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# Nuclear Matrix Proteins Specific for Subtypes of Human Hematopoietic Cells

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**Abstract** Nuclear matrices were prepared from isolated subtypes of human hematopoietic cells and from cultured leukemia cells. The nuclear matrix proteins were analyzed by high-resolution two-dimensional gel electrophoresis and computer-assisted image analysis. While more than 200 protein spots were shared among the cells, about 50 distinct spots were found characteristic for individual cells or groups of related cells. This allowed to differentiate between hematopoietic cells and nonhematopoietic cells, lymphocytes and myeloid cells, monocytes, neutrophils, and promyelocytic leukemia cells. B and T lymphocytes could not be differentiated. Myeloid cells with their polymorph nuclei were characterized by the presence of 13 and by the absence of seven distinct spots, as well as by low concentrations of nuclear lamins and of heterogeneous nuclear ribonucleoproteins. Neutrophils with multilobular nuclei displayed six additional spots, while lacking 18 nuclear matrix protein spots. The nuclear matrix of proliferating cells showed three distinct spots in addition to proliferating cell nuclear antigen, increased concentrations of numatrin (B23), and heterogeneous nuclear ribonucleoproteins. The described cell-specific nuclear matrix proteins may represent new markers for hematopoietic cells. J. Cell. Biochem. 72:470–482, 1999. 

**Key words:** nuclear matrix proteins; leukocytes; lymphocytes; monocytes; neutrophils; leukemia cells; heterogeneous nuclear ribonucleoproteins; nuclear lamins; two-dimensional polyacrylamide gel electrophoresis

On the basis of biochemical and morphological studies, the nuclear matrix has been described as a nonchromatin scaffold that maintains the domain organization of the interphase nucleus. Consisting of a filamentous protein network binding to DNA loops, the nuclear matrix has been suggested to determine higher chromatin organization and to play a role in DNA replication, RNA synthesis, RNA processing, and RNA transport. The occurrence of cell-, tissue-, tumor-, and cell state-specific nuclear matrix proteins has been reported [for review, see Berezney and Jeon, 1995; Stuurman et al., 1992; Stein et al., 1996].

In this study, the nuclear matrix proteins of human hematopoietic cells, namely B lymphocytes, T lymphocytes, monocytes, neutrophils, and lymphoid and promyelocytic leukemia cell lines, were investigated. This allowed comparison of nuclear matrix proteins of highly differentiated cells originating from one type of stem cell, but differing by function, proliferation capability, and nuclear morphology.

Lymphocytes differentiate in two distinct hematopoietic microenvironments—that of the thymus and of the bone marrow—leading to cells of very distinct function. B lymphocytes are antibody-forming cells mediating humoral immunity, while T lymphocytes act as helper cells and as effector cells in cell-mediated immunological responses. Though differing significantly in respect to cellular function and signal processing, they appear morphologically identical. Monocytes are myeloid cells, which act as phagocytes and secretory cells. Monocytes are still capable of mitosis and are characterized by their eccentric reniform nucleus.

Neutrophils also belong to the myeloid lineage, but are nondividing short-lived phagocytes characterized by a relatively heterochromatic nucleus divided into three to five lobes. Promyelocytic leukemia cells do not show the polymorph nuclei of the highly differentiated monocytes and neutrophils, their morphology resembling that of lymphoid leukemia cells [for review, see Williams et al., 1990].

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For purposes of biochemical analysis, the nuclear matrix has been defined as the residual insoluble material obtained by treatment of isolated nuclei with detergents, nucleases and high ionic strength buffers [Berezney and Coffey, 1977]. In this study, the nuclear matrices were prepared by a modified method designed to preserve morphological characteristics of the nuclear structures [Gerner et al., 1998b]. The data demonstrate nuclear matrix proteins specific for leukocyte subtypes, for cell proliferation, and potentially for the nuclear morphology of cells.

## MATERIALS AND METHODS

### Isolation of B and T Lymphocytes

Preparation of cells was accomplished essentially as described in Current Protocols in Immunology [Coligan et al., 1997]. In short, fresh human tonsils were cut into small pieces, and lymphoid cells pushed through a 250-µm steel sieve. The cell suspensions were layered over Ficoll Paque (Pharmacia, Biotech, Uppsala, Sweden) and centrifuged. The cells of the interface were washed in Hanks' balanced salt buffer (HBSS) and subsequently suspended in RPMI-1640 supplemented with 10% fetal calf serum (RPMI-10). To obtain T-lymphocyte rosettes, the cell suspension was mixed with fetal calf serum (FCS) and with a S-(2-aminoethyl)isothiouroniumbromidehydrobromide (AET)-treated sheep red blood cell suspension, centrifuged, and incubated at 4°C for 1 h. The pellet was resuspended, layered onto Ficoll Paque, and centrifuged. The interface consisting of B lymphocytes was isolated and washed in HBSS. The pellet, consisting of T lymphocytes, was suspended in hemolysis buffer (150 mM $NH_4Cl$, 10 mM $KHCO_3$, 1 nM EDTA). After addition of RPMI-10, the pelleted T lymphocytes were resuspended and washed in HBSS. T-cell rosetting was repeated once. Flow cytometric analysis revealed the purity of B and T lymphocytes to be 95% at minimum.

### Isolation of Monocytes and Neutrophils

Heparinized human blood was mixed with 2 vol HBSS, layered over Ficoll Paque, and centrifuged. The interface cells were washed in HBSS and subsequently suspended in Dulbecco's modified Eagle's medium (DMEM)(suspension A). To isolate neutrophils, the resulting pellet was resuspended in phosphate-buffered saline (PBS) and mixed with Macrodex 6%. After allowing the erythrocytes to settle, the supernatant was again layered over Ficoll Paque and centrifuged. After the addition of RPMI-10, the pelleted cells were resuspended and washed in HBSS.

Monocytes were isolated according to the methodology of Hassan et al. [1986, 1990]. Suspension A was incubated in gelatin-coated tissue culture flasks at 37°C, 5% $CO_2$, for 1 h. The adherent cells were washed three times with DMEM and the loosely coadherent B lymphocytes released by short incubation with 1 mM EDTA in HBSS. The adherent monocytes were released by incubation with 5 mM EDTA in DMEM, 10% horse serum at 37°C for 15 min and finally washed in PBS. Flow cytometric analysis demonstrated the purity of monocyte and neutrophil preparations to be 98% at minimum.

### Leukemia Cell Lines

Cells were grown in RPMI-1640 medium containing 10% (20% in case of Daudi cells) FCS at 37°C in a humidified atmosphere, 5% $CO_2$. Daudi (American Type Cell Culture [ATCC] CCL213) is a human B-lymphoblast cell line of a Burkitt lymphoma, HL-60 (ATCC CRL-1964), a promyelocytic leukemia; Jurcat (ATCC TIB 152), an acute T-cell leukemia; K-562 (ATCC CCL 243), a chronic myeloid leukemia; MOLT-4 (ATCC CRL-1582), an acute T-lymphoblastic leukemia; and Reh (ATCC CRL-8286), a B-lymphoblast cell line of an acute lymphoid leukemia.

