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Apoptotic State of Colostral/Milk Cells

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It has been proposed that the population of leukocytes released in the mother's milk penetrates the newborn's body through the gut and populates it's lymphoid organs. In order to test this hypothesis we first studied the state of apoptosis of the cells in the milk population. For this purpose we used a method measuring the fragmentation of DNA (by means of the ^{3}H-thymidine incorporation in DNA located in the different cellular compartments — nucleus and cytoplasm). When working with whole milk cell population we found different percent of DNA fragmentation from 40 to 70%. We next separated the milk cell population into nearly pure fractions of macrophages and lymphocytes. The level of the DNA fragmentation in these experiments was much better. The macrophages and the lymphocytes were enough viable to penetrate the mucosal immune system of the newborn and to influence its development and maturation.

Key words: — milk cells, apoptosis, DNA fragmentation, lymphocyte, macrophage.

Introduction

In the colostrum and milk of all mammalian species a huge number of viable cells are released consisting of macrophages, neutrophils and lymphocytes. It has been shown for different species including monkeys that these cells penetrate the gut of the newborn and populate its lymphoid organs[2]. On the basis of these results a hypothesis has arisen that the cells of the milk population, especially lymphocytes and macrophages are immunocompetent cells and they could influence the immature mucosal immune system of the new born[3]. To test this hypothesis we have to be sure that these otherwise vital cells do not undergo an apoptotic process.

The aim of the present study was to investigate the apoptotic state of the cells in the human colostrum and milk.

Materials and Methods

Apoptosis assay

Milk samples were taken from 14 healthy human donors. They were washed 3 times in PBS and resuspended in 1 ml RPMI 1640 containing 5% FCS. 1 μl ^{3}H- thy-

midine was added to each sample. The next step was an incubation of the samples at 37 °C for approximately 16 hours. After spinning down the cells were washed 3 times with PBS (or serum free media) to remove any free radiolabel, then the cells were resuspended at a concentration of 5×10^5 cells/ml in serum free medium. After 1 hour the cells were gently pelleted and supernatant count: supernatant — A. The pellet was resuspended in lysis buffer (1× PBS; 0.2% Triton-X100; 2Mm EDTA) and centrifuged. The collected pellet and supernatant were counted in scintilation counter (Beckman): supernatant — B and pellet — C. To The per cent fragmentation was calculated after the equation:
Per cent fragmentation = (A + B) / (A + B + C)
All the samples were in triplicates and the results were expressed as mean per cent DNA fragmentation[6, 1].

Separation of the milk cells

In respect to the significant differences observed after performed DNA fragmentation test with whole milk cell population and for more precise image of apoptotic events among the different subpopulation of milk cells, we separated the milk cells to macrophage and lymphocyte fractions. The separation protocol includes the next chain of laboratory manipulations: washing 3 times in PBS; resuspension of cells in RPMI 1640 (serum free media), preparation of Ficol-RPMI 1640 gradient (11%, 20%, 30% Ficol); centrifugation on this gradient; collection of macrophage or lymphocyte fractions and performance of the assay for apoptosis.

Microscopic approach used

For a visual demonstration of the apoptosis in the milk cells the microscopic observations of the fresh milk samples was performed. After 3 times washing in PBS, ethidium bromide was added to the cells at 0.5 μg/ml final concentration and the cells were observed under microscope at UV and visible light. The photos of stained cells were taken in parallel at UV and visible light [6].

