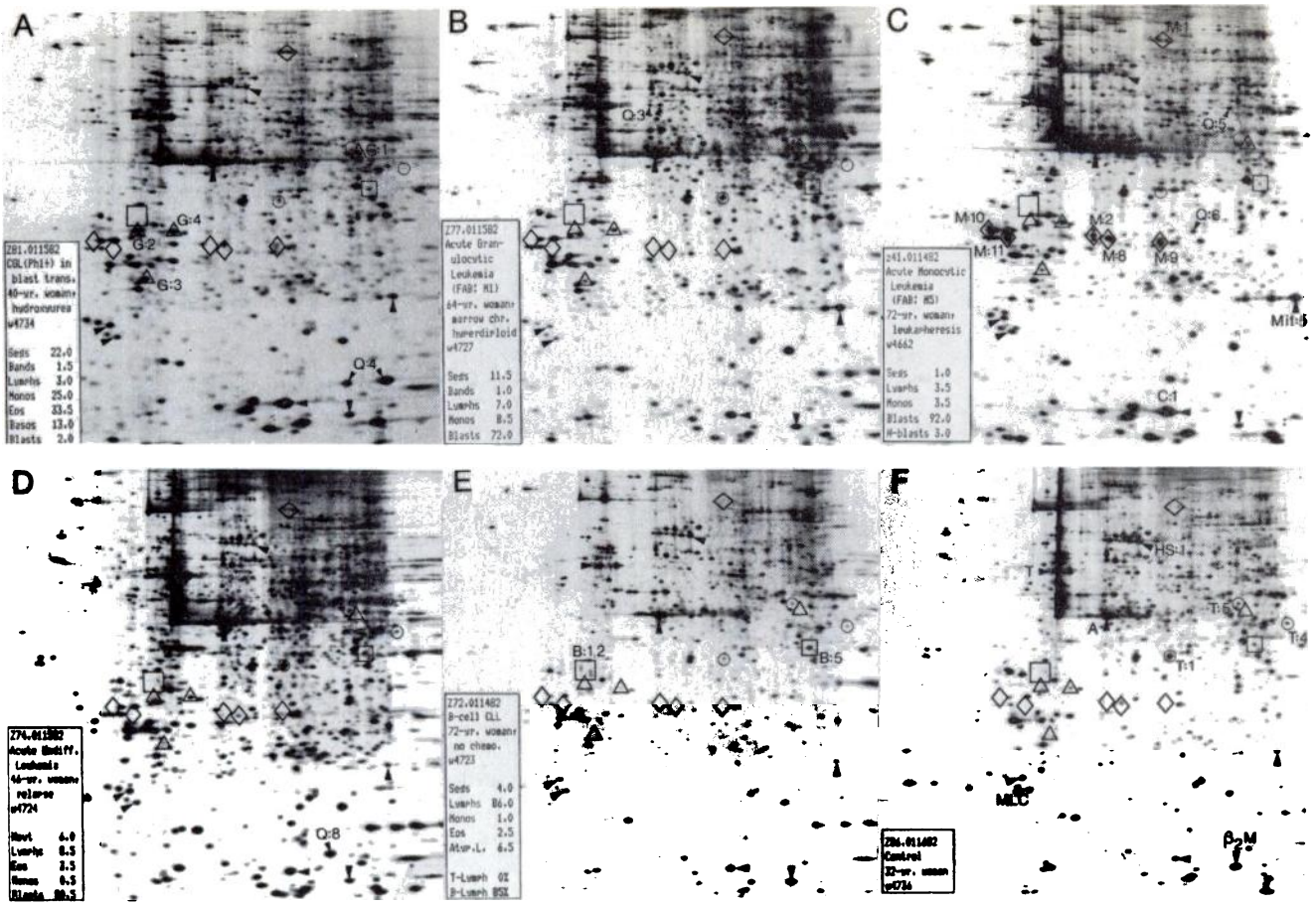

Fig. 1. Two-dimensional patterns of [^{35}S]methionine-labeled mononuclear leukocytes

