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DENUCLEATION IN ERYTHROCYTES: B-DNA, SINGLE-STRANDED DNA AND Z-DNA

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During the last several decades research on cell death has been proliferating at a fast rate. Regulated cell death is important not only in morphogenesis, but also in the removal of damaged or neoplastic cells and the optimization of immunological factors [1]. Three types of cell death can occur among a variety of tissue types: apoptosis , necrosis and terminal differentiation (denucleation). Terminal differentiation may be considered a very specialized form of apoptosis that has evolved to serve tissue-specific function [1]. DNA is no longer considered a static molecule, but rather a dynamic entity [5]. Alternative forms of the more widely present B-DNA can exist in cells, such as Z-DNA [5]. The expulsion of the mammalian nucleus from erythroblasts has been the focus of many studies over the past forty-five years [2,3]. The final product of mammalian erythropoiesis, a very complicated process, is a non-nucleated red blood cell. The mammalian reticulocyte lacks a nucleus and is incapable of further cell division or differentiation to another cell type. It should be noted that many other species produce nucleated red blood cells. The precise mechanism of mammalian erythropoiesis (terminal differentiation) is not yet fully understood, especially on a nucleic acids level: B-DNA, Z-DNA, Z-RNA [6]. Controversy still exists as to whether the loss of nuclei from mammalian erythroblasts occurs by extrusion, by karyolysis or a combination of both mechanisms. The specific purpose of this research project was to examine the molecular biology of the immature red blood cells, and specifically examine the role of denucleation on the different types of nuclear DNA helical structures: right-handed double-stranded (ds-) B-DNA, left-handed ds-Z-DNA and denatured single-stranded (ss-) DNA.

All animal surgery was performed under sterile conditions. The spleens were removed from 3- to 7-day old mice and processed for the microscopic visualization of enucleating blood cells after 40 min *in vitro* [6,7]. The mice were then sacrificed by lethal injection, which conforms to the guidelines of the Institutional Animal Use Committee. Fixation was performed for either 1,3,6,10,23 or 27 hrs. Anti-ds-B-DNA (2C10) monoclonal antibodies (MAb) and polyclonal antibodies (B11) (PAb), anti-ss-DNA (F7-26) MAb, and anti-Z-DNA (Z22) MAb were used as immunohistochemical nucleic acid probes [4]. We employed the avidin-biotin immunostaining method for all procedures [4]. Only molecular biological grade reagents and ultra pure water were used. A new computerized image analysis system was employed to quantify anti-DNA antibody and nucleic acid binding protein probes, and specific blood cell dimensions [4]. For orientation of all the cellular components and as an additional control the Wright-Giemsa stain was used.

Our data reveal that there is a gradual increase of denatured ss-DNA and a gradual decrease of right-handed ds-B-DNA sequences beginning during the end portion of nuclear extrusion. This process increases during the nuclear constriction stage. Limited fixation times employing precipitating fixatives, such as Methacam, Clarke's and Carnoy's produced superior results. Longer exposure times

especially with cross-linking fixatives such as 10% Neutral Buffered Formalin, and 1% Glutaraldehyde resulted in poor data, and required the use of antigen retrieval procedures.

Concerning the maturation of these blood cells, nuclear extrusion occurs late in the maturation of mammalian erythroid cells. During this process the erythroid nucleus undergoes a gradual process of condensation. Very little decrease in ds-Z-DNA immunoreactivity is observed within the erythroblast, late erythroblast, extruding nucleus, or early to middle stages of the nuclear constriction process. Concerning Z-DNA a precipitous decrease occurs just prior to the end of nuclear extrusion (very late nuclear constriction stage), and especially within the extruded nucleus of the erythroblast. Our group speculates that a karyolytic process may start very late in the maturation process of erythroid cells with the direct destruction of Z-DNA. Another possibility is the inhibition of the B-DNA to left-handed Z-DNA transition within specific genes. A gradual loss of ds-B-DNA was observed during the maturation of erythrocytes, which was accompanied by a gradual increase in denatured ss-DNA. At this point we observed a great deal of DNA fragmentation, which also contained nicks. Our group also observed a decrease in both B-DNA and Z-DNA binding proteins, which correlates directly with the loss of the nuclear nucleic acids. We interpret these data, along with the precipitous loss of Z-DNA during the late constriction stage as a major event in the deterioration of the genome of the mature red blood cell [8,9].

Denucleation of mammalian erythroblasts reveals two special features, active expression of the globin gene and nuclear extrusion that give rise to anucleated reticulocytes. The present study reveals that during the early and middle stages of erythrocyte maturation little cell death occurs via terminal differentiation. However, during the last stages, we observed a significant increase in ss-DNA content and DNA fragmentation. Additionally, we observed a gradual decrease in the presence of ds-B-DNA and DNA binding proteins. We also detected a decrease in the potential for DNA to undergo negative DNA supercoiling. A precipitous loss of left-handed Z-DNA content and Z-DNA binding proteins occurred in the stage just prior to nuclear extrusion, the very late nuclear constriction stage. The cellular and molecular basis of certain blood diseases can be seen in a different context when terminal differentiation is emphasized. Understanding the specific molecular mechanisms that control terminal differentiation regulation points of the denucleation pathway will have an enormous impact on future strategies for therapeutic intervention in treating blood disease.

References

[1] Liu, Y.J., et al., *Nature* **342**:21-28 (1989).
[2] Arends, M.J., Morris, R.G., Willy, A.H. *Am. J. Pathol.* **136**:593-608 (1990).
[3] Awai, M., et al., *Acta. Haematol. Jpn.* **39**:139-202 (1968).
[4] Gagna C.E., et al., *J. Histochem. Cytochem.* **45**: 1511-1521 (1997).
[5] Rich, A. *Ann. N.Y. Acad. Sci.* **726**:1-17 (1994).
[6] Repasky EA, Eckert BS. *Cell Tissue Res.* **221**:85-91 (1981).
[7] Skutelsky, E., Danon, D. *J. Cell. Biol.* **33**: 625-635 (1967).
[8] Gagna, C.E., Kuo, H.-R., Lambert, W.C. *Cell Biol. Int.* **23**: 1-5 (1999).
[9] Gagna, C.E., et al., *Cell Biol. Int.* **24**: 657-659 (2000).

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