Types of Nuclear Endonuclease Activity Capable of Inducing Internucleosomal DNA Fragmentation Are Completely Different Between Human CD34+ Cells and Their Granulocytic Descendants

By Naoyuki Anzai, Hiroshi Kawabata, Toshiyasu Hirama, Hiroshi Masutani, Yasunori Ueda, Yataro Yoshida, and Minoru Okuma

A hallmark of apoptosis is internucleosomal DNA fragmentation resulting from the activation of endonucleases. We characterized the endonuclease activity of human myeloid cell nuclei that cleaved their own nuclear chromatin to oligonucleosomal length fragments. Polymorphonuclear leukocytes (PMNs) of normal peripheral blood contained both Ca2+/Mg2+-dependent and DNase II-like acidic endonuclease activities in their nuclei. Immature myeloid cells of normal bone marrow at various stages of granulocytic maturation had similar nuclease activities. In contrast, a clear difference was shown in the circulating CD34+ cells, in that only Mg2+-dependent, Ca2+-independent endonuclease activity was detected. Consistent with these findings is the emergence of the Ca2+/Mg2+-dependent and acidic endonuclease concomitantly with the disappearance of the Mg2+-dependent endonuclease when CD34+ cells were induced to differentiate in vitro toward granulocytes. Leukemic cell lines of all lineages also had Mg2+-dependent nuclease activity. Our results suggest an association of the Mg2+-dependent endonuclease with hematopoietic progenitor cells and that the relative activities of the nuclear nuclease in human myeloid cells change substantially during granulocytic differentiation.

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A POPTOSIS IS A common mode of cell death and is activated under many physiologic conditions.1 With respect to hematopoiesis, hematopoietic factors provide signals for proliferation and differentiation, and deprivation of these factors causes apoptosis of cells, the survival of which is dependent on hematopoietic factors.2,3 Thus, apoptosis is believed to play a major role in controlling the size of cell populations.3 Furthermore, apoptosis has been implicated in the elimination of senescent terminally differentiated hematopoietic cells, such as aging neutrophils.6,7

Activation of endonuclease(s) is believed to be a critical event in the apoptotic process,8 and several candidates have been proposed.9-14 However, no direct evidence of the participation of these endonucleases in apoptosis has been presented to date. In addition, characterization and purification of these endonucleases have been attempted mainly in lymphoid cells, but rarely in myeloid cells. Human leukemic HL-60 cells have been reported to contain a Ca2+/Mg2+-dependent endonuclease.15,16 We have previously shown the presence of a Mg2+-dependent, Ca2+-independent endonuclease in myeloid leukemia cell nuclei.17 Assuming that apoptosis occurs without new protein synthesis in myeloid cells,18 identification of constitutive endonucleases is important in elucidating the mechanism of endonuclease activation. In this study, we examine the characteristics of nuclear endonucleases in myeloid cells at various stages of granulocytic differentiation. We show that types of nuclear endonuclease activity capable of producing DNA fragmentation characteristic of apoptosis differ between progenitor cells and differentiated cells.

MATERIALS AND METHODS

Reagents

All chemicals were obtained from Nacalai Tesque (Kyoto, Japan), except where otherwise indicated.

Cell Separation

Polymorphonuclear leukocytes (PMNs). Peripheral blood was obtained from healthy volunteers. PMNs were isolated by dextran sedimentation followed by centrifugation over a Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient. Contaminating red blood cells were eliminated by hypotonic lysis. The percentages of PMNs were above 95% on cytocentrifuge cell preparations.

Imature myeloid cells. Bone marrow cells were obtained from hematologically normal patients with informed consent. Light-density mononuclear cells were depleted of adherent cells by incubation in RPM1 1640 medium (Nikken Bio Laboratory, Kyoto, Japan) supplemented with 10% fetal calf serum (FCS; Irvine Scientific, Santa Ana, CA) in plastic dishes at 37°C for 2 hours. Then, the cells were incubated with monoclonal antibodies (MoAbs) CD3, CD11b, CD14, CD19, and CD56 (UCHT1, 44, C7, B-C3, and B-A19, respectively; from Serotec, Oxford, UK); CD11d (Leu-11b; Becton Dickinson, San Jose, CA); and glycoporphin A (JC159; Dako A/S, Glostrup, Denmark) in phosphate-buffered saline (PBS; Nikken Bio Laboratory) containing 5% FCS for 30 minutes at 4°C. After three washes, the labeled cells were removed by incubation in PBS containing 5% FCS once in plastic dishes coated with goat IgG to mouse Igs (Cappel, Durham, NC) and once with immunomagnetic beads (Dynabeads M-450 Tosylactivated; Dynal, Oslo, Norway) coated with the same goat IgG at 4°C for 1 hour, respectively. The resulting antibody-negative fractions were used as immature myeloid cells. The cellular compositions of these fractions were largely (at least 80%) granulocytic cells at various stages of maturation from myeloblasts to metamyelocytes.