### Preparation of the Nuclear Matrix

Preparation of nuclear matrices was performed as described previously [Gerner et al., 1998b]. Briefly, cells were washed twice in HBSS and lysed by suspension in hypotonic buffer supplemented with 0.05% Nonidet P-40 (NP-40)(Sigma Chemical Co., St. Louis, MO). The nuclei were pelleted through a 300-mM sucrose cushion, resuspended in 1.5 M sucrose, sheared with a tight-fitting potter and centrifuged through a 2 M sucrose cushion. The pellet was resuspended in 1 M sucrose adjusted to 2 mM vanadyl ribonucleoside complex (Gibco-BRL, Gaithersburg, MD), and incubated for 3 min. Subsequently, the nuclei were treated with 0.2% sodium deoxycholate (Sigma) and 0.4% Tween 40 (Sigma). DNA was digested with 100 U/ml deoxyribonuclease I (DNase I, from bovine pan-

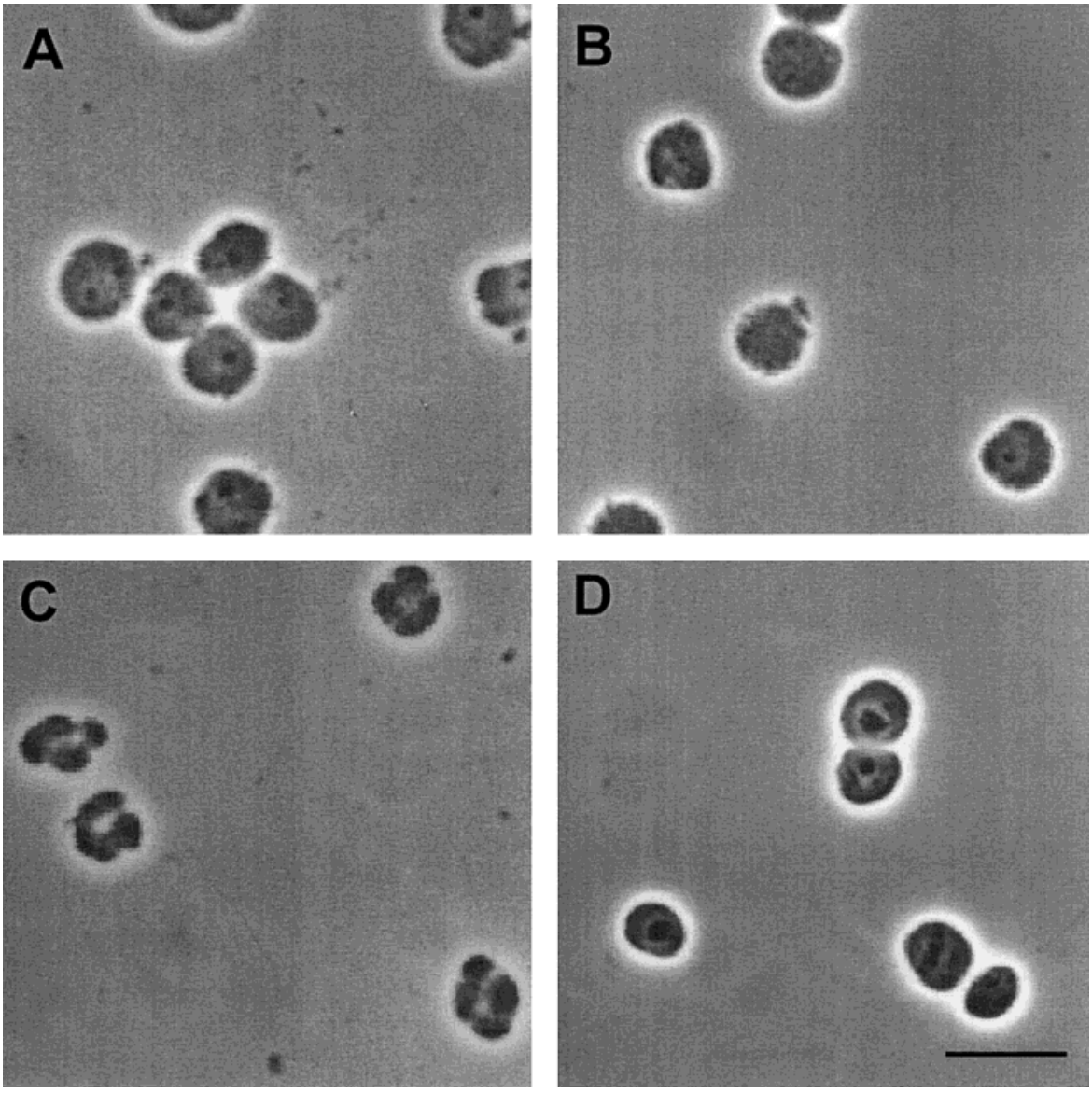


**Fig. 1.** Phase-contrast microscopy of nuclear matrices. **A:** Lymphocytes. **B:** Monocytes. **C:** Neutrophils. **D:** HL-60 cells. Scale bar = 10 μm.

creas, EC 3.1.21.1, Sigma) at 22°C for 30 min. After centrifugation, the residual nuclear pellet was extracted with 250 mM ammonium sulfate and washed, finally yielding the nuclear matrix.

### Two-Dimensional Gel Electrophoresis

High-resolution two-dimensional gel electrophoresis was carried out by the method of Hochstrasser et al. [1988], using the Protean II xi electrophoresis system (Bio-Rad, Richmond, CA) as described [Holzmann et al., 1997]. One-dimensional isoelectric focusing was performed at 15,500 V-h in 1.5-mm × 16-cm tube gels in a stepwise fashion (2 h at 200 V, 3 h at 500 V, 17 h at 800 V), using 4% acrylamide (Gerbu, Gaiberg, Germany)/0.1% piperazine di-acrylamide, 0.035% P-40, and 2% ampholytes (pH 3.5–10: pH 4–8: pH 5–7 = 1: 1: 2; Merck, Darmstadt, Germany) with degassed 20 mM NaOH as catholyte, and 6 mM $H_3PO_4$ as anolyte. The tube gels were placed on top of 1.5-mm 10% sodium dodecyl sulfate (SDS)-polyacrylamide slab gels. After 3-min equilibration with 3% SDS, 70 mM Tris base, 0.001% bromphenol blue, the second dimension was run at 15°C, using 0.1% SDS, 25 mM Tris base, and 200 mM glycin as electrode buffer [Laemmli, 1979]. Gels were silver stained

