BIOGERONTOLOGY

Age-Related Differences in Expression of Signal Differentiation Factors for Human Thymic Epithelial Cells

V. O. Polyakova, I. M. Kvetnoy, S. S. Konovalov, P. N. Zezulin, and I. V. Knyazkin

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 144, No. 8, pp. 202-204, August, 2007
Original article submitted March 20, 2007

Expression of transcription proteins PAX1, Hoxa3, and TLP regulating differentiation of thymic epithelial cells is detected in human thymus starting from gestation week 22 until the age of 95 years. Expression of transcription factors significantly decreased during aging. Apart from the decrease in the expression of signal differentiation factors in cultured thymic epithelial cells, proliferative activity of T lymphocytes cocultured with thymic epithelial cells also decreased in aging cultures, which demonstrated the important regulatory effect of transcription proteins on maturation and maintenance of T lymphocytes. Taking into account the important role of transcription proteins in the regulation of proliferation and function of T lymphocytes, whose number sharply decreases during aging, the maintenance of the level of expression of transcription factors during aging is a promising trend in modern biogerontology.

Key Words: *thymus; aging; transcription factors; confocal microscopy*

Normal development and function of thymic epithelial cells (TEC) play an important role in the maintenance of immunocompetent activity of T lymphocytes. Expression of signal factors for TEC differentiation in experimental animals at the early stages of ontogeny is well studied. Transcription proteins PAX1, Hoxa3, and TLP are expressed in mice starting from the embryonic stage of thymus development [2,3]. Expression starts in the early endodermal epithelium lining the foregut region and includes the epithelium of the third pharyngeal pouch, a structure giving rise to the thymus epithelium. [4]. Expression of these proteins (particularly of PAX1) decreases with age. In adult animals, only small number of TEC intensively express differentiation factors. The ratio of PAX1-immunopositive cells sharply decreases [1,5].

Taking into account the key role of protein expression in the thymic microenvironment that determines maturation of T lymphocytes, we studied age-related differences in the expression of signal differentiation factors of human TEC.

MATERIALS AND METHODS

We examined thymuses from people of different age groups died from somatic diseases. Autopsy specimens were obtained at Departments of Pathoanatomy in the D. O. Ott Institute of Obstetrics and Gynecology (Russian Academy of Medical Scien-

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

0007-4888/07/1442-0235

TABLE 1. Expression of Pax1, Hoxa3, and TLP in Human TEC during Ontogeny and Aging (*M*±*m*)

Group	Pax1		Hoxa3		TLP	
	OD, arb. units	S_{EX}, %	OD, arb. units	S_{EX}, %	OD, arb. units	S_{EX}, %
1	1.46±0.03	3.24±1.45	1.37±0.03	3.46±0.52	2.11±0.15	4.26±1.73
2	2.05±0.06*	3.36±1.54	1.44±0.02	5.28±1.13*	4.02±0.11*	5.76±1.18*
3	0.54±0.02*	1.24±0.06*	1.36±0.02	1.97±0.08*	2.02±0.09	3.12±0.07*
4	0.39±0.01*	0.99±0.03*	1.47±0.03	1.54±0.06*	0.97±0.02*	2.11±0.04*

Note. OD, optical density; S_{EX}, area of expression, *$p<0.05$ compared to group 1.

ces), St. Petersburg Municipal Hospital No. 3, and St. Petersburg Municipal Bureau of Pathoanatomy. Thymus samples from children (from 6 months to 4 years) were obtained during surgeries for congenital heart diseases at the V. I. Burakovskii Institute of Cardiosurgery (A. N. Bakulev Research Center of Cardiovascular Surgery, Russian Academy of Medical Sciences).

The samples were divided into 4 groups by ontogenetic characteristics: group 1, embryonic thymus (death on week 22 through 33 of intrauterine development); group 2, thymus from children (from 6 months to 4 years); group 3, thymus area from elderly people (65-79 years); and group 4, thymus area from old people (80-95 years).

In vitro studies of cell aging were performed on TEC cultures of passages 1, 4, and 7 (young, mature, and old cell cultures, respectively). VTEC2.H/S suspension cells obtained by transformation of embryonic TEC with SV40 virus and cloning of transformed cells were used as TEC. Thymocytes were isolated from fragments of human embryonic thymus (gestation week 20, abortion specimens). TEC were cultured individually (monocultures) or with thymocytes (1:10 cocultures). Cell count in tissue cultures was estimated by calculating the mean number of cells per 1 mm^2.

Immunofluorescence confocal microscopy was performed on sections obtained on a Leica CM 1850 cryostat (*in vivo* study) or nonfixed cell suspensions (*in vitro* study). Cryostat sections (7 μ) and smears of cell suspensions were dried overnight, fixed in acetone at -20°C for 10 min, and incubated with primary monoclonal antibodies against PAX1 (1:200), Hoxa3 (1:150), and TLP (1:100) for 1 h. Expression of signal molecules was studied using Vector Red kits for immunofluorescent visualization of alkaline phosphatase (Vector Lab.) according to manufacturer's recommendations. Levamisole (1.25 mM) was added during incubation with alkaline phosphatase to block endogenous enzyme activity.

The preparations were examined under a Leica TCS SP5 confocal microscope (×400, ×1000) using a MRC-1024 system and LaserSharp 5.0 software for computer processing of confocal microscopic images and construction of 3D images (Bio-Rad) at the Philip Biomedical Research Center (Spain).