CD34+ cells. Peripheral blood cells were collected by leukapheresis after informed consent from patients with leukemia in complete remission. Light-density mononuclear cells were isolated by Ficoll-Paque gradient centrifugation. Adherent cells were removed by two cycles of plastic adherence. The cells were incubated in IgG-coated PBS (Nikken Bio Laboratory) containing 5% FCS for 30 minutes at 4°C. After three washes, the labeled cells were removed by incubation in PBS containing 5% FCS once in plastic dishes coated with goat IgG to mouse Igs (Cappel, Durham, NC) and once with immunomagnetic beads (Dynabeads M-450 Tosylactivated; Dynal, Oslo, Norway) coated with the same goat IgG at 4°C for 1 hour, respectively. The resulting antibody-negative fractions were used as immature myeloid cells. The cellular compositions of these fractions were largely (at least 80%) granulocytic cells at various stages of maturation from myeloblasts to metamyelocytes.

From the First Division, Department of Medicine, Faculty of Medicine, Kyoto University, Saky, Kyoto, Japan.

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Address reprint requests to Naoyuki Anzai, MD, First Division, Department of Medicine, Faculty of Medicine, Kyoto University, Saky, Kyoto 606, Japan.

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CD11b, CD14, CD16, CD19, CD56, CD57 (Leu-7; Becton Dickinson), and glycophorin A. Negative selection was performed using IgG-coated dishes and immunomagnetic beads. Finally, the cells were incubated with CD34-coated immunomagnetic beads (Dynabeads M-450 CD34; Dynal) for 1 hour at 4°C. To evaluate the percentage of CD34⁺ cells, portions of microsphere-rosetting cells were treated with 100 U/mL chymopapain (Sigma Chemical Co, St Louis, MO) at 37°C for 30 minutes. Then, the microsphere-free cells were labeled with CD34 (Tük-3; Dako A/S) MoAb and with fluorescein isothiocyanate (FITC)-conjugated F(ab')₂ fragment of rabbit antimouse IgGs (Dako A/S). The labeled cells were analyzed by FACScan (Becton Dickinson).

Culture of CD34⁺ Cells

Isolated CD34⁺ cells were cultured in Iscove's modified Dulbecco's medium (Gibco, Grand Island, NY) supplemented with 30% FCS at 37°C in a humidified 5% CO₂ atmosphere. At initiation and every 48 hours thereafter, cultures received 100 U/mL interleukin (IL)-3 (Krin Angen, Tokyo, Japan), 50 ng/mL granulocyte colony-stimulating factor (G-CSF; Chugai, Tokyo, Japan), and 50 ng/mL stem cell factor (Krin Angen). The differentiation of CD34⁺ cells was assayed by FACScan for the expression of the following surface markers: CD13 (MCS-2; Nichirei, Tokyo, Japan), CD14, CD15 (Leu-M-1; Becton Dickinson), CD33 (VM-54; Dakopatts), and CD34.