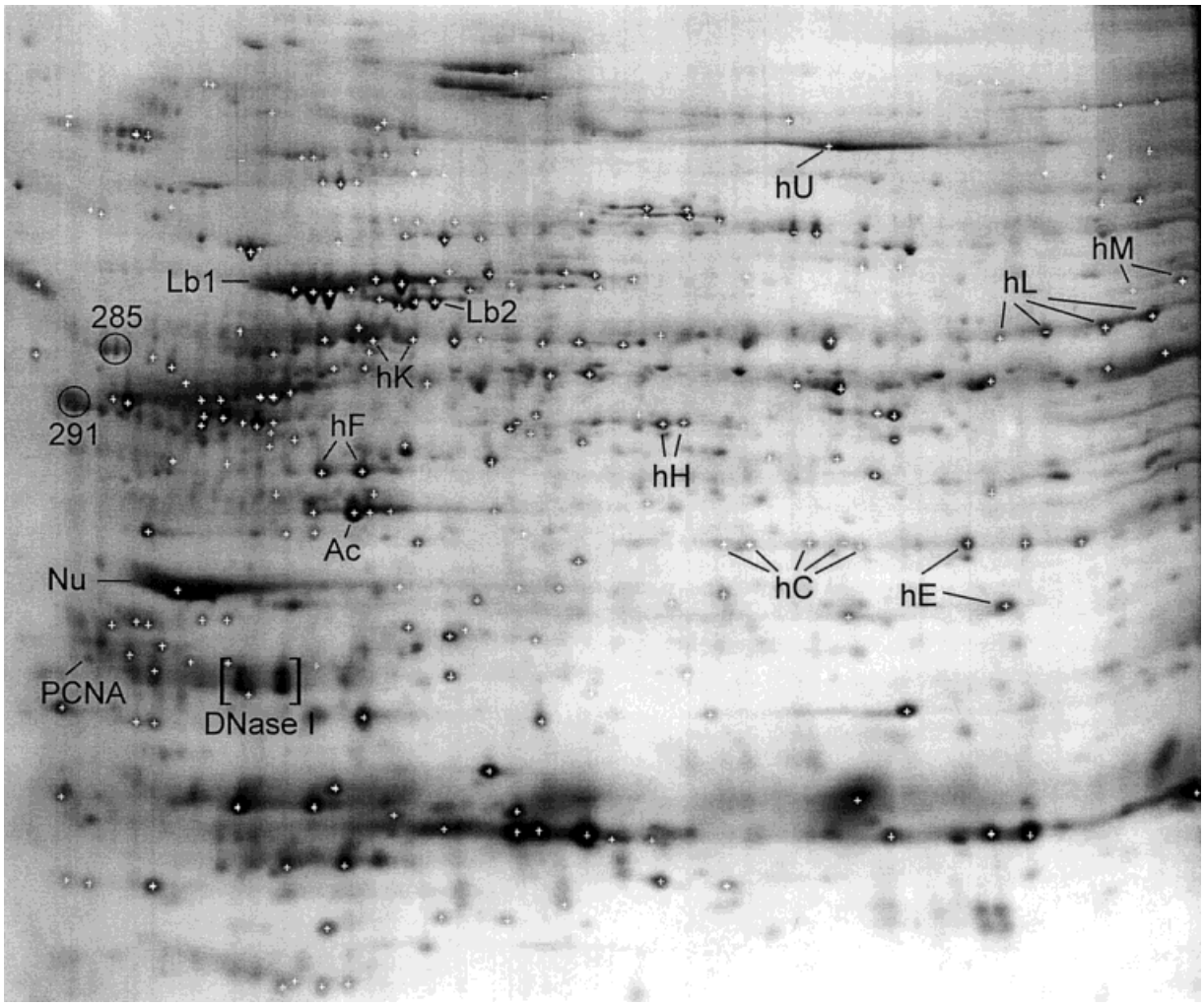


**Fig. 2.** Nuclear matrix proteins of human B lymphocytes. hNMP 291, leukocyte-specific; hNMP 285, lymphocyte-specific (Table I). Plus signs (+), nuclear matrix proteins also present in nonhematopoietic cells. 2D electrophoresis and protein identification as described under Materials and Methods. DNase I was added during preparation. Ac, actin; hC, hE, hF, hH, hK, hL, hM, hU, heterogeneous nuclear ribonucleoproteins C, E, F, H, K, L, M, U; Lb1, Lb2, nuclear lamins B1, B2; Nu, numatrin (B23); PCNA, proliferating cell nuclear antigen.

by the method of Wray et al. [1981]. Proteins detectable in this system are within the range of 20–240 kDa, pI 3.5–8.0.

### Identification of Protein Spots

The proteins annotated in Figure 2 were identified as described [Gerner et al., 1998b]. In short, hnRNP H, L and nuclear lamin B2 were identified by sequencing and matrix-assisted laser-desorption ionization-time-of-flight (MALDI-TOF) mass spectrometry. Actin, vimentin, nuclear lamins A, B1, C; hnRNPs F, H, L, U, and proliferating cell nuclear antigen (PCNA) were identified immunologically, hnRNPs C, E, K, and M were recognized by their pI/molecular mass and comparison with published nuclear matrix spot patterns [Mattern et al., 1996].

### Evaluation of Two-Dimensional Data

Scanning of gels, spot editing, and evaluation of data were accomplished with the BioImage Investigator system (BioImage, Ann Arbor, MI), using the 2-D Analyzer™ (V 6.1) software package. Gels were edited to define spots, spot boundaries and anchors as described [Korosec et al., 1997]. At least six independent nuclear matrix samples were prepared of each cell type. Three of the resulting six images were selected for computer analysis. The specific spots indicated in Figures 2–6 were found in at least six gels

