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CHANGES IN THYMOCYTES UNDERGOING PROGRAMMED DEATH

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Differentiation of pre-thymocytes in the thymus gives rise to the cortical cell line of the peanut agglutinin-positive (PNA^+) dexamethasone-sensitive cells. The surface phenotype of the cells has been relatively well characterized, and their frequency in the thymus has been found to reflect animal's age. It is also known that the cells undergo the so called programmed death (apoptosis), the cause and the mechanism of which have not yet been elucidated. Apoptosis is an active process of cellular self destruction, which often serves a biologically meaningful homeostatic function [1, 2]. It is morphologically and biochemically distinct from necrosis and, in the early stages of its evolution, it can be distinguished from necrosis by its characteristic pattern of chromatin condensation and by the absence of cytoplasmic degeneration. Biochemically, the distinctive internucleosome cleavage of DNA in apoptosis contrasts with the random DNA degradation observed in necrosis.

In this study we have decided to examine whether thymic PNA^+ cells and PNA^- cells differ from each other in the DNA structure and protein spectrum in various periods of animal's life.

Thymuses were taken from male Wistar rats aged 1, 7, 14 and 28 days and from 2 months old rats, which had been given i.p. 100 μg dexamethasone or saline (control rats) 24 h and 48 h earlier. PNA^+ and PNA^- cells were isolated by applying a modification of Refiner's technique [3]. Thymus DNA was isolated using a modified technique of Fife *et al.* [4].

In this modification the amount of proteinase K was doubled and the incubation time was prolonged to 12 h. The purity of the DNA preparation was checked by electrophoresis in 0.8% agarose gel [5]. Nick translation labeling and nucleic acid hybridization were performed as described earlier [5]. Protein was isolated according to [6]. Protein fractions precipitated by ammonium sulfate (final concentration 0.8 M) were separated in 7% polyacrylamide gel, containing 0.1 % SDS [7]. The obtained autoradiograms and electrophoregrams were analyzed using the computer program designed by W. Warchoł (Department of Biophysics, Medical School, Poznań). DNAse activity was analyzed as described in [8].

Studies on apoptosis in thymocytes from rat thymus treated earlier with dexamethasone revealed two peaks of nucleolytic activity, probably containing two distinct nucleases. One of them has the molecular weight of about 35 - 40 kDa (DNAse I) and the other about 70 kDa (DNAse II) (Fig.1). After 2 days of dexamethasone treatment, when only medullary thymocytes are still present, activity of DNAse I was not detected. The relationship of the endonuclease activity, with apoptosis in unclear.

Electrophoretic analysis of DNA, derived from PNA^+ and PNA^- cells, performed in 0.8% agarose gel, demonstrated that DNA of PNA^+ was not homogeneous and it was more often in a degraded form than DNA of PNA^- cells. DNA isolated from either cell fraction hybridized less efficiently (by approximately 10%) with DNA of the other cell fraction than with the homologous DNA.

The results suggest that PNA^+ and PNA^- cells contain different DNA species. The differences may reflect a deletion or modification of some

Fig. 3. SDS gel electrophoresis of proteins from PNA^+ and PNA^- cells from rat thymus. 30 - 35 μg, proteins precipitated with 0.8 M ammonium sulfate, were electrophoresed through a 7% polyacrylamide gel, containing 0.1% SDS. Line 1 proteins were isolated from PNA^- cells of 7 days old rat. Line 2 proteins were isolated from PNA^+ cells of 7 days old rat. Line 3 proteins were isolated from PNA^- cells of 14 days old rat. Line 4 proteins were isolated from PNA^+ cells of 14 days old rat. Line 5 proteins were isolated from PNA^- cells of 28 days old rat. Line 6 proteins were isolated from PNA^+ cells of 28 days old rat. Line 7 marker of molecular size in kDa

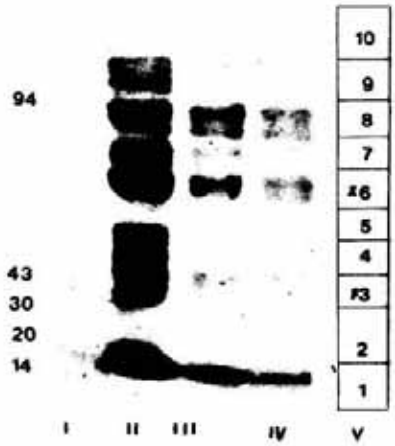


Fig. 1

Fig. 1. SDS gel electrophoresis of proteins from rat thymus. I, marker of molecular size in kDa, II, proteins from thymus of the control rats; III, proteins from thymus after 1 day dexamethasone treatment; IV, proteins from thymus after 2 days dexamethasone treatment stained with Comassie Blue*. DNAse activity: 50 -70 µg proteins precipitated with 0.8 M ammonium sulfate were electrophoresed in a 7% polyacrylamide gel, containing 0.1% SDS. The gel was cut into 10 slices as indicated in the line V. Proteins were eluted from each of the slices, denatured, allowed to renature and tested for the DNAse activity

