Tactivin in the Regulation of Dexamethasone-Induced Apoptosis in Thymocytes

V. Ya. Arion*, V. I. Mel'nikova, M. A. Afanas'eva, S. N. Moskvina, L. A. Zakharova

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Supplement 2, pp. 109-111, April, 2007
Original article submitted February 18, 2006

The level of natural apoptosis in rat thymus on day 18 of embryo development attained 25%, while at subsequent terms it was about 5%. In the spleen, this parameter gradually decreased from 15 to 37% starting from day 18 of embryo development to postnatal day 30. Tactivin prevented the development of dexamethasone-induced apoptosis in thymocytes of 30-day-old rats, but had no effect on spontaneous apoptosis. Tactivin can be used as a modulator of apoptotic processes.

Key Words: *tactivin; apoptosis; thymocytes; rats; flow cytofluorometry*

Apoptosis, a genetically-controlled cell response, is the basis for the natural mechanism of positive and negative selection of lymphocytes in the thymus. Apoptosis maintains quantitative and qualitative composition of cells in the thymus during its development. This process is mediated via signal molecules: cytokines, gangliosides, and hormones, in particular, glucocorticoids [5,7,8,11,13]. They enhance or inhibit apoptosis at different stages. At the same time, the mechanisms underlying changes in the lifetime of lymphoid cells of the thymus are poorly understood, which necessitates the search for regulators of this phenomenon. There are published data on the influence of various bioregulatory peptides, *e.g.* thymic peptides, on the development of apoptosis in animal lymphocytes and human peripheral blood leukocytes [2,4]. Tactivin, a polypeptide preparation from cattle thymus, exhibits immunocorrective activity and regulates some key biological processes [6]. A modulating effect of Tactivin preparation [6], a natural complex of thymic peptides, on apoptosis of neutrophils from patients with systemic lupus erythrematous was demonstrated [3].

Here we studied the dynamics of natural apoptosis in cells of rat thymus and spleen during perinatal ontogeny and the effect of Tactivin on apoptosis induced by dexamethasone (DM).

MATERIALS AND METHODS

Experiments were carried out on Wistar rats (Stolbovaya nursery, Russian Academy of Sciences) on days 18 and 21 of embryonic development (E18 and E21) and on postnatal days 7, 14, and 30 (P7, P14, and P30). Pregnant rats were maintained under standard conditions with control illumination regimen (light from 5:00 to 19:00).

For isolation of cell suspension from the thymus and spleen, the rats of different age were sacrificed by cervical dislocation. Not less than 50 animals were used. The thymuses and spleens were homogenized in ice-cold phosphate buffered saline (PBS, 0.1 M phosphate buffer with 0.9% NaCl, pH 7.2-7.4) with glucose (10 mM), filtered through a capron filter, and centrifuged at 400*g* for 10 min. Thymus cells were incubated in 24-well plates (1×10^6 per well in 1 ml medium) in RPMI-1640 medium (Serva) containing 10% FCS (Gibko),

N. K. Kol'tsov Institute of Developmental Biology, Russian Academy of Science; Institute of Physicochemical Medicine, Federal Agency on Health Care and Social Development, Moscow ***Address for correspondence:*** svetlana.moskvina@ripcm.org.ru. S. N. Moskvina.

0007-4888/07/1443-0479 © 2007 Springer Science + Business Media, Inc.

2 mM glutamine (ICN), 10 mM HEPES buffer (ICN), and 50 μg/ml gentamicin (Gibko) for 6, 8, or 10 h at 37°C and 5% CO_2.

Apoptosis in thymocytes from 30-day-old rats were *in vitro* induced with DM. Thymocytes were precultured with Tactivin (0.01-50.00 μg/ml) for 2 h, then DM (10^{-6} M, Sigma) was added and culturing was continued for 4, 6, or 8 h. For evaluation of the effect of Tactivin on spontaneous apoptosis, thymocytes were incubated with the preparation for 6, 8, or 10 h without DM. Culture of intact or DM-induced cells without Tactivin served as the control.