A, chronic granulocytic leukemia undergoing change to acute myeloblastic (Z81; treated with hydroxyurea beginning one month before the study, with blasts up to 7.5% three days after this sample). *B*, acute granulocytic leukemia (Z77, classified FAB:M_1). *C*, acute monocytic leukemia (Z41, classified FAB:M_5). *D*, acute undifferentiated leukemia (Z74). *E*, B-cell chronic lymphocytic leukemia (Z72, 85% B-cells, 0% T-cells). *F*, control (Z86). Patterns are shown with acid end to the left and high relative molecular mass at the top. *Triangles* G:1–4 (panel *A*) are granulocyte markers (*9*); *diamonds* M:1,2,8–11 (panel *C*) are monocyte markers; Mit:5 is a major monocyte-/granulocyte-specific mitochondrial protein (panel *C*); C:1 is Calgon:1 (also known as L1, ref. *14*); *squares* B:1,2, and -5 (panel *E*) are B-lymphocyte markers; *circles* T:1,4,5 (panel *F*) are T-lymphocyte markers (T:1 is the LDH-B chain); *MLC* are non-muscle myosin light chains; β_2M is β_2-microglobulin; *A* is β-actin; *T* is tubulin; and *HS:1* is the major human heat-shock protein. *Insert* panels in each case show the patient number in the Z-study series; an abbreviated diagnosis; age, sex, and status of the patient; gel number; and differential count on the blood sample from which the mononuclear cells were obtained

(including Mitcon:5) and high amounts of the major indicators of interferon induction (Interf:1 and :2). The second case (Z105; an acute granulocytic leukemia classified FAB M_1) shows no appreciable monocyte or granulocyte markers, but high amounts of Inmono:1 (a protein thought to be closely associated with infectious mononucleosis) and the Metmin proteins. The latter set of proteins is induced by methionine starvation (during labeling) in the promyelocytic leukemic cell line HL-60 (N. L. Anderson and M. A. Gemmell, manuscript in preparation) and in the lymphoblastoid cell line CCL119.

Both Z90 and Z105 express a set of proteins (Q:7) that also appear in some promyelocytic leukemias, an acute monocytic leukemia (Z41; Figure 1*C*), and to a lesser extent in an acute undifferentiated leukemia (Z74, Figure 1*D*). The Q:7 set is not found in normal lymphocytes, monocytes, or granulocytes.

Discussion

The results presented here are intended to elucidate two principal questions: (*a*) Are the results of two-dimensional electrophoretic analyses of well-differentiated leukemias interpretable in terms of normal cell-type-specific markers? (*b*) Are there novel features in the patterns of less-well-differentiated leukemias that might prove useful in improving the classification of different disease forms? We have obtained evidence that both questions can be answered in the affirmative.

Chronic lymphocytic leukemia appears to involve the proliferation of rather mature lymphocytes. Because monoclonal antibodies are available that can distinguish lymphocyte subsets and fluorescence-activated cell sorters are available that can sort these subsets preparatively, CLL represents one of the best systems with which to investigate the behavior of multiple differentiation markers in human cancer cells. Recent studies by Willard-Gallo and Loken (manuscript in preparation) have led to the identification of a panel of 30 proteins that differ quantitatively between normal human T and B lymphocytes. Using this panel, we have examined the fidelity of almost pure T- and B-cell CLL's to the phenotypic patterns of their normal counterparts. The cases we used to represent T and B CLL's were characterized by high leukocyte counts (60 and 150 $\times$ 10^3/μL, respectively). The leukocytes consisted mainly of lymphocytes (lymphocytes + atypical lymphocytes represented 92.5 and 98%, respectively), indicating a large preponderance of leukemic cells, and showed very high type-specificity, as determined by staining with enzyme-labeled monoclonal antibodies (77% T + 0% B and 0% T + 85% B, respectively). Of the 29 T and B markers we could unequivocally identify in the leukemic cell patterns, 27 differed quantitatively in the same sense (by increase or decrease) as