Cell Lines

HL-60, a promyelocytic leukemia cell line; KG-1, a myeloblastic leukemia cell line; K562, a chronic myelogenous leukemia cell line; THP-1 and U937, monocytoïd cell lines; HEL, an erythroleukemia cell line; P39, a myelomonocytoid cell line; Raji, a Burkitt's lymphoma cell line; RPMI-8226, a myeloma cell line; LC4-1, a B-cell lymphoma cell line; and Jurkat and Molt-4, T-lymphoblastoid cell lines were kindly provided by Drs. K. Tohyama (Fukui Medical School, Fukui, Japan), respectively. The medium required for the cell lines was supplemented with 0.6 mmol/L NaCl, 10 mmo/L CaCl₂, 5 mmo/L MgCl₂, 1 mmol/L dithiothreitol (DTT); buffer 2: 10 mmo/L TRIS-HCl pH 7.6, 1 mmo/L MgCl₂, 2 mmo/L EGTA, 1 mmo/L EDTA; buffer 3: 50 mmo/L bis-TRIS-HCl pH 6.0, 5 mmo/L EDTA, 1 mmo/L EDTA. Buffer conditions below 0.1 mU of MN, and the single-nucleosomal size area (area S) containing DNA smaller than 300 bp. We then calculated the integration of the intensity of each area (IL, IM, and IS, respectively). The reaction volume was increased to 100 μL, and the reaction was terminated by adding 5 μL of 0.5 mmo/L EDTA pH 8.0, 2 μL of 10% sodium dodecyl sulfate, and 10 μL of 20 mg/mL proteinase K. After incubation of the mixture at 50°C for 1 hour, the samples were precipitated with an equal volume of 2 mmo/L sodium iodide and 2 vol of 2-propanol. After centrifugation at 14,000g for 15 minutes and rinsing with 70% ethanol, the pellets were dissolved in 10 mmo/L TRIS-HCl pH 8.0, 1 mmo/L EDTA containing 1 μg/mL of RNase A. The samples were incubated at 37°C for 1 hour, and the DNA concentration was estimated by measuring the optical density (OD) at 260 nm. DNA (2 μg per lane) was electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining, and the gels were photographed under UV illumination.

Quantitative Analysis of Nucleosome Activities

The method for quantitative analysis of the degree of nucleosomal-size DNA fragmentation has been described elsewhere. Briefly, each lane of the electrophoretic image was divided into three areas: the large-molecular-size area (area L) containing DNA larger than 10 kbp, the middle-molecular-size area (area M) containing DNA between the sizes of 10 kbp and 300 bp, and the single-nucleosomal-size area (area S) containing DNA smaller than 300 bp. We then calculated the intensity of each area (IL, IM, and IS, respectively). The reaction volume was increased to 100 μL, and the reaction was terminated by adding 5 μL of 0.5 mmo/L EDTA pH 8.0, 2 μL of 10% sodium dodecyl sulfate, and 10 μL of 20 mg/mL proteinase K. After incubation of the mixture at 50°C for 1 hour, the samples were precipitated with an equal volume of 2 mmo/L sodium iodide and 2 vol of 2-propanol. After centrifugation at 14,000g for 15 minutes and rinsing with 70% ethanol, the pellets were dissolved in 10 mmo/L TRIS-HCl pH 8.0, 1 mmo/L EDTA containing 1 μg/mL of RNase A. The samples were incubated at 37°C for 1 hour, and the DNA concentration was estimated by measuring the optical density (OD) at 260 nm. DNA (2 μg per lane) was electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining, and the gels were photographed under UV illumination.

Acid Phosphatase Measurement

The nuclei pellets were incubated in 10 mmo/L TRIS-HCl pH 7.5, 0.6 mmo/L NaCl, and 0.2% NP-40 for 1 hour on a rotating platform and centrifuged at 183,000g for 1 hour at 4°C. The acid phosphatase activity of the supernatants was measured using a commercially available kit (Wako, Osaka, Japan), based on the Bessey-Lowry method using p-nitrophenyl phosphate as a substrate.

Autodigestion

The isolated nuclei were incubated in digestion buffer at 37°C for 16 hours. The incubation buffers for determining the activities of Ca²⁺/Mg²⁺-dependent, Mg²⁺-independent, and acidic endonucleases, respectively, were as follows: buffer 1: 10 mmo/L TRIS-HCl pH 7.6, 10 mmo/L CaCl₂, 5 mmo/L MgCl₂, 1 mmo/L dithiothreitol (DTT); buffer 2: 10 mmo/L TRIS-HCl pH 7.6, 1 mmo/L MgCl₂, 2 mmo/L EGTA, 1 mmo/L EDTA (this condition was determined previously using P39 cells); and buffer 3: 50 mmo/L bis-TRIS-HCl pH 6.0, 5 mmo/L EDTA, 1 mmo/L EDTA. Buffer conditions 1 and 3 were determined primarily using PMNs (see Results). The reaction volume was increased to 100 μL, and the reaction was terminated by adding 5 μL of 0.5 mmo/L EDTA pH 8.0, 2 μL of 10% sodium dodecyl sulfate, and 10 μL of 20 mg/mL proteinase K. After incubation of the mixture at 50°C for 1 hour, the samples were precipitated with an equal volume of 2 mmo/L sodium iodide and 2 vol of 2-propanol. After centrifugation at 14,000g for 15 minutes and rinsing with 70% ethanol, the pellets were dissolved in 10 mmo/L TRIS-HCl pH 8.0, 1 mmo/L EDTA containing 1 μg/mL of RNase A. The samples were incubated at 37°C for 1 hour, and the DNA concentration was estimated by measuring the optical density (OD) at 260 nm. DNA (2 μg per lane) was electrophoresed on 1.5% agarose gels and visualized by ethidium bromide staining, and the gels were photographed under UV illumination.