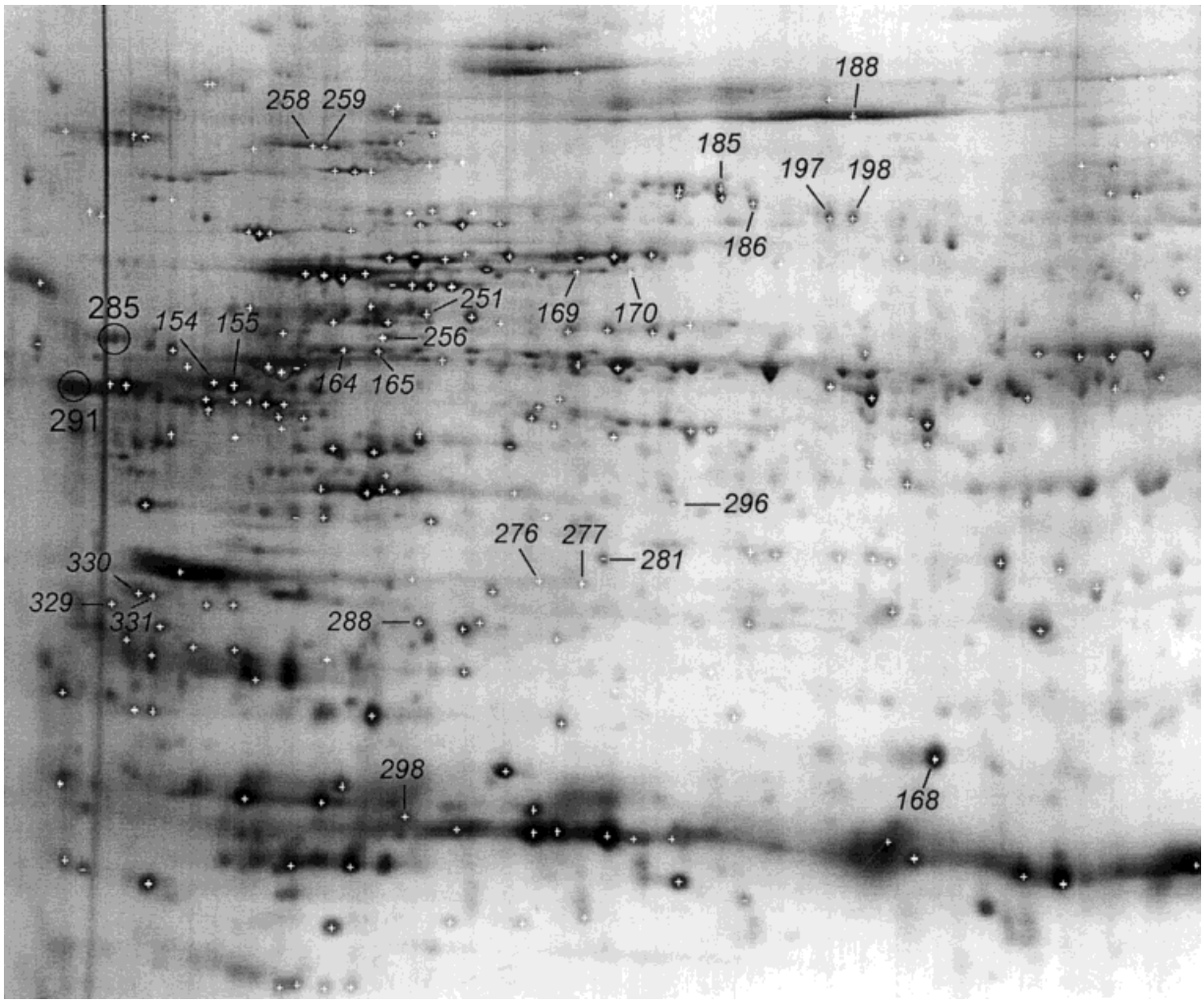


**Fig. 3.** Nuclear matrix proteins of human T lymphocytes. Spots missing in other leukocyte subtypes are annotated by their spot number. Plus signs mark nuclear matrix proteins also present in nonhematopoietic cells. Note that no characteristic difference was observed between the NM proteins of B and T lymphocytes.

per cell type. Spots marked by plus (+) signs in Figures 2–6 were found in lymphocytes and various other human cell types (not shown) and match with the spots, which have been described previously to occur in lymphocytes, UAC cells, and liver tissue [Gerner et al., 1998b]. The dendrogram (Fig. 7) was generated by the BioImage software, using the average linkage (UPGMA) method. The relative integrated spot intensities related to the total integrated spot intensities (%IOD) were determined, using the BioImage software. Of protein isoforms, the intensities were summarized. For calibration cellular proteins were used that are described in the human keratinocyte cell two-dimensional protein database [Celis et al., 1995]. The cells were kindly donated by Dr. J. Celis (Danish Centre for Human Genome Research, University of Aarhus, Denmark).

## RESULTS

To investigate nuclear matrix proteins of distinct subtypes of hematopoietic cells, B and T lymphocytes were isolated from inflamed palatine tonsils and the myeloid cells, monocytes and neutrophils, from peripheral blood. In addition, lymphoid and promyeloid leukemia cell lines were analyzed. From these cells, nuclear matrices were prepared by a recently described method [Gerner et al., 1998b]. As illustrated in Figure 1, morphological characteristics of nuclear structures were maintained during the preparation procedure. For example, nuclear matrices from neutrophils show the striking

lobular shape that is typical of nuclei of this cell type (Fig. 1C).

After separation of the nuclear matrix proteins by two-dimensional electrophoresis, the resulting spot patterns were analyzed with the aid of the BioImage Investigator system (see under Materials and Methods). The results of the comparative analysis are presented as follows. Spots shared by B and T lymphocytes and various other human cells (not shown) were marked by plus (+) signs. This applies to 240 spots in Figures 2 and 3. By contrast, nuclear matrix protein spots specific for individual types of cells were encircled (Figs. 2–6). Spots that were present in lymphocytes, but absent in myeloid cells were labeled by their spot numbers in Figure 3.

Their corresponding position in Figures 4 and 5 was indicated by open squares.

As summarized in Tables I and II, the subtypes of the hematopoietic cells investigated displayed distinct nuclear matrix proteins, and were characterized by the presence and absence of a number of individual spots.

### Spots Characteristic for Leukocytes, Lymphocytes, and Myeloid Cells

Remarkably, one spot (spot hNMP 291, Figs. 2–5, Table IA) was detected that occurred in B and T lymphocytes, monocytes and neutrophils, but not in the nonhematopoietic cells investigated in this laboratory. These controls included various normal and tumor tissues and epithelial, endothelial, and neuronal cell lines