Freshly isolated thymus and spleen cells or thymic cells obtained after culturing were washed with ice-cold PBS with glucose (10 mM) and fixed with 70% ethanol. The material was stored at -20°C until cytofluorometric analysis (not longer than for 7 days). Spontaneous and induced apoptosis in the thymus of pre- and postnatal rats was evaluated by flow cytofluorometry using DNA-specific dye propidium iodide. For evaluation of the level of apoptosis, fixed cells were washed with PBS (300*g*, 10 min). The pellet was resuspended in 1 ml PBS containing RNase A (100 μg/ml, Sigma) for RNA degradation and prevention of its binding with propidium iodide. The cells were incubated at 37°C for 20 min and then 1 μg/ml propidium iodide was added to the samples. Staining was carried out at room temperature in darkness for 20 min. Analysis of intracellular content of DNA was performed on a Coulter EPICS XL-MCL flow cytofluorometer. Fluor-

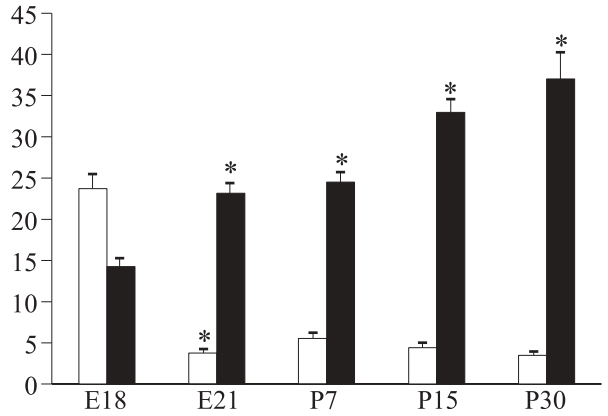
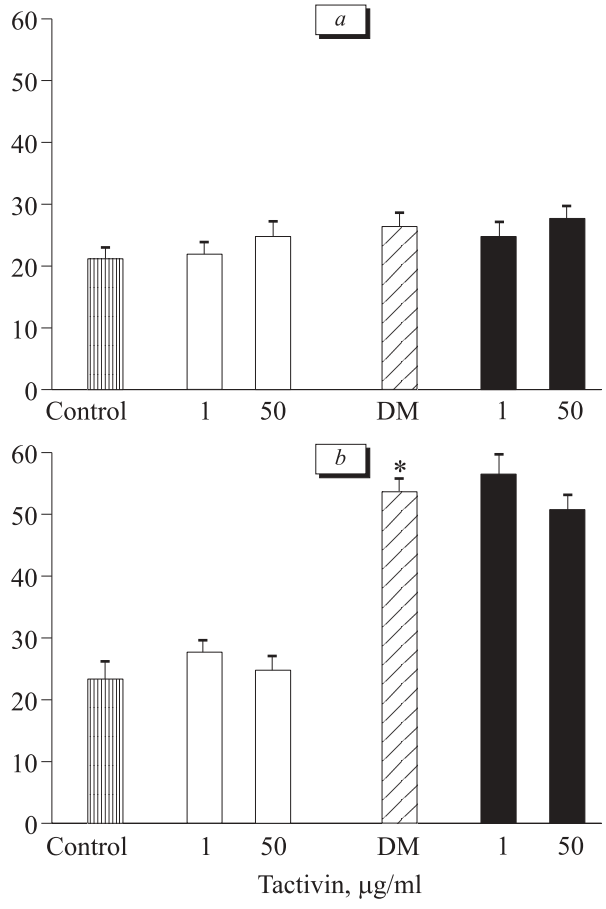


Fig. 1. Spontaneous apoptosis of cells in rat thymus and spleen in prenatal and early postnatal ontogeny. Ordinate: number of apoptotic cells, % from total number of analyzed cells. Light bars: thymus, dark bars: spleen. **p*<0.05 compared to E18.



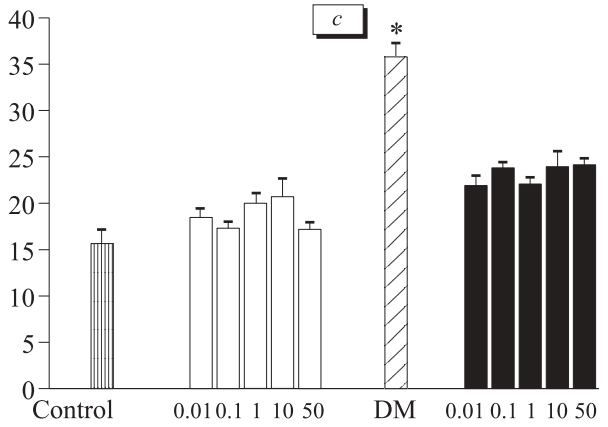


Fig. 2. Spontaneous and DM-induced (10^{-6} M) apoptosis of thymocytes in culture in the absence or presence of Tactivin (0.01-50.00 μg/ml). Cells were incubated with Tactivin for 2 h and then DM was added for 4 (*a*), 6 (*b*), and 8 h (*c*). Ordinates: number of apoptotic cells, % from total number of analyzed cells. Light bars: intact cells+Tactivin, dark bars: DM+Tactivin. *p*<0.05 compared to: *control, +DM

escence of at least 40,000 viable cells (according to sight scatter characteristics) was recorded. The number of apoptoic cells (forming a hypodiploid area on the histigram) was determined using Multi-Cycle software.