Characterization of Nuclear Endonuclease of PMNs

We examined the characteristics of endonuclease activity of PMNs. Figure 1 shows that the activity at alkaline pH was dependent on Ca²⁺ and Mg²⁺, and the optimal concentra-
tion of both cations was 5 to 10 mmol/L, respectively. In addition to the Ca\(^{2+}\)/Mg\(^{2+}\)-dependent endonuclease activity, nuclei of PMNs also had a cation-independent acidic nuclease activity. In the presence of EDTA, the optimal pH for the endonuclease activity was 6 to 6.5, and no activity was observed at pH above 7. The endonuclease activity was observed in the presence of Ca\(^{2+}\) and Mg\(^{2+}\) ions at a wide pH range from 4 to 8 (Fig 2). For subsequent experiments, we fixed pH at 6.0 and 7.6 as the buffer conditions for the acidic and the Ca\(^{2+}\)/Mg\(^{2+}\)-dependent endonucleases, respectively. The cation concentrations for the Ca\(^{2+}\)/Mg\(^{2+}\)-dependent endonuclease were determined as 10 mmol/L Ca\(^{2+}\) and 5 mmol/L Mg\(^{2+}\), because 10 mmol/L Ca\(^{2+}\) almost completely blocked the Mg\(^{2+}\)-dependent endonuclease activity of the myeloid cell line. Table 1 shows the effects of known inhibitors\(^{1,2,9}\) on the Ca\(^{2+}\)/Mg\(^{2+}\)-dependent and acidic endonuclease activity. Zn\(^{2+}\) and N-ethylmaleimide inhibited the Ca\(^{2+}\)/Mg\(^{2+}\)-dependent but not the acidic endonuclease activity. Both types of activity were inhibited by NaCl and KCl at concentrations greater than 50 mmol/L. Adenosine triphosphate (ATP), an inhibitor of the Mg\(^{2+}\)-dependent endonuclease,\(^{12}\) also inhibited the activity of the acidic endonuclease. The Mg\(^{2+}\)-dependent, Ca\(^{2+}\)-independent activity, which was found in myeloid leukemia cell nuclei,\(^{17}\) was not detected in PMN nuclei (Fig 3A). The nuclei of PMNs separated by method 1 contained approximately 15% to 20% of total cellular acid phosphatase (lysosomal marker) activity. Thus, to obtain purer nuclei, we used method 2.

The endonuclease and acid phosphatase activities of the nuclei prepared by method 2 are compared with those by method 1 in Table 2. Both the Ca\(^{2+}\)/Mg\(^{2+}\)-dependent and the acidic nuclease activities were reduced considerably, but to a lesser extent than the corresponding change in acid phosphatase activity. With respect to the Mg\(^{2+}\)-dependent endonuclease, the activity determined by method 2 was 115%
Table 1. Effects of Several Compounds on Activities of Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-Dependent and Acidic Endonucleases

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mmol/L)</th>
<th>Relative Activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-ethylmaleimide</td>
<td>0.1</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>80</td>
</tr>
<tr>
<td>Aurintricarboxylic acid</td>
<td>0.5</td>
<td>1.8</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td>NaCl</td>
<td>100</td>
<td>19</td>
</tr>
<tr>
<td>KCl</td>
<td>100</td>
<td>26</td>
</tr>
<tr>
<td>ZnCl\textsubscript{2}</td>
<td>0.1</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>97</td>
</tr>
<tr>
<td>ATP</td>
<td>2</td>
<td>55</td>
</tr>
</tbody>
</table>

PMN nuclei were incubated in buffer 1 (for Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-dependent endonuclease) or buffer 3 (for acidic endonuclease) with or without inhibitors for 16 hours at 37°C. Relative activity is expressed in terms of percent MN equivalent of DNA fragmentation (%FR) compared with that without inhibitor as 100%.