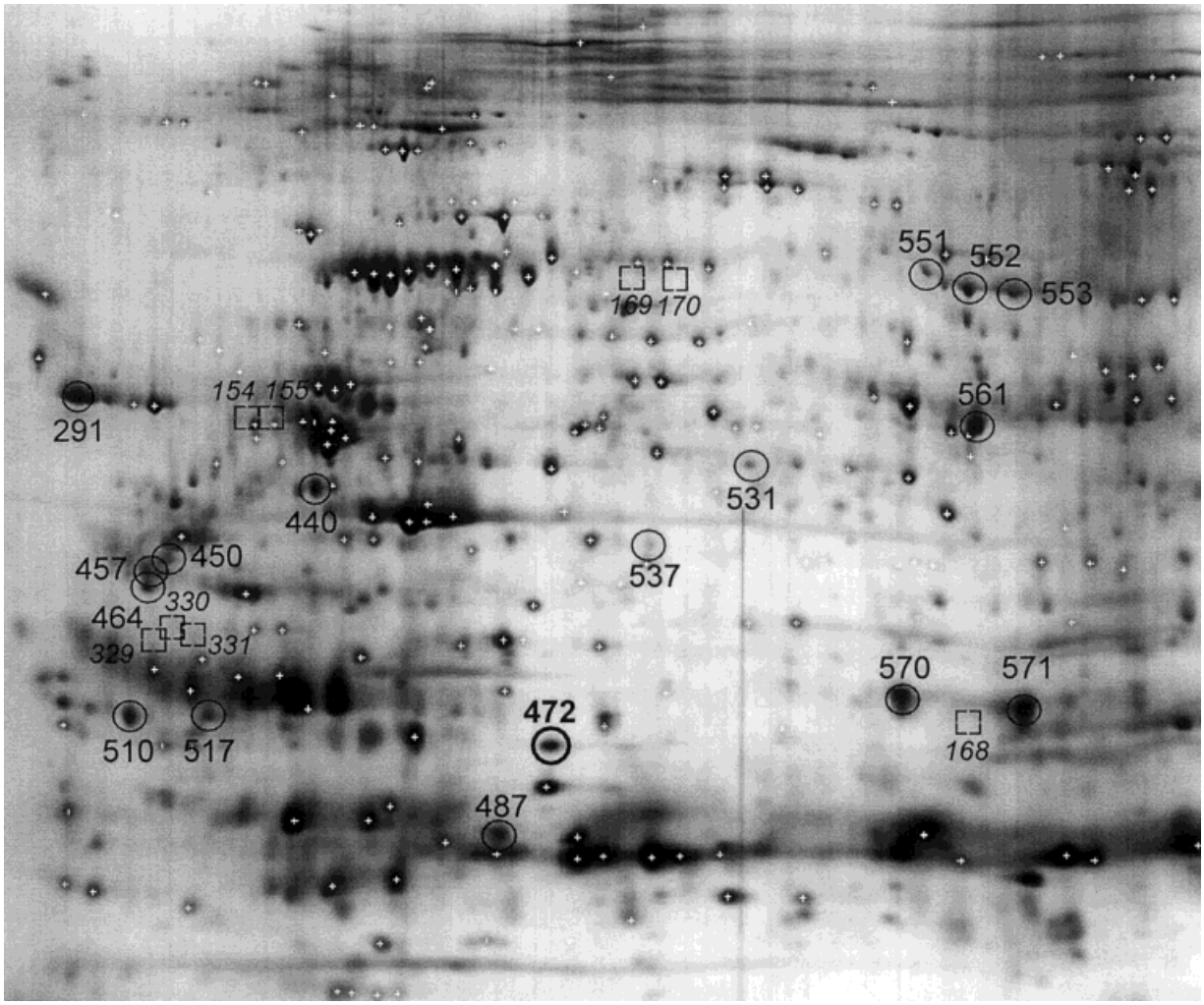


**Fig. 4.** Nuclear matrix proteins of human monocytes. hNMP 472, specific for monocytes. The other encircled spots occurred exclusively in myeloid cells. Squares, absence of spots present in the other cells investigated (Tables I, II). Plus signs (+), nuclear matrix proteins also present in nonhematopoietic cells.

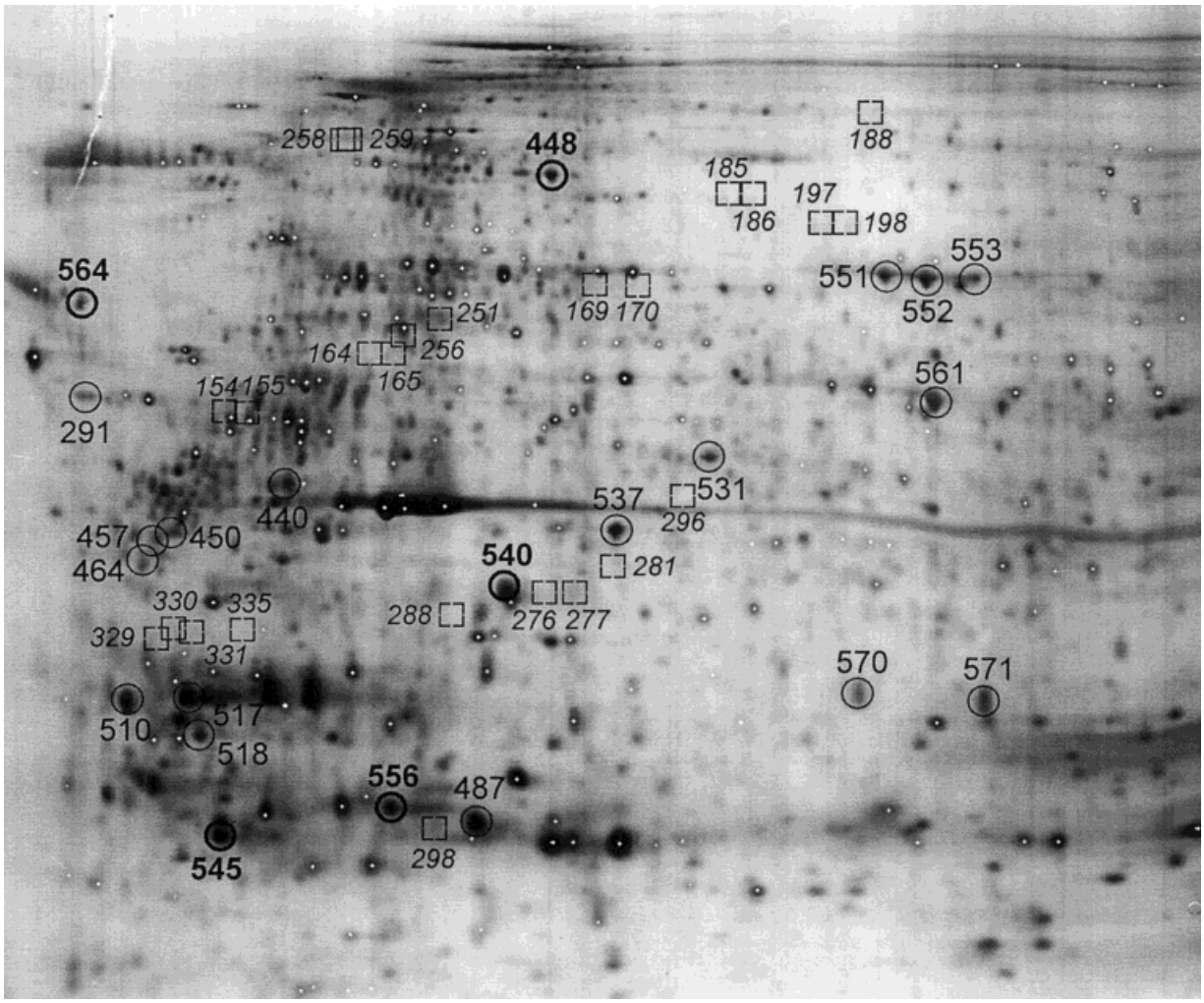


**Fig. 5.** Nuclear matrix proteins of human neutrophils. hNMP 448, 540, 545, 556, 564, specific for neutrophils. The other encircled spots occurred exclusively in myeloid cells. Squares indicate the absence of spots present in the other cells investigated (Tables I, II). Plus signs (+), nuclear matrix proteins, also present in nonhematopoietic cells.

(not shown). Thus, hNMP 291 imposes as a nuclear matrix protein specific for nucleated hematopoietic cells.

Furthermore, a spot was present in nuclear matrices of B and T lymphocytes, but missing in nuclear matrices of myeloid cells and nonhematopoietic cells (hNMP-285, Figs. 2 and 3, Table IB). Spot hNMP 285 apparently represents a lymphocyte specific protein. However, no significant difference between the nuclear matrix protein composition of B and T lymphocytes was detected.

Fifteen spots occurred only in monocytes and neutrophils, but not in other human cells (Figs. 4 and 5, Table IC,D). These spots appeared to be group specific for myeloid cells. In addition, it was observed that seven spots were missing in the myeloid cells (Figs. 4 and 5, Table IE), spots that were present in other human cells investigated. Their positions and spot numbers are depicted in Figure 3.

### Nuclear Matrix Proteins Specific for Monocytes and Neutrophils

Within the group of myeloid cells, monocytes were characterized by alteration of two nuclear matrix proteins. One spot occurred in monocytes, but not in other cell types (hNMP 472, Fig. 4, Table IIA). By contrast, one spot was missing in monocytes, which occurred in various other human cells (hNMP 168, Fig. 4, Table IIB).