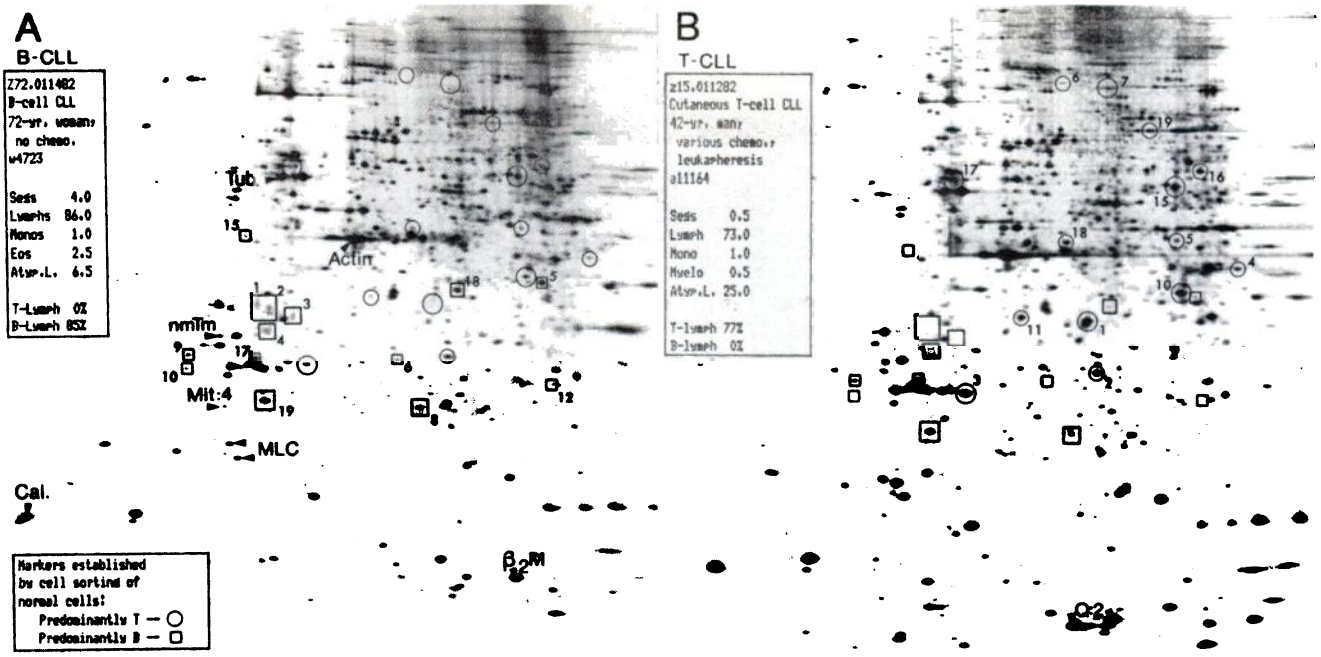

Fig. 2. Two-dimensional patterns of a B-cell (panel *A*) and a T-cell (panel *B*) chronic lymphocytic leukemia, chosen for compositional purity on the basis of antigenic markers (monoclonal antibodies to HLA-DR and T-cell surface antigen)

Circles indicate T-cell predominant proteins; *squares* indicate B-cell proteins, numbered in each case according to the nomenclature of Willard-Gallo and Loken (manuscript in preparation) for sorted normal T and B cells. *Tub.* is tubulin; β_2-M is β_2-microglobulin; *MLC* are non-muscle myosin light chains, *Mit:4* (also T-cell marker 12) is a mitochondrially encoded protein; and *nmTm* is non-muscle tropomyosin. *Q:2* indicates the position of two proteins normally associated with phagocytic or attached cells, not normally seen in lymphocytes