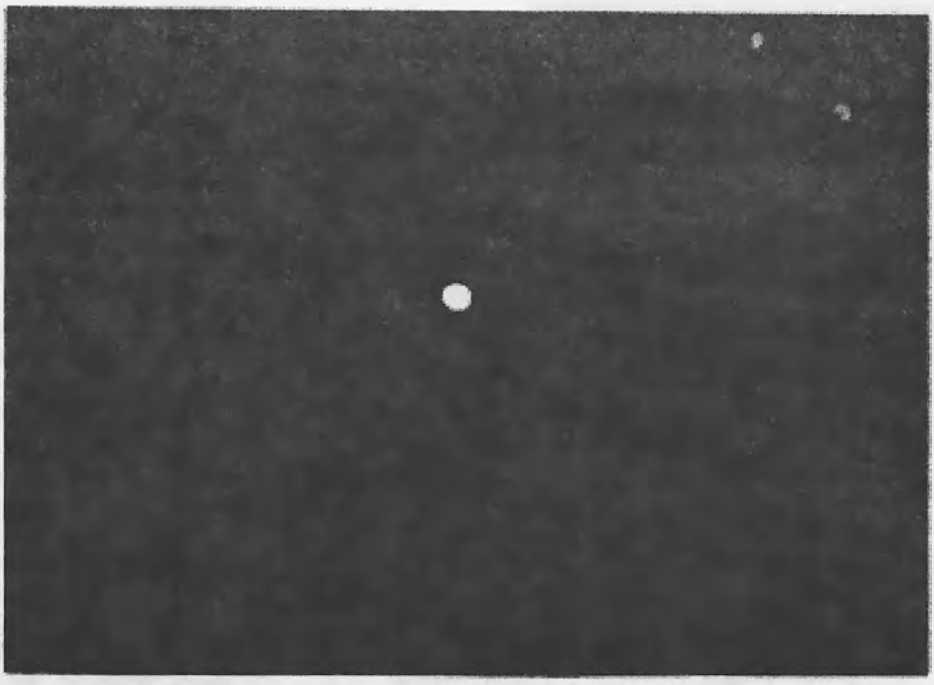
Results and Discussion

Apoptosis is widely distributed process affecting all animal cells, organs and tissues. Here we present another approach into the application of knowledge about the apoptosis. The hypothesis, that we have tested in the present study, is that the leukocytes, that are released in the mother's milk penetrate the gut of the newborn and populate its lymphoid organs. It is possible that these cells could influence the maturation of the mucosal immune system of the newborn[4]. To be involved in a transfer of immunological competence, a certain level of viability of the leukocyte cells is required[5]. We examined the apoptotic status of cells isolated from human mother's colostrum and milk. A variable count of DNA fragmentation was observed. As Table 1 shows, the per cent of apoptotic events (presented as a mean percent fragmentation of DNA in whole milk population) varied from 40 to 70% approximately. We might explain these differences with

Table 1. Per cent fragmentation in whole milk leukocyte population

Dónor, No	Per cent fragmentation
1	43
2	87
3	97
4	95
5	70
6	40

A



B

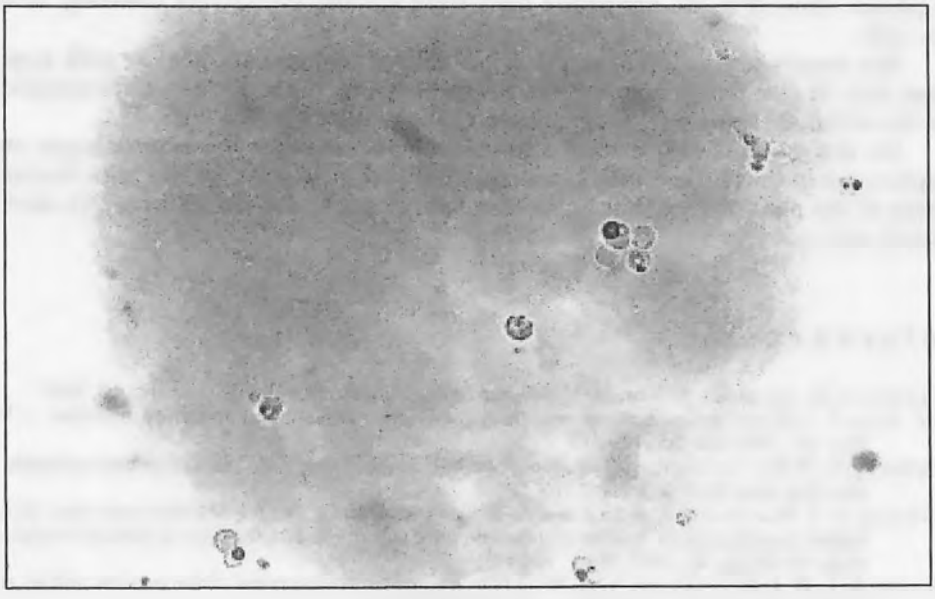


Fig. 1. Apoptotic cells from milk's population, treated with ethidium bromide under UV light (*A*) and visible light (*B*)

Table 2. Per cent fragmentation in mother's milk after separation of macrophage and lymphocyte subpopulations

Donor, No	Fraction	Percent fragmentation	Fraction	Percent fragmentation
1	Macrophages	42	Lymphocytes	59
2	Macrophages	94	Lymphocytes	91
3	Macrophages	83	Lymphocytes	74
4	Macrophages	74	Lymphocytes	68
5	Macrophages	80	Lymphocytes	71
6	Macrophages	80	Lymphocytes	65
7	Macrophages	62	Lymphocytes	77
8	Macrophages	69	Lymphocytes	83

genetic and environmental influences on the donor.

For more precise investigation of apoptotic status of milk cells a separation of milk population into macrophage and lymphocyte fractions was performed. The level of DNA fragmentation in these experiments was much better as shown in Table2. The macrophages showed about 40% fragmentation and lymphocytes showed about 60% fragmentation.

For visualizing apoptotic cells in mother's milk, the ethidium bromide inclusion test was used. As the photos show (Fig. 1), apoptotic cells were stained intensively contrary to viable cell.

Our results suggest that the higher per cent of fragmentation in the milk population may be due to the neutrophils, which are in the final stage of differentiation and die within 24 hour after their release in the mother's milk.

On the grounds of our results we might conclude that the macrophages and lymphocytes in the mother milk are enough viable to penetrate the mucosal immune system of the newborn and not apoptotic enough so they could influence its development and maturation.

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