Fig. 2

Fig. 2. Agarose gel electrophoresis of DNA isolated from PNA^+ and PNA^- cells of rat thymus. 4 - 5 µg DNA were applied to 0.8% agarose gel. DNA visualized by ultraviolet fluorescence after staining the gel with ethidium bromide. **A.** DNA was isolated from the PNA^+ cells of rat thymus. Line 1, 2 ,the rat was: 1 day old. Line 3, 4, the rat was: 7 days old. Line 5, 6, the rat was: 14 days old. Line 7, 8, 9, the rat was: 28 days old. Line 10, DNA from wheat as a control. **B.** DNA was isolated from PNA^- cells of the rat thymus. Line 11, the rat was: 1 day old. Line 12, the rat was: 7 days old. Line 13, the rat was: 14 days old. Line 14, the rat was: 28 days old

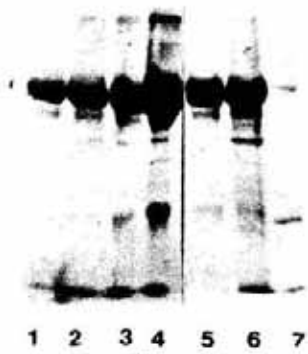
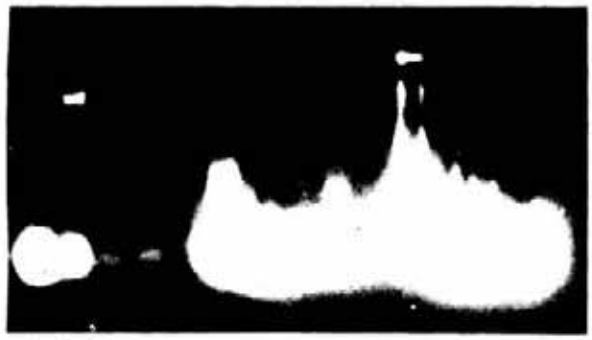


Fig. 3



1 2 3 4 5 6 7 8 9 10 11 12

Fig. 4A

1 2 3 4 5 6 7 8 9 10 11 12

Fig. 4B

Fig. 4. Northern blot hybridization of RNA isolated from PNA^+ and PNA^- cells of rat thymus. **A**. 50 - 55 μg of rat thymus RNA isolated from PNA^- and PNA^+ cells were electrophoresed in 1% agarose denaturing gel and transferred onto a nitrocellulose filter. The filter was hybridized with ^{32}P-labeled DNA which was isolated from PNA^+ cells. RNA was isolated from the PNA^+ cells of rat thymus. Line 1, 2, the rat was: 7 days old. Line 5, 6, the rat was: 14 days old. Line 9, 10, the rat was: 28 days old. RNA was isolated from PNA^- cells of the rat thymus. Line 3, 4, the rat was: 7 days old. Line 7, 8, the rat was: 14 days old. Line 11, 12, the rat was: 28 days old. **B**. Autoradiogram of hybridization.

DNA fragments which results in a limited cross-hybridization between the two DNA preparations. The differences may also arise in the course of α, β, γ and Δ T cell receptor (TCR) gene rearrangements accompanying thymocyte differentiation.

The observations also pose the question whether the observed differences in the DNA structure are directly responsible for apoptosis. Apoptosis may occur as a results of activation of a gene set, coding information for proteins of various types, among other, for enzymes responsible for DNA synthesis and degradation. Therefore, we have performed an analysis of proteins of PNA^+ and PNA^- cells obtained in different periods of rat's life (7, 14 and 28 days of life), using electrophoresis in 7% polyacrylamide gel, containing 0.1% SDS. Significant changes in protein level of PNA^+ cells were observed between the 14th and 28th day of life and they were accompanied by an appearance of proteins not observed at the earlier stages of rat's life (Fig. 3). An increase in RNA level was also found, pointing to enhanced transcription and translation in PNA^+ cells (Fig. 4).

The results may suggest that PNA^+ cell, in response to specific signals, begins to synthesize specific proteins, thus triggering the chain of events leading to cell death. Identification of the proteins which are responsible for DNA degradation and for regulation of transcription and translation is essential to deciphering of the molecular mechanisms involved in apoptosis.

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