The nuclear matrix protein pattern of neutrophils displayed great particularities. In spe-

**TABLE I. Nuclear Matrix Proteins Characteristic of Hematopoietic Cells, Lymphocytes, and Myeloid Cells**

| | Spots | | | Occurrence in cells | | | | |
|---|---|---|---|---|---|---|---|---|
| | hNMP no. | Mw (kDa) | pI | Lymphocytes | Monocytes | Neutrophils | HL-60, K-562 | Nonhematopoietic |
| Leukocytes | | | | | | | | |
| A | 291 | 50 | 4.20 | + | + | + | − | − |
| Lymphocytes | | | | | | | | |
| B | 285 | 58 | 4.40 | + | − | − | + | − |
| Myeloid cells | | | | | | | | |
| C | 552 | 65 | 6.50 | − | + | + | + | − |
| | 553 | 65 | 6.65 | − | + | + | + | − |
| D | 440 | 46 | 4.95 | − | + | + | − | − |
| | 450 | 41 | 4.45 | − | + | + | − | − |
| | 457 | 40 | 4.40 | − | + | + | − | − |
| | 464 | 39 | 4.40 | − | + | + | − | − |
| | 487 | 28 | 5.45 | − | + | + | − | − |
| | 510 | 32 | 4.30 | − | + | + | − | − |
| | 517 | 32 | 4.55 | − | + | + | − | − |
| | 531 | 48 | 5.90 | − | + | + | − | − |
| | 537 | 41 | 5.65 | − | + | + | − | − |
| | 551 | 65 | 6.40 | − | + | + | − | − |
| | 561 | 56 | 6.55 | − | + | + | − | − |
| | 570 | 35 | 6.35 | − | + | + | − | − |
| | 571 | 35 | 6.70 | − | + | + | − | − |
| E | 154 | 52 | 4.75 | + | − | − | + | + |
| | 155 | 52 | 4.80 | + | − | − | + | + |
| | 169 | 66 | 5.60 | + | − | − | + | + |
| | 170 | 66 | 5.70 | + | − | − | + | + |
| | 329 | 36 | 4.45 | + | − | − | + | +/−[a] |
| | 330 | 36 | 4.50 | + | − | − | + | +/−[a] |
| | 331 | 36 | 4.55 | + | − | − | + | +/−[a] |

[a]Proliferation dependent.

cific, six spots were found that did not occur in the nuclear matrix of any other cell type (Fig. 5, Table IIC). On the other hand, 18 nuclear matrix protein spots shared by various other cells were absent (Fig. 5, Table IID).

Thus, the nuclear matrix protein pattern of the myeloid cells, and especially of the neutrophils, is very exceptional. In none of the other human cells investigated in our laboratory were such characteristic alterations from the general pattern observed. This may signify the high degree of differentiation and functional specialization of these cells.

### Nuclear Matrix Proteins of Cultured Leukemia Cells

To detect potential alteration of nuclear matrix proteins in malignant cells, proteins of two lines of B- lymphoid leukemias (Daudi, Reh), two lines of T-lymphoid leukemias (MOLT-4 and Jurkat), and two lines of promyeloid leukemias (HL-60 and K-562) were analyzed.

As observed with normal lymphocytes, no characteristic difference between B and T lymphoid leukemia subtypes was detectable. In all four lymphoid leukemia cell lines, the abovementioned spot hNMP-285 characteristic for lymphocytes was present. However, the protein spot hNMP 291 characteristic for normal hematopoietic cells was absent in the leukemia cell lines.

In the promyelocytic HL-60 and K-562 cells, indeed some of the nuclear matrix proteins characteristic for myeloid cells (hNMP 552 and 553) and neutrophils (hNMP 518) were found (Fig. 6). Surprisingly, however, hNMP 285 characteristic for lymphocytes was also detected (Fig. 6). Two spots (hNMP 557 at 30 kDa, pI 5.30; and hNMP 560 at 56 kDa, pI 6.50) were found that did not occur in any other cells.

**TABLE II. Nuclear Matrix Proteins Differentiating Monocytes and Neutrophils**

| | Spots | | | Occurrence in cells | | | | |
|---|---|---|---|---|---|---|---|---|
| | hNMP no. | Mw (kDa) | pI | Lymphocytes | Monocytes | Neutrophils | HL-60, K-562 | Nonhematopoietic |
| Monocytes | | | | | | | | |
| A | 472 | 33 | 5.50 | − | + | − | − | − |
| B | 168 | 33 | 6.55 | + | − | + | + | + |
| Neutrophils | | | | | | | | |
| C | 448 | 99 | 5.55 | − | − | + | − | − |
| | 518 | 31 | 4.65 | − | − | + | + | − |
| | 540 | 38 | 5.45 | − | − | + | − | − |
| | 545 | 27 | 4.75 | − | − | + | − | − |
| | 556 | 29 | 5.20 | − | − | + | − | − |
| | 564 | 62 | 4.10 | − | − | + | − | − |
| D | 164 | 56 | 5.15 | + | + | − | + | + |
| | 165 | 56 | 5.20 | + | + | − | + | + |
| | 185 | 93 | 5.90 | + | + | − | + | + |
| | 186 | 90 | 6.00 | + | + | − | + | + |
| | 188 | 120 | 6.35 | + | + | − | + | + |
| | 197 | 87 | 6.20 | + | + | − | + | + |
| | 198 | 87 | 6.30 | + | + | − | + | + |
| | 251 | 60 | 5.35 | + | + | − | + | + |
| | 258 | 110 | 5.10 | + | + | − | + | + |
| | 259 | 110 | 5.12 | + | + | − | + | + |
| | 265 | 58 | 5.20 | + | + | − | + | + |
| | 276 | 38 | 5.50 | + | + | − | + | + |
| | 277 | 38 | 5.55 | + | + | − | + | + |
| | 281 | 39 | 5.65 | + | + | − | + | + |
| | 288 | 37 | 5.35 | + | + | − | + | + |
| | 296 | 44 | 5.85 | + | + | − | + | + |
| | 298 | 27 | 5.35 | + | + | − | + | + |
| | 335 | 36 | 4.80 | + | + | − | − | + |

Whether these spots represent nuclear matrix proteins specific for promyeloid leukemia cells in general has to be further established. Individually differing, some of the generally occurring spots were missing in the leukemia cell lines (not shown).