Table 2. Comparison of Acid Phosphatase Activity and Endonuclease Activities in Nuclei Prepared by Two Methods

<table>
<thead>
<tr>
<th></th>
<th>Method 1</th>
<th>Method 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid phosphatase activity/DNA (IU/g)</td>
<td>358 ± 188</td>
<td>23 ± 14</td>
</tr>
<tr>
<td>Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-dependent endonuclease activity (MN equivalent mU)</td>
<td>1.06 ± 0.19</td>
<td>0.36 ± 0.15</td>
</tr>
<tr>
<td>Acidic endonuclease activity (MN equivalent mU)</td>
<td>7.43 ± 3.52</td>
<td>0.91 ± 0.52</td>
</tr>
</tbody>
</table>

Acid phosphatase activity of nuclear extracts was measured as described in Materials and Methods. PMN nuclei were incubated in buffer 1 (for Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-dependent endonuclease) or buffer 3 (for acidic endonuclease) for 16 hours at 37°C. The activities were expressed as MN equivalent of DNA fragmentation after autodigestion of PMNs. Results are means ± SD of four experiments.

Features of Nuclear Endonuclease of Immature Myeloid Cells and Hematopoietic Progenitor Cells

Two types (the Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-dependent and the acidic) of activity were observed with no detectable level of the Mg\textsuperscript{2+}-dependent endonuclease activity in nuclei of immature bone marrow myeloid cells. Thus, the pattern of nuclease activity in these cells was similar to that in PMNs (Fig 4), although DNA fragmentation tended to form a smear rather than a ladder on electrophoresis.

In contrast, the Mg\textsuperscript{2+}-dependent endonuclease activity, with no apparent activity of the Ca\textsuperscript{2+}/Mg\textsuperscript{2+}-dependent or the acidic endonuclease found in PMNs, was detected unequivocally in nuclei of CD34\textsuperscript{+} cells (Fig 3B). In the presence of EGTA but without Mg\textsuperscript{2+} ions, no DNA fragmentation was observed in CD34\textsuperscript{+} cells (data not shown), thus confirming
the dependence of the nuclease activity on Mg\(^{2+}\) ions. The percentages of CD34\(^+\) cells were consistently above 98% as assessed by flow cytometry, and DNA from 2 \times 10^5 to 4 \times 10^5 cells per lane was electrophoresed in three experiments, all of which yielded similar and reproducible results. During induced differentiation of CD34\(^+\) cells, the Ca\(^{2+}/Mg\(^{2+}\)-dependent activity appeared first, followed by the acidic activity. Furthermore, concomitant with the emergence of the new activities, the Mg\(^{2+}\)-dependent activity was reduced (Fig 5). CD13, CD14, CD15, CD33, and CD34 expression on days 7 and 10 were 83%, 0, 70%, 58%, 5%, respectively, and 88%, 1%, 65%, 41%, and 1%, respectively. Phenotypic analysis indicated that these cells differentiated toward granulocytes. These results were in agreement with the distribution of endonuclease activities in myeloid cells.

Next, we investigated whether leukemic cells had Mg\(^{2+}\)-dependent endonuclease activity. Regardless of the cell lineage, all the leukemia cell lines examined had varying degrees of this activity in their nuclei (Fig 6). Ladder formation on agarose gel electrophoresis was confirmed in all experiments.

**DISCUSSION**

The present study showed that three types of endonucleases exist in myeloid cell nuclei, and the pattern of nuclease activity expression is strikingly different between CD34\(^+\) cells and mature neutrophils. The three types of endonucleases (Ca\(^{2+}/Mg\(^{2+}\)-dependent, Mg\(^{2+}\)-dependent, and acidic) could be distinguished from each other with regard to their cation requirement, optimal pH, and response to inhibitors.

Several candidate endonucleases involved in apoptosis have been proposed. These include (1) a Ca\(^{2+}/Mg\(^{2+}\)-dependent endonuclease, which has been investigated most intensively, such as NUC18 and DNase I\(^{11}\); (2) a Ca\(^{2+}\)-independent endonuclease activity appeared first, followed by the acidic activity. Furthermore, concomitant with the emergence of the new activities, the Mg\(^{2+}\)-dependent activity was reduced (Fig 5). CD13, CD14, CD15, CD33, and CD34 expression on days 7 and 10 were 83%, 0, 70%, 58%, 5%, respectively, and 88%, 1%, 65%, 41%, and 1%, respectively. Phenotypic analysis indicated that these cells differentiated toward granulocytes. These results were in agreement with the distribution of endonuclease activities in myeloid cells.

