BIOGERONTOLOGY

Age-Related Features of Expression of Cell Renewal Factors in the Intestinal Peyer's Patches

L. A. Grabezhev, N. N. Sevostjanova, A. N. Kolmakov, S. S. Konovalov, V. O. Polyakova, and I. M. Kvetnoy

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 150, No. 10, pp. 440-442, October, 2010
Original article submitted June 28, 2009

Immunohistochemical study with markers of proapoptotic protein P53 and proliferation protein Ki-67 revealed an age-related decrease in proliferative activity of lymphocyte in the intestinal Peyer's patches and an increase in the ratio of apoptotic cells in humans. These changes aggravate atrophy and involution of the intestinal epithelium during aging.

Key Words: *Peyer's patches; aging; proliferation marker Ki-67; apoptotic marker P53*

According to modern notions, the digestive tract is a large neuroendocrine organ. Epithelial cells of the digestive tract produce more than 100 hormones and growth factors, which are involved in digestion and regulation of homeostasis [6]. The intestine has a strong system of immune surveillance, whose activity is provided by numerous clusters of lymphocytes in the submucosal layer forming Peyer's patches (PP) [5]. PP mainly consist of B cells. They secrete various cytokines and signal molecules, which trigger and regulate the local immune response.

It should be emphasized that the maintenance of the sufficient pool of active lymphoid cells provides the adaptive barrier [7]. Studying of the structural and functional features, proliferative activity, apoptosis and elimination of PP lymphocytes during aging is important for the development of new approaches to hormonal and immune prevention of age-related abnormalities.

St. Petersburg Institute of Bioregulation and Gerontology, Northwestern Division of the Russian Academy of Medical Sciences, Russia.
Address for correspondence: vopol@yandex.ru. V. O. Polyakova

MATERIALS AND METHODS

Experiments were performed on autopsy specimens of the small intestine (n=47) from humans of various age groups. The samples were dived into 3 groups (according to the WHO classification): group 1, elderly individuals (60-74 years); group 2, old individuals (75-89 years); and group 3, long-lived individuals (90 years or more).

PP fragments were fixed in neutral formalin (pH 7.2), treated with alcohols and xylenes, and embedded into paraffin (standard method). Paraffin blocks were used for preparing sections (~3 μ) on a Leica 54OM microtome.

The sections were stained with hematoxylin and eosin for morphological study. Immunohistochemical study was conducted with primary monoclonal antibodies to the proapoptotic protein P53 (1:100, Dako) and proliferation factor Ki-67 (1:100, Dako). Biotinylated anti-mouse antibodies (Dako) were used as the secondary antibodies. Staining was visualized with a complex of avidin with biotinylated peroxidase (ABC-kit). Horseradish peroxidase was developed with diaminobenzidine (all reagents were from Dako).

0007-4888/11/1504-0465

The intensity of staining was estimated with Nikon Eclipse 400 systems for a computer analysis of microscopic images and VideoTesT-Morfologiya 5.0 software. The optical density and percentage of the total area of immunostained structures were estimated.

The results were analyzed by Statistica 7.0 software. The significance of differences was evaluated by nonparametric Kruskal–Wallis (ANOVA) and Mann–Whitney test.

RESULTS

Phosphoprotein P53 plays a key role in cell transition from G1 to G2 stage of the cell cycle. It belongs to a group of nuclear oncoproteins. A short-lived protein P53 is rapidly degraded in the ATP-dependent system of protein degradation. Various disturbances are followed by termination of P53 degradation and accumulation of this protein [3]. Therefore, P53 protein is the major factor in the recognition of DNA damages and subsequent induction of apoptosis. Insertion of the *p53* gene can be followed by cell cycle arrest, which results in DNA reparation or apoptosis [2]. P53 forms a complex during the interaction with various cell proteins. This complex plays a key role in the maintenance of a cell cycle and regulation of gene activity, cell differentiation, and apoptosis [4]. *p53* gene mutations are accompanied by inactivation of protein expression, which leads to progressive accumulation of aberrations in cell genome [1].

Statistical analysis showed that optical density of P53-immunostained cells significantly increases with age (p=0.0048; Fig. 1).

We calculated the percentage of the total area of stained structures. The area of P53 protein expression in long-lived individuals was 6-fold higher (p<0.01) than in elderly subjects (group 1, 1.999±0.157; group 2, 8.921±1.103; and group 3, 12.642±1.574).

It is probably associated with age-related increase in the number of genetic errors in cells. These changes are followed by accumulation of proapoptotic protein P53.

Immunohistochemical study of Ki-67 expression was performed with PP lymphocytes from individuals of various age groups. The Ki-67 protein was identified in active phases of the cell cycle (G(1), S, G(2), and mitosis), but not in resting cells (G(0).

Expression of the Ki-67 marker in human PP was shown to decrease significantly with age (Fig. 2). The significant decrease in the optical density of this marker with age (p=0.0039, ANOVA) reflects the age-related reduction of proliferative activity. Similar changes were found in the area of Ki-67 expression (decrease with age, p=0.0004). The expression area in group 3 (2.485±0.289) was 2-fold lower than in groups 2 and 1.