### Nuclear Matrix Proteins Indicating Cell Proliferation

The cells analyzed in this study differed by their state of proliferation. While cultured cells were proliferating, as parts of a tonsillar lymphocyte population, monocytes and neutrophils were nondividing [Metcalf, 1989]. This allowed to discriminate nuclear matrix proteins potentially correlated with cell proliferation. In accordance with previous observations [Feuerstein and Mond, 1987; Gerner et al., 1998b], numatrin (B23) concentrations were low in the nondividing myeloid cells, especially in neutrophils, and PCNA absent (Figs. 3–6). Furthermore, a spot was found with relatively high concentration in dividing cells, which according to its position in the 2D gels appears to be identical with the proliferation-specific nuclear matrix protein mitotin (hNMP 356, 125 kDa, pI 5.5–6.0; Fig. 6) [Zhelev et al., 1990]. A new finding was that three spots occurred at high concentrations in proliferating cells and at low concentrations in slowly dividing cells, which were absent in the nonreplicating myeloid cells (hNMP 329, 330, and 331; Figs. 3–6, Table I). Moreover, these proteins (numatrin, PCNA, mitotin, hNMP 329, 330, and 331) were strongly induced in phytohemagglutinin- stimulated T lymphocytes (not shown). Recently, nuclear matrix proteins have been described as being specific for the proliferative and the quiescent state of lymphocytes [Estanol et al., 1997]. According to our experience, however, the first are residu-

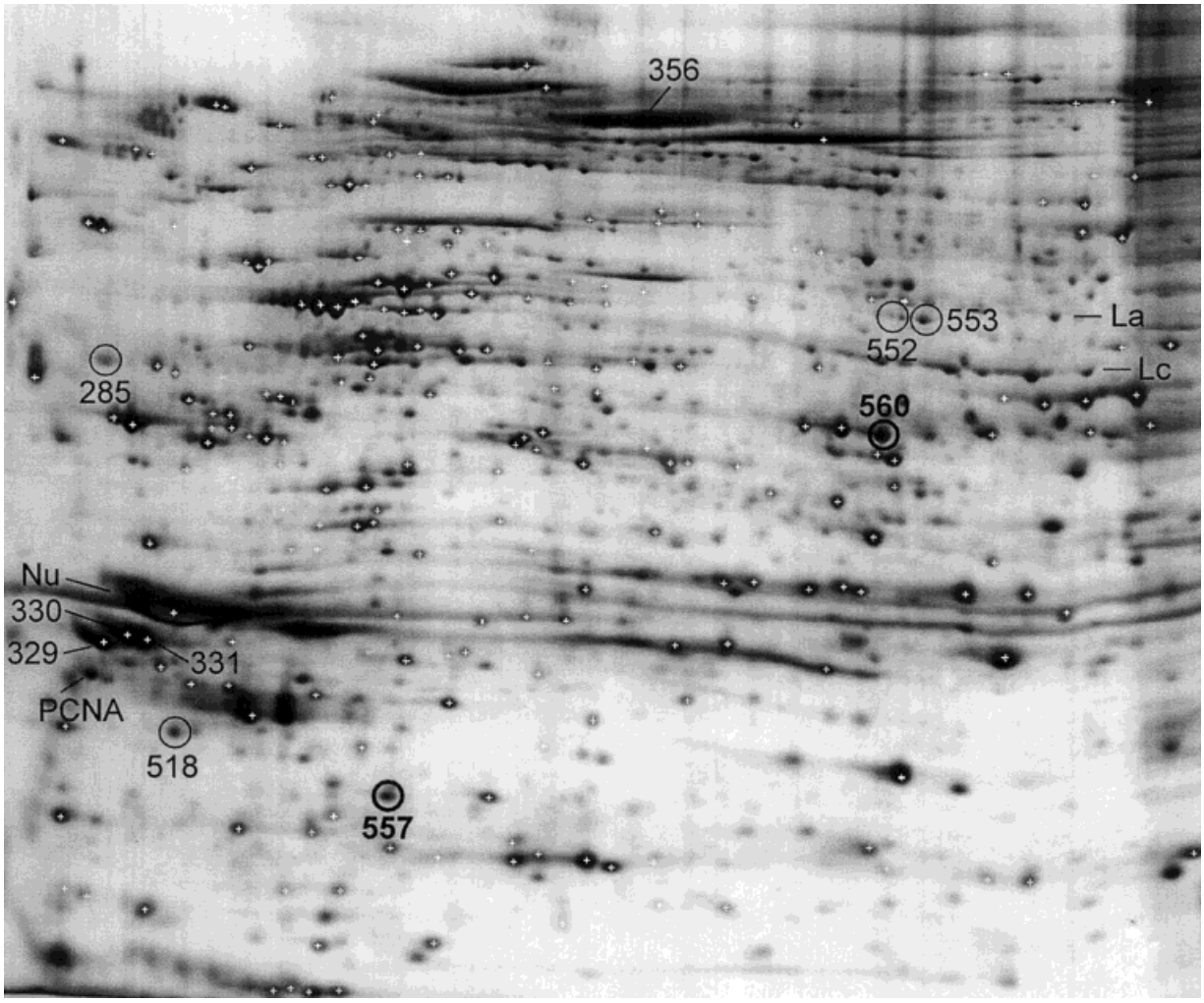


**Fig. 6.** Nuclear matrix proteins of human HL-60 cells. hNMP 557, 560, specific for HL-60 and K-562 cells. Other encircled spots were found to occur exclusively in myeloid cells. High concentrations of hNMPs 329, 330, 331, numatrin (B23), PCNA, and mitotin (hNMP 356) correlated with proliferation. La, Lc; nuclear lamins A, C; Nu, numatrin (B23); PCNA, proliferating cell nuclear antigen. Plus signs (+), nuclear matrix proteins also present in nonhematopoietic cells.

als of the mitogen phytohemagglutinin, whereas some of the latter proteins originate from contaminating monocytes (data not shown).

### Relation of the Hematopoietic Cells by Comparison of Their Nuclear Matrix Proteins

As demonstrated above, a characteristic set of nuclear matrix proteins was determined for each cell type. Computer-assisted image analysis of the 2D electropherograms allowed to generate a dendrogram visualizing the similarity of the protein patterns (Fig. 7). According to this scheme, the resulting relation between cells is similar to the relation resulting from differentiation studies, otherwise determined with the aid of the cluster of differentiation (CD) surface antigens. However, an exception is posed by the myeloid leukemia cell lines, also differing by their nuclear morphology from normal myeloid cells.

### hnRNPs and Nuclear Lamins

Heterogeneous nuclear ribonucleoproteins (hnRNPs) have been found to play a role in pre-mRNA processing, mRNA transport and in the interaction of hnRNA with nuclear structures [Dreyfuss et al., 1993; Weighardt et al., 1996]. The hnRNP content of cells and isolated nuclear matrices has been described to vary depending on the cell and tissue type [Fey and

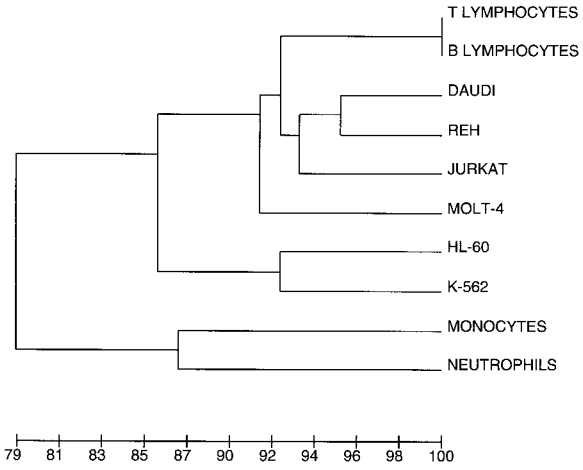


**Fig. 7.** Dendrogram relating hematopoietic cells by similarity of their nuclear matrix protein patterns. Daudi and Reh, B-lymphoid leukemias; MOLT-4 and Jurkat, T- lymphoid leukemias; HL-60 and K-562, promyeloid leukemias. See under Leukemia Cell Lines, for further description.