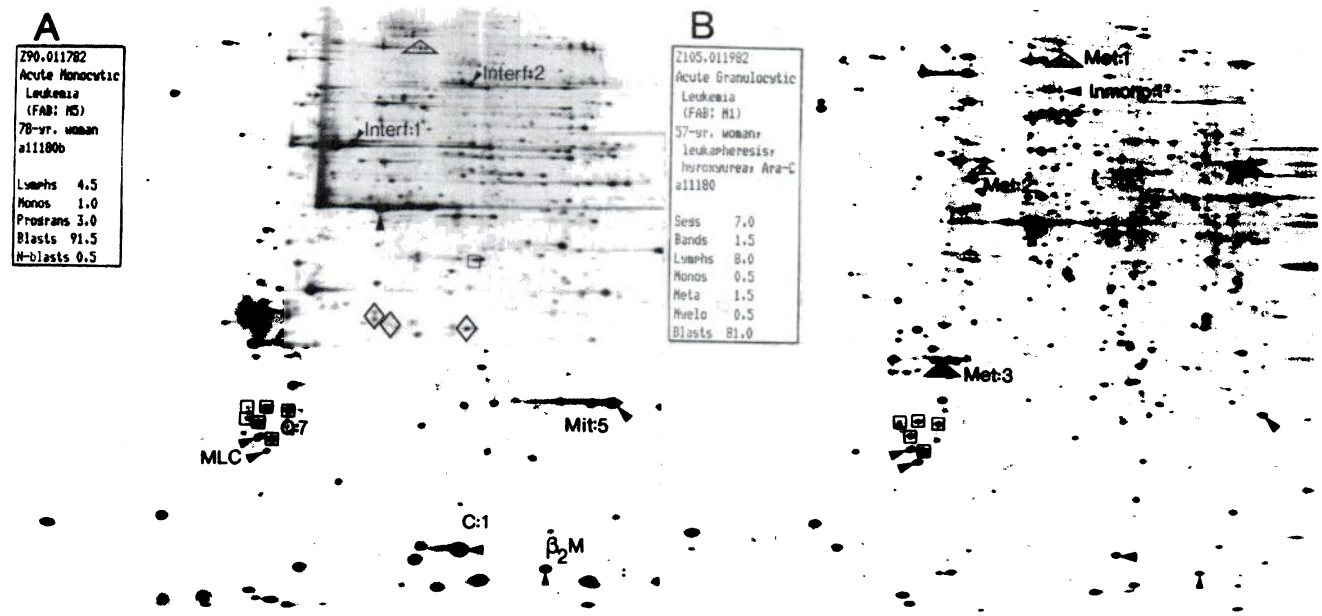

Fig. 3. Two-dimensional patterns from a second case of acute monocytic leukemia (Z90; FAB:M_5), showing preponderance of monocyte markers (*diamonds*) and interferon-induced proteins (*Interf:1,2*; N.L.A., unpublished observations), and an acute granulocytic leukemia (Z105; FAB:M_1) showing expression of the Inmono:1 protein (ref. *15*) thought to be associated with active mononucleosis and the Metmin proteins (*triangles* labeled Met:1–3).

Mit:5 is the mitochondrial protein Mitcon:5; β_2M is β_2-microglobulin; and *C:1* is Calgon:1. Both samples show expression of Q:7 proteins (*squares*)

they do between normal T and B cells. Thus in these two cases of chronic lymphocytic leukemia the complex and subtle features of the normal T and B lymphocyte phenotypes have been largely preserved. The remaining CLL's we examined all showed clear evidence of substantial B-cell increases, in agreement with monoclonal antibody typing results. These results make it appear quite likely that further advances in the development of panels of markers, particularly those associated with pre- and immature B-cells, will allow further subdivision of the B-cell malignancies.