Intergroup differences were evaluated by Student's *t* test.

RESULTS

Expression of transcription proteins PAX1, Hoxa3, and TLP regulating differentiation of TEC and maintaining maturation and function of T lymphocytes was verified in human thymus during embryogenesis (gestation week 22) and persisted until 95 years of life. Age-related differences were revealed in the localization of transcription protein expression. During the prenatal period and in children, expression of PAX1, Hoxa3, and TLP was identified in cortical and medullar TEC. During aging (groups 3 and 4) these proteins were expressed only in cortical TEC.

TABLE 2. Expression of TEC Differentiation Factors in Aging Cultures (*M*±*m*)

Number of TEC passages	Area of expression			Number of T lymphocytes per 1 mm^2
	Pax1	Hoxa3	TLP	
Passage 1 (young cultures)	2.24±0.08	1.74±0.06	2.06±0.16	23 254±1272
Passage 4 (mature cultures)	1.16±0.04*	0.72±0.03*	2.17±0.08	18 675±1178*
Passage 7 (old cultures)	0.39±0.01*	0.30±0.01*	2.38±0.12	14 242±927*

Note. *$p<0.005$ compared to passage 1.

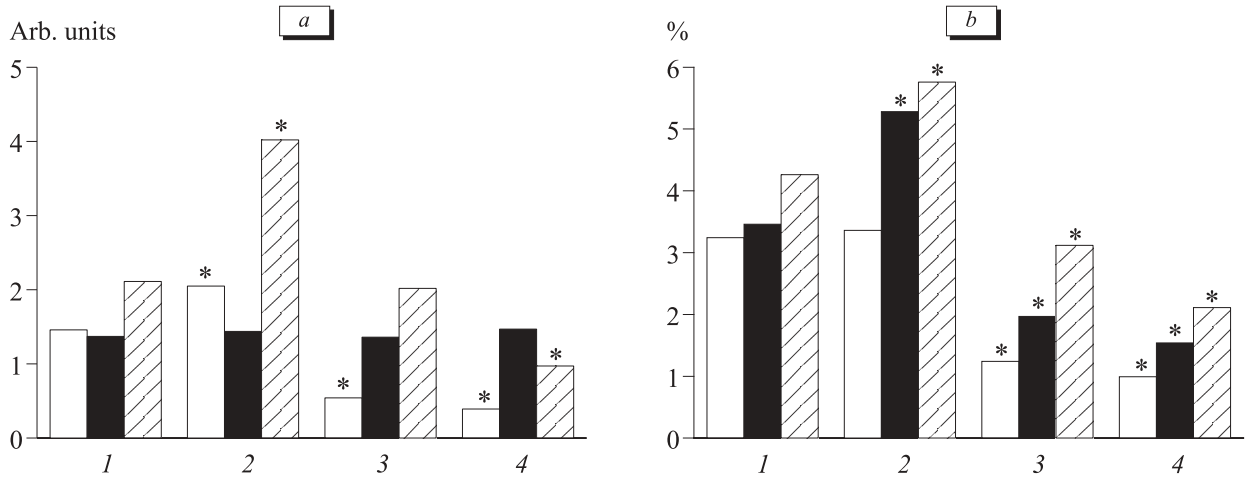

Fig. 1. Morphometric parameters of expression of transcription factors of human TEC differentiation during aging. Optical density (*a*) and area of expression (*b*). Light bars, Pax1; dark bars, Hoxa3; shaded bars, TLP. *1-4*, groups 1-4. **p*<0.005 compared to group 1.

Expression of transcription factors decreased with age (Table 1). Optical density and area of expression of PAX1 and TLP in TEC decreased during ontogeny and aging (Table 2). However, the synthesis of protein Hoxa3 in individual cells remained unchanged during aging. At the same time, we revealed a significant decrease in the total number of TEC expressing this factor (Fig. 1, *a*, *b*).

Apart from the decrease in the expression of signal factors for differentiation of cultured TEC, proliferative activity of T lymphocytes cocultured with TEC also decreased in aging cultures (Table 2). These data illustrate the regulatory effect of transcription proteins on maturation and maintenance of T lymphocytes.

Confocal microscopy verified the expression of signal factors for TEC differentiation in human thymus at the early stage of embryogenesis (proteins PAX1, Hoxa3, and TLP). The synthesis of these proteins significantly decreased during aging, which is consistent with the results of animal experiments.

Transcription proteins play a key role in the regulation of proliferation and function of T lymphocyte, whose number sharply decreases during aging. Hence, the development of new approaches to the maintenance of transcription factor expression is an urgent problem of modern biogerontology.

REFERENCES

1. V. O. Polyakova, I. M. Kvetnoi, and A. A. Yarilin, *Neuroimmunoendocrinology of the Thymus* [in Russian], St. Petersburg (2005).
2. L. P. Hale, *Ann. Diagn. Pathol*., **8**, No. 1, 50-60 (2004).
3. J. Gill, M. Malin, G. A. Hollander, and R. Boyd, *Nat. Immunol*., **3**, No. 7, 635-642 (2002).
4. H. Min, E. Montecino-Rodriguez, and K. Dozschkind, *Immunol. Rev*., **205**, 7-17 (2005).
5. C. Ramsen, S. Hassler, P. Marits, *et al*., *Eur. J. Immunol*., **36**, No. 2, 305-317 (2006).