Penman, 1988; Faura et al., 1995; Kamma et al., 1995; Holzmann et al., 1997]. Therefore, it was of interest to measure the hnRNP content in the present nuclear matrix samples. Figure 8 shows the relative integrated spot intensities of hnRNPs C, E, F, H, K, L, M, and U, while Figure 2 indicates their position in the 2D gels. Remarkably, the concentration of hnRNPs, especially of hnRNPs C, E, F, and K, was low in monocytes and neutrophils. hnRNP-U (hNMP-188), which has also been attributed the function of DNA binding protein designated scaffold attachment factor A [Göhring et al., 1997] was missing entirely in neutrophils. On the other hand, in cultured proliferating HL-60 cells, the hnRNP concentrations were very high, with hnRNP K, E, and C concentrations exceeding 5- to 15-fold those of nondividing myeloid cells.

The nuclear lamins A/C and B1/B2, underlying the inner surface of the nuclear envelope [Gerace and Burke, 1988], are known constituents of the nuclear matrix, appearing in 2D gels as trains of spots differentially modified by phosphorylation and ADP-ribosylation. In the present experiments, it was observed that the nuclear lamin A/C concentrations were high in promyelocytic leukemia cells, very low in lymphocytes, and under the detection limit in myeloid cells and cultured leukemia cells [Rober et al., 1990]. Remarkably, nuclear lamin B1/B2 concentrations were low in neutrophils, while being high, at comparable levels, in the other cells investigated.

## DISCUSSION

Although the leukocyte subtypes descend from one species of stem cells, their function and morphology greatly differs [for review, see Quesenberry, 1990]. As shown, these cells may be differentiated by the presence or absence of about 50 distinct nuclear matrix protein spots. This finding is remarkable, when comparing the data with those of other cell types, where less differences between the respective nuclear matrix protein compositions were observed. This results from our ongoing studies on nuclear matrix proteins of various human normal and tumor cell species, including those of bladder, kidney, liver, lung, the nervous system, prostate, spleen, testes, and thyroid, and various cell lines.

Evidently, great differences between the nuclear matrix proteins of lymphocytes on one side and myeloid cell on the other side are paralleled by significant differences in the nuclear morphology of the cells. Though suggestive, it remains further to be investigated whether or not the presence and the absence of some of the described nuclear matrix proteins is causally related with nuclear architecture [see also Nickerson et al., 1995]. In this context, it should be mentioned that significant alterations of nuclear matrix proteins have been observed after induction of apoptosis, a process greatly affecting the organization of the nucleus [Gerner et al., 1998a]. Considering the role that has been attributed to these proteins as structural elements of the nuclear matrix [Moir et al., 1995; Weighardt et al., 1996], the presently observed lack of nuclear lamins A/C, the low amounts of nuclear lamins B1/B2 and the decreased concentration of hnRNPs in neutrophils may relate with the nuclear morphology of these cells, also evidenced by the observed fragility of the isolated nuclei.

Confirming previous observations [Feuerstein and Mond, 1987; Zhelev et al., 1990; Gerner et al., 1998b], increased levels of PCNA, numatrin (B23), and mitotin were found in the nuclear matrix of dividing cells. In addition, three new nuclear matrix protein spots were detected that appeared in high concentrations in proliferating cells (hNMP 329, 330, and 331). It remains to be investigated whether these proteins represent general markers for cell proliferation.

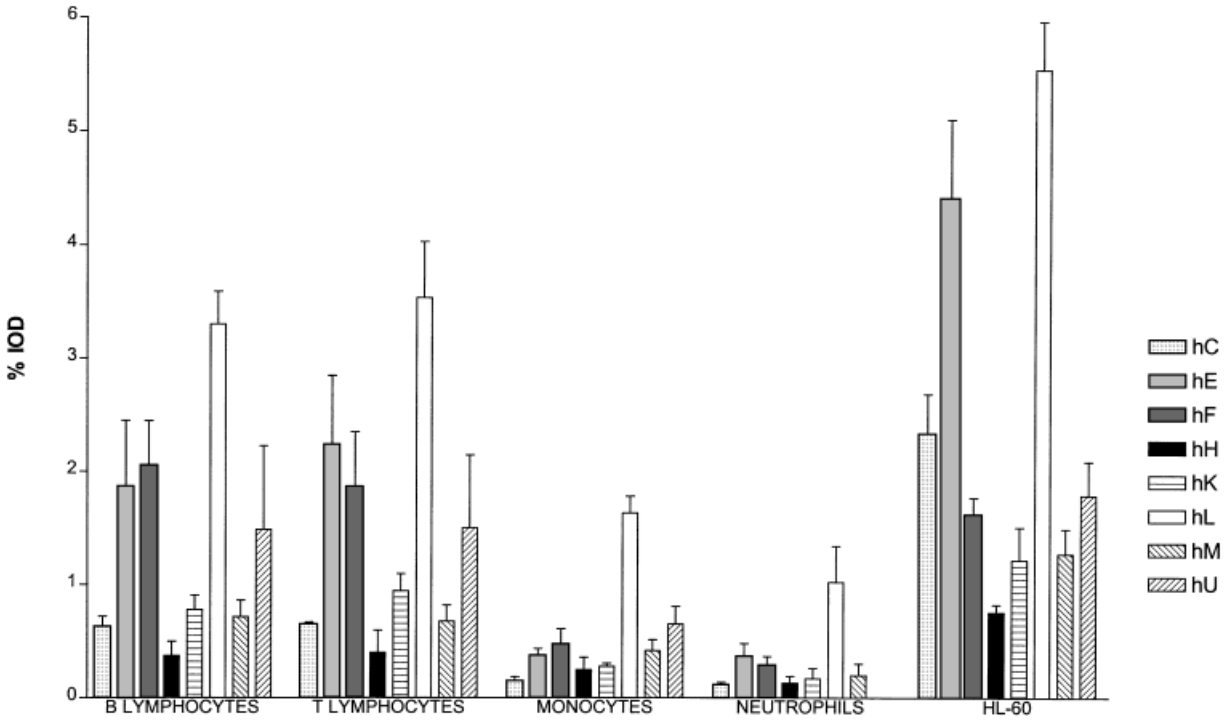


**Fig. 8.** Relative amounts of hnRNP proteins in human leukocyte nuclear matrix preparations. The relative integrated spot intensities (%IOD) were determined. Results ±SEM of three independent experiments. hC, hE, hF, hH, hK, hL, hM, hU, heterogeneous nuclear ribonucleoproteins C, E, F, H, K, L, M, U.

In practice, analysis of nuclear matrix proteins by 2D electrophoresis permitted detection of the presence of hematopoietic cells in isolated tissue samples. Thus, invasion of tumor tissues by myeloid cells, and contributions of myeloid cells to the spleen, were apparent. Evidently, the presently described nuclear matrix proteins may serve as new protein markers for hematopoietic cells.

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