The data were processed statistically using non-parametric Wilcoxon's test.

RESULTS

The level of apoptosis in E18 fetuses attained 25%, but in E21 fetuses this parameter decreased to 4-5% and remained at this level until P30 (Fig. 1). In the spleen of E18 fetuses, the level of apoptosis was 15%; this parameter increased to 25% and 37% by E21 and P30, respectively (Fig. 1). Thus, apoptosis in the thymus of E18 fetuses was 5-fold more intensive than at other terms. This was probably related to migration of lymphoid precursors into the thymus from the liver in E16 [12] and, hence, activation of apoptotic processes. At the same time in the spleen, the percent of dying cells gradually increased to P30, probably due to hemopoietic cells. It is well known, that hemopoiesis in rat spleen is active during the first month after birth [10].

The level of natural apoptosis in thymocytes after 6-, 8-, and 10-h culturing was 15-20%. Tactivin added in various concentrations (0.01-50.00 μg/ml) to cultured thymus cells had no effect on cell death (Fig. 2). We previously showed that Tactivin did not modulate apoptosis of spleen cells in mature animals [1].

DM did not affect apoptosis during culturing with thymocytes for 4 h (Fig. 2, *a*), but 2-fold increased it during culturing for 6 and 8 h (Fig. 2, *b, c*). It is known that DM triggers cascade reactions of cell apoptosis; its effect develops after 12 h [9] *in vivo* and according to our data not less than after 6 h *in vitro*.

Addition of Tactivin prevented the development of DM-induced apoptosis only after 8-h culturing with thymocytes (Fig. 2, *c*).

Some synthetic peptides, *e.g.* low-molecular-weight peptide thymodepressin and neogen, modulate the development of spontaneous apoptosis of peripheral blood leukocytes from healthy donors [2]. Authors believe that cells carry specific sites on their surface, which mediate the influence of thymic peptides on the apoptosis program.

Our findings suggest that Tactivin prevents the development of induced apoptosis and does not affect processes mediating physiological cell death.

The work was supported by Russian Foundation for Basic Research (grant No. 05-04-48830).

REFERENCES

1. L. A. Zakharova, V. Ya. Arion, and I. Yu. Ermilova, *Alergol. Immunol.*, **5**, No. 4, 542-547 (2004).
2. A. A. Ivanova, E. V. Deigin, E. I. Vladimirskaya, *et al., Gematol. Transfusiol.*, **45**, No. 4, 9-10 (2000).
3. V. A. Romanov, A. G. Borodin, and I. L. Krylov, *Arkh. Ter.*, **5**, 65-69 (1992).
4. V. Kh. Khavinson and I. M. Kvetnoi, *Byull. Eksp. Biol. Med.*, **130**, No. 12, 657-659 (2000).
5. S. Aiello, M. Noris, G. Piccinini, *et al*., *J. Immunol.*, **164**, No. 9, 4649-4658 (2000).
6. V. Ya. Arion, *Sov. Ved. Rev. B. Phisicochem. Asp. Med.*, **2**, 1-57 (1989).
7. J. J. Cohen, *Semin. Immunol.*, **4**, No. 6, 363-369 (1992).
8. R. Dimri, Y. Sharabi, and J. Shoham, *J. Immunol.*, **164**, No. 5, 2479-2486 (2000).
9. K. J. Donner, K. M. Becker, B. D. Hissong, and S. A. Ahmed, *Cytometry*, **35**, No. 1, 80-90 (1999).
10. *M. A .S .Moore, Adv. Biosci*., **16**, 87-103 (1975).
11. N. Moulian, J. Bidault, C. Planche, and S. Berrih-Aknin, *Blood*, **92**, No. 4, 1297-1307 (1998).
12. E. P. Van Rees, C. D. Dijkstra, and T. Sminia, *Dev. Comp. Immunol.*, **14**, No. 1, 9-18 (1990).
13. J. Zhou, H. Shao, N. R. Cox, *et al., Cell. Immunol.*, **183**, No. 2, 90-98 (1998).