The use of large marker panels appears especially important in view of the fact that almost none of the T vs B markers we used is unique to either T or B lymphocytes

Table 1. Quantitative Differences between a B and a T Chronic Lymphocytic Leukemia in the Synthesis of B and T Cell Markers

	B-CLL (Z72) W4723	T-CLL (Z15) a11164	B/T (CLL)	B/T (normal)	Same sense?
T:1	N[a]	60 000	<.02	.28	+
2	19 000	25 500	.74	.54	+
3	10 700	64 200	.17	.21	+
4	5 060	8 100	.62	.20	+
5	4 000	7 200	.56	<.07	+
6	N	3 600	<.21	.11	+
7	N	2 200	<.34	.05	+
10	12 300	72 000	.17	.25	+
11	3 900	30 500	.13	.42	+
12	6 900	N	>9.2	.22	−
15	31 100	47 000	.66	.55	+
16	6 550	28 100	.23	.95	+
17	42 400	71 000	.60	.94	+
18	N	20 000	<.04	.36	+
19	2 130	7 800	.27	.26	+
					14+, 1−
B:1	14 700	N	>20.	66.	+
2	18 700	N	>25.	12.	+
3	13 000	N	>17.	7.5	+
4	16 000	1 600	10.	19.	+
5	25 600	N	>34.	2.8	+
6	6 900	N	>9.2	16.	+
8	18 100	9 400	1.9	1.9	+
9	5 400	8 000	.67	2.6	−
10	4 400	3 100	1.4	3.2	+
12	3 000	N	4.0	13.	+
15	2 400	1 100	2.2	4.3	+
17	52 000	17 900	2.9	2.0	+
18	35 400	2 000	18.	3.9	+
19	52 200	33 300	1.6	2.5	+
					13+, 1−

[a] N = not detected under conditions such that a spot of integrated density 750. is usually detected.

The integrated amounts of various T and B cell marker proteins (T:1, etc.) synthesized in a B-cell CLL and a T-cell CLL (same gels as illustrated in Figure 2) are shown here. Quantitative results were obtained by using the TYCHO I computer system (*4*) to analyze autoradiographic films and are on an arbitrary linear scale (grey levels × mm^2 × 100) set up by making the sums of the densities of all the identified spots equal for the two gels. Also shown is the ratio of abundances in B and T CLL's and in normal (sorted) B and T cells for each marker, and an indication of whether the abundances differ in the same sense (i.e., increase or decrease) in CLL and normal cells.

(B:1–3, which may comprise part of the DR antigen, and a few of the others being possible exceptions). Most occur in other leukocyte types as well, suggesting that particular cell subtypes may be defined more by *patterns* of gene expression than by a few unique proteins. Although a large panel of both T and B leukemias will be needed to confirm the reliability of individual markers, it seems highly likely that panels such as we have used can distinguish T and B leukemias without recourse to antibody staining.

Only one of the proteins in the T vs B panel we used has yet been identified as a previously known marker; T:1 (Figure 2) is the LDH-B polypeptide that, when expressed alone, gives rise to the LDH-1 phenotype. The LDH-A isoenzyme has not yet been identified in the two-dimensional pattern, and may have an isoelectric point too basic to appear on these gels. Nevertheless, the results are consistent with the generally accepted preponderance of forms containing LDH-B in normal T cells and forms containing LDH-A in normal B cells (*16*). In the CLL cases compared in Figure 2, chosen for purity based on monoclonal antibody markers, the difference in LDH-B content is dramatic (no detectable LDH-B in the B-CLL). This result contrasts with those of Rambotti and Davis (*17*), who found as much LDH-1 (B_4) as LDH-5 (A_4) in five cases of B-CLL. Our finding may be the result of choosing a particularly pure, particularly B-like CLL for this detailed comparison. The LDH-A polypeptide will have to be identified on the two-dimensional map in order to relate our results reliably to the earlier isoenzyme work.

