INTERNALUCLEOSOMAL CLEAVAGE OF DNA IS INSUFFICIENT EVIDENCE TO CONCLUDE THAT CELL DEATH IS APOPTOTIC

To the Editor:

In a recent volume of Blood, Yuan et al demonstrated internucleosomal cleavage of DNA in erythroid precursors obtained from the bone marrow (BM) of a patient with β-thalassemia major, “lesser” (but unquantitated) amounts of internucleosomal cleavage in those from a β-thalassemia trait donor, and no evidence of such cleavage in erythroid precursors separated from normal BM. Although no other evidence was presented, the investigators concluded that β-thalassemic progenitors exhibit apoptosis, or “programmed cell death.” Apoptosis is a form of cell death distinct from necrosis. Morphologic criteria for the definition of cell death as apoptotic include rapid chromatin condensation with aggregation of chromatin along the nuclear envelope and plasma membrane blebbing followed by nuclear condensation and separation into small, apoptotic bodies. When it accompanies these features, internucleosomal fragmentation of chromatin DNA is useful as a criterion to help define cell death as apoptotic.

We recently identified artifactual internucleosomal cleavage of DNA after its extraction from normal rat kidneys. Extraction of DNA from tissue processed in buffer containing low concentrations of EDTA resulted in extensive internucleosomal fragmentation of DNA (Fig 1) despite the fact that these tissues showed no evidence of apoptosis morphologically. This artifact was not prevented simply by the addition of a protease inhibitor (phenylmethyl-sulfonylfluoride [PMSF]), of 25 mmol/L EGTA to inhibit endogenous calcium-dependent nuclease activity, or of spermidine to stabilize chromatin structure. However, the artifact was prevented by an increased NaCl concentration combined with 25 mmol/L EDTA. It is of note that the buffer used by Yuan et al for isolation of fragmented DNA (10
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following DNA extraction buffer: Lane 1, 10 mmol/L Tris, 1 mmol/L EDTA, 10 mmol/L sodium chloride. Lanes 2, 3, 5, and 6, as in lane 1 with the addition of 25 mmol/L EGTA (lane 2), 100 mmol/L NaCl (lane 3), 0.2 mmol/L phenylmethylsulfonyl fluoride (lane 5), and 2.5 mmol/L spermidine (lane 6). Lane 4 shows DNA from tissue processed in 25 mmol/L EDTA, 10 mmol/L Tris, and 100 mmol/L sodium chloride.

mmol/L Tris, 1 mmol/L EDTA, 0.2% Triton X-100) is unlikely to prevent artifactual internucleosomal cleavage of DNA, as demonstrated by these data.

Thus, the findings of Yuan et al easily could reflect artifactual nuclease activity occurring at a number of stages during DNA isolation from the erythroid progenitors. Firstly, all thalassemic patient samples were shipped on ice from Italy, and a number of the normal samples that were obtained from Italy, it is probable that these are not adequate as controls for thalassemic cells that have never showed ladder patterns either from normal marrows.

REFERENCES


RESPONSE

We thank Drs Enright, Nath, and Hebbel for their interesting critique of our cited report. Their letter can be broken down to consist of two somewhat different issues. One issue is the possibility that the internucleosomal cleavage of DNA is an artifact that can be prevented by increasing the concentration of NaCl and EDTA. The extraction and analytic method we used is essentially the “standard” method used in many laboratories making these sorts of analyses. If the nucleosomal breakdown was artifactual we should have seen it in the now 17 normal subjects studied (half shipped from Italy and half from Stanford) or in the normal murine erythroid marrow we have studied. Furthermore, in our report we pointed out that an important control was to analyze the CD45-positive fraction from the samples by the identical methods. This fraction, enriched in white blood cell precursors and depleted of erythroid precursors, never showed ladder patterns either from normal or Cooley’s Anemia marrows.

The gel photo submitted by Drs Enright, Nath, and Hebbel consists of an analysis performed on rat kidney. The pattern of DNA breakage is peculiar in that three to four distinct bands are visible rather than the more typical multiple ladder pattern. Did Enright et al perform this extraction and analysis on purified erythroid precursors from rat femoral marrow, a tissue that would be more relevant for comparison than kidney?

We agree that morphologic changes are important in identifying apoptosis. In making these now 20-year-old electron microscopic observations the investigators obviously did not specifically search for those changes now known to be indicative of apoptosis. However, nuclear condensation was observed, as well as loss of nuclear membrane and duplication of nuclear membranes. The second part of the letter consists of an idea we are much interested in, that is, the accumulation of α globin chains with attached iron could produce oxidant damage that might lead to a switch on of apoptotic programs. An association between oxidants and apoptosis has, in fact, been proposed.

Lastly, apoptosis may be a more general mechanism for hematologic conditions characterized by intramedullary erythroid cell death.
Koury et al. recently presented an abstract showing that enhanced apoptosis occurred in erythroid precursors of folate-deficient mice. Apoptosis has also been identified as a component of anemia in the Diamond-Blackfan syndrome.

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Internucleosomal cleavage of DNA is insufficient evidence to conclude that cell death is apoptotic [letter; comment]

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