Next, we investigated whether leukemic cells had Mg\(^{2+}\)-dependent endonuclease activity. Regardless of the cell lineage, all the leukemia cell lines examined had varying degrees of this activity in their nuclei (Fig 6). Ladder formation on agarose gel electrophoresis was confirmed in all experiments.
dent form, and (3) a cation-independent acidic form such as DNase II. However, there have been few reports on the endonucleases of PMNs. Only one report suggested the presence of an ion-insensitive endonuclease in PMN nuclei, but no detailed characterization was reported. In this study, we determined the characteristics of the activities of the Ca\(^{2+}/Mg\(^{2+}\)-dependent and the acidic cation-independent endonuclease of PMN nuclei. Similar enzyme activities with regard to ion requirement, pH range, and inhibitors to each have been reported previously in other tissues. Further characterization and isolation of these nucleases will be necessary to determine their identities.

Some investigators have not detected endonuclease activity in leukemia cell nuclei. This discrepancy from our results may be attributed to different experimental conditions for examining nuclease activity, as well as the possible uneven intracellular distribution of the nucleases. It has been reported that three types of endonuclease activities (Ca\(^{2+}/Mg\(^{2+}\)-dependent, Mg\(^{2+}\)-dependent, and acidic) were recovered mainly from cytoplasmic and nuclear sap fractions in rat thymocytes. Our observation that nuclease activities were reduced to a great extent with the strict nuclear isolation method (method 2) indicates that the endonuclease activities in PMNs are distributed mainly in the cytoplasmic fraction. Therefore, our assay system (method 1) may overestimate nuclear endonuclease activities. The acidic nuclease of PMNs, like DNase II, may be primarily of lysosomal origin. The precise subcellular localization of apoptotic endonucleases remains controversial. However, the present results strongly suggest that at least a certain proportion of these nucleases exist in PMN nuclei. In contrast, substantial amounts of the Mg\(^{2+}\)-dependent nuclease may be present in nuclei of myeloid cell lines. In PMNs, the Mg\(^{2+}\)-dependent nuclease activity was identified in neither nuclear nor cytoplasmic fractions (data not shown).

Examination of three types of endonuclease activities revealed a clear-cut difference between CD34\(^+\) cells and immature myeloid cells or PMNs. Ca\(^{2+}/Mg\(^{2+}\)-dependent and acidic, but not Mg\(^{2+}\)-dependent, endonuclease activities were found in immature myeloid cell and PMN nuclei. In contrast, only Mg\(^{2+}\)-dependent nuclear endonuclease activity could be detected in CD34\(^+\) cells. Furthermore, leukemic cell lines of all lineages have been found to contain the same Mg\(^{2+}\)-dependent activity as that of CD34\(^+\) cells. These results suggest that hematopoietic progenitor cells contain the Mg\(^{2+}\)-dependent endonuclease activity in their nuclei. CD34\(^+\) cells may lose the Mg\(^{2+}\)-dependent endonuclease activity and acquire new activities along the differentiation pathway to granulocytes. Alternatively, de novo synthesis of the enzyme, or modification of activity of endonuclease such as degradation of inhibitors or access of the enzyme to the nucleus from the cytoplasm, may be necessary for apoptosis.

Ca\(^{2+}\) has been postulated to trigger endonuclease activation and apoptosis in some systems. In contrast, Ca\(^{2+}\) appears to inhibit apoptosis of the progenitor cell line caused by factor withdrawal. CD34\(^+\) cells require growth factor(s) for their survival, as supported by the results of a recent study in which IL-3 and c-kit ligand suppressed apoptosis of CD34\(^+\) cells depending on their differentiation stage. Collectively, our observations favor the hypothesis that the Mg\(^{2+}\)-dependent endonuclease is activated in apoptosis arising from factor deprivation.

There are two possible explanations for why the Mg\(^{2+}\)-dependent endonuclease activity is not detected in PMN nuclei. One possibility is that the protein itself is lost, or its amount is markedly diminished. Alternatively, the activity may be inhibited by other nuclear components. Indeed, we have confirmed the presence of inhibitors of the Mg\(^{2+}\)-dependent endonuclease in nuclear extracts of PMNs. We are currently attempting to isolate the nuclease and its inhibitor(s), with the hope of validating one or other of the above possibilities.

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