On a coarser level, the main granulocyte and monocyte markers (*9*) convincingly identify cells from a chronic granulocytic leukemia in blast transformation and two acute monocytic leukemias (Figure 1*C* and Figure 3*A*). Although several major proteins are present in both granulocytes and monocytes but not in substantial amounts in lymphocytes (Calgon:1 and the mitochondrial protein Mitcon:5), there are proteins that appear to be specific to either granulocytes (G:2,3) or monocytes (M:2, 8–11). The monocyte markers appear to be proteins involved in phagocytosis or lysosomal degradative processes (N.L.A., unpublished observations), and hence are likely to be associated with fairly mature cells of that lineage. The granulocyte markers are likely to be proteins in or associated with granules, and thus also representative of rather mature forms. If these assumptions prove to be true, then the occurrence of the granulocyte or monocyte markers in less-well-classified leukemias may provide an additional measure of the maturity of these cells. Two acute monocytic leukemias show pronounced monocyte markers (Z41 and Z90) while two acute granulocytic leukemias (Z77, Z105) fail to show high amounts of the granulocytic markers observed in chronic granulocytic leukemia (Z81).

That unusual proteins are observed in leukocytes from particular types of leukemia is of special interest, because of the possibility that such proteins may be markers of early precursors to the normally observed mature cell types. We have observed several such proteins [Q:3 in acute granulocytic leukemia Z77 (Figure 1*B*), Q:8 in acute undifferentiated leukemia Z74 (Figure 1*D*), Q:5 and Q:6 in acute monocytic leukemia Z41 (Figure 1*C*)]. These proteins are seen against a background of recognizable proteins, and thus are unlikely to be the result of general sample-preparation problems. Additional groups of proteins may have significance in the leukemias, even though they can also be detected elsewhere. The set marked Q:7 [Figure 3, acute monocytic and granulocytic leukemias, also occurring in acute monocytic leukemia Z41 (Figure 1*C*), and to some extent in acute undifferentiated leukemia Z74 (Figure 1*D*)] can be induced in normal lymphocytes under some stressful conditions, but it seems never to occur in normal lymphocytes that have been handled like the leukemic cells in this study (they do not occur in our parallel control samples, for instance). The main spots of Q:7 show striking similarities to the non-muscle myosin light chains (MLC) near which they are situated; possibly they represent expression of a set of normally repressed MLC genes. The Metmin spots (Figure 3*B*) include three apparently different proteins that are induced in some cells during culture in methionine-free medium. Metmin:2 and -3 in particular seem to be substantially expressed only in one acute granulocytic leukemia (Z105) and the acute undifferentiated leukemia (Z74) examined in this study. Other work (N. L. Anderson and M. A. Gemmell, manuscript in preparation) indicates that these proteins are strongly expressed in the promyelocytic cell line HL-60 and the lymphoblastic line CCL119 when cultured without methionine, but are repressible, even in methionine-free medium, by treatment with phorbol ester tumor promoters that cause the cells to differentiate. Because cell lines that cannot be made to differentiate in vitro generally do not express Metmin:2 or -3, these proteins may be markers of cells that are susceptible to some differentia-

tion signal. The involvement of methionine metabolism may indicate some relationship to methylation of protein, lipid, or DNA. Further studies on fresh leukemic cells treated with phorbol esters should help clarify this point.

Taken together, the results obtained in this study support the view that a detailed examination of gene expression in leukemic cells is likely to yield information of diagnostic and, perhaps, prognostic significance. Similar conclusions have been drawn in two recent two-dimensional electrophoretic studies of various types of leukemia performed without the advantage of numerous cell-type-specific marker proteins (*18, 19*). Here we have shown that the limited knowledge we have so far concerning differences among cell types and among subtypes appears to be directly applicable to the typing of some leukemias (as in CLL). As this knowledge is expanded its usefulness in diagnostic typing will probably increase. In addition, the observation of novel proteins, particularly in the acute leukemias, may provide markers capable of further dissecting the pathways of leukocyte differentiation. As the function of such marker proteins are elucidated, new targets for therapeutic agents may be discovered.

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