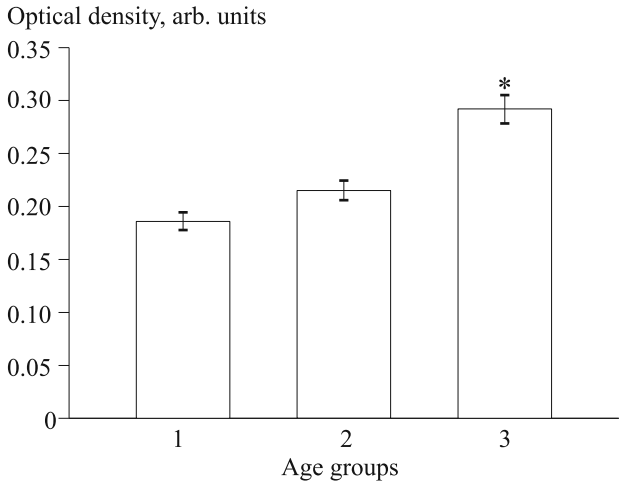

Fig. 1. Optical density of the P53 protein in PP lymphocytes of elderly, old, and long-lived people. *p=0.0048.

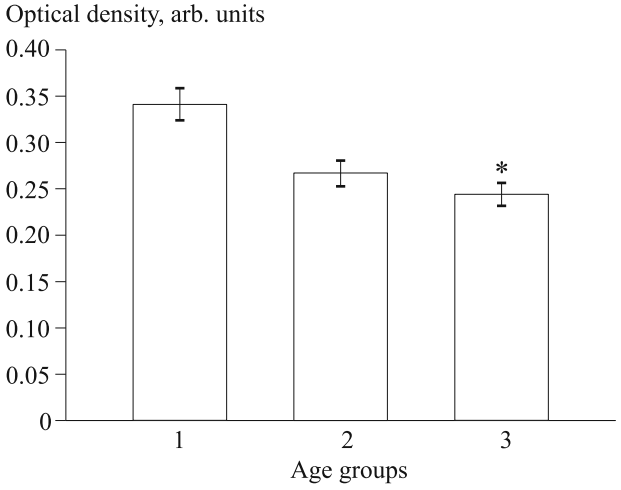

Fig. 2. Optical density of the Ki-67 protein in PP lymphocytes of elderly, old, and long-lived people. *p=0.0039.

We performed general study of P53 and Ki-67 in various age groups. The optical density of Ki-67 decreased with age. However, optical density of P53 was shown to increase under these conditions. Optical density of P53 and Ki-67 was estimated in group 1 (0.186±0.026 and 0.341±0.081 respectively) and group 3 (0.292±0.056 and 0.244±0.027).

Similar changes were found in the expression area of these markers. This parameter increased for the P53 marker, but decreased for the Ki-67 marker. No correlation was revealed between optical densities of expression of these markers in various age groups. Our results attest to independence of these factors. Therefore, these processes can be described independently of each other.

Differences between the optical densities of expression of P53 and Ki-67 became less significant with age. The opposite ratio was found between these markers. Therefore, the process of proliferation pre-

vails over the process of apoptosis in elderly people of group 1. These differences were less pronounced in old people of group 2. However, the intensity of proliferative processes was greater than that of apoptotic processes in this group of subjects. The apoptosis/proliferation ratio was modified in long-lived people of group 3. The degree of apoptosis was higher than that of proliferation in group 3 subjects.

The absence of correlation between the expression areas of P53 and Ki-67 attests to independent expression of these markers.

Differences between the expression area of P53 and Ki-67 were shown to become more significant with age. Proliferative activity was higher than apoptotic activity in group 1. The opposite ratio was observed in group 2. The intensity of apoptosis was much greater than that of proliferation in group 3.

Our results indicate that aging is associated with enhanced apoptotic death and reduced proliferative activity of human PP lymphocytes. These changes result in partial degradation of PP and play an important role in aging of the immune system.

We conclude that aging is accompanied by a decrease in proliferative activity of lymphocytes and increase in the ratio of apoptotic cells. These changes aggravate atrophy and involution of the intestinal epithelium in aging.

Our results hold much promise for the development of new approaches to the prevention and treatment of age-related "bowel syndrome". These approaches are based on activation of immunotropic signal mechanisms for the maintenance and restoration of differentiated lymphocytes in human PP.

REFERENCES

1. N. V. Zelenina, A. T. Mar'yanovich, and V. N. Tsygan, *Programmed Cell Death* [in Russian], St. Petersburg (1996), pp. 89-103.
2. N. Debard, F. Sierro, and J. P. Kraehenbuhl, *Semin. Immunol*., **11**, No. 3, 183-191 (1999).
3. B. J. Dobson, C. G. Mackintosh, and J. F. Griffin, *Vet. Immunol. Immunopathol*., **136**, No. 4, 143-148 (2010).
4. L. Ginaldi, M. De Martines, A. D'Ostilio *et al*., *Immunol. Res*., **21**, No. 1, 31-38 (2000).
5. J. Krege, S. Seth, S. Hardtke, *et al*., *Eur. J. Immunol*., **39**, No. 10, 2765-2778 (2009).
6. G. McGovern, S. Martin, L. Gonzales, *et al*., *Vet. Pathol*., **46**, No. 2, 233-240 (2009).
7. Y. J. Zhou, J. Gao, H. M. Yang, *et al*., *World J. Gastroenterol*., **15**, No. 10, 1246-1253 (2009).