GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Adaptation to Heat Limits Stress-Induced Activation of Caspases in the Thymus

S. V. Kruglov*, L. A. Baida, M. G. Pshennikova, N. P. Larionov*, E. B. Manukhina, and I. Yu. Malyshev

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 134, No. 10, pp. 374-378, October, 2002
Original article submitted February 6, 2002

We demonstrated selective activation of caspases in the thymus during heat shock, *i.e.* primary activation of initiator caspase 9 (but not caspase 8) and effector caspase 3 (but not caspase 6). Preadaptation to heat improved animal survival after heat shock and reduced heat shock-induced activation of both initiator and effector caspases. Hence, adaptation to heat produced an antiapoptotic effect, which was not selective towards receptor-dependent or mitochondrial pathway of the caspase cascade activation.

Key Words: *apoptosis; caspases; thymus; heat shock; adaptation to heat*

The thymus is a central lymphoid organ with important endocrine (production of thymosine, thymuline, and peptide hormones) and immune (differentiation of T precursors into Th1 and Th2 cells responsible for cellular and humoral immune responses, respectively) functions. Maturation of T cells is accompanied by selection and elimination of potentially autoreactive T cells via activation of the apoptotic program leading to death of undesirable cells [10]. In this case apoptosis performs an important defense function and prevents the development of autoimmune processes in the organism. Inhibition of apoptosis in the thymus can cause tumor transformation of normal immune cells and induce many diseases, including lymphadenopathy, hepatosplenomegalia, hypersplenic syndrome, and autoimmune lymphoproliferative syndrome. Positively selected mature thymocytes migrate into the peripheral lymphoid organs and play the key role in the formation of adaptive immunity in response to exogenous bacterial or viral antigens.

Physiological functions of the thymus are regulated by the neuroendocrine system. Binding of pituitary hormones or acetylcholine to their receptors on thymic epithelial cells stimulates the release of thymic peptide hormones, while increased level glucocorticoid hormones during stress causes thymus atrophy via stimulation of thymocyte apoptosis [4]. Hypophysectomy leads to atrophy of the thymus with impairment of the immune response. Peptides and hormones produced by the thymus, in turn, modulate the functions of the neuroendocrine system. Thymectomy not only empairs the immunity, but also causes dysplasia of the anterior pituitary and promotes learning and memory disturbances during aging. This can be explained by impairment of thymus-dependent adrenoreceptor functions of the brain and suppressed regulatory function of the hypothalamus [9]. Thus, physiological regulatory relationships between the thymus and central systems of hormone and neuropeptide production constitute the key prerequisite for normal functioning of the immune and neuroendocrine systems.

Stress induces pronounced neuroendocrine and metabolic shifts and suppresses the physiological functions of the thymus. G. Selie considered the decrease of thymus weight as one of the major manifestations of stress reaction. Stress suppresses the immune system through activation of the hypothalamic-

Institute of General Pathology and Pathophysiology, Russian Academy of Medical Sciences, Moscow; *Vladimir State Pedagogical Institute

0007-4888/02/1344-0321$27.00

pituitary-adrenal system associated with increased blood levels of glucocorticoids and catecholamines, suppressed production of immunostimulatory hormones (prolactin, growth hormone, insulin-like growth factor-1), and intensification of apoptosis in the thymus [4].

Fever is an important and often obligatory component of the immune response to bacterial stress factors. On the one hand, hyperthermia leads to activation of endogenous defense systems, primarily to activation of heat shock protein (HSP70) synthesis. On the other hand, intense hyperthermia (heat shock, HS), being a potent stress factor, causes stress reaction, increases blood corticosteroid and catecholamine concentrations [1], and thus creates conditions for activation of apoptosis in immune organs. Previous studies demonstrated intensification of apoptosis, progressive decrease of the total thymocyte count, and imbalance between various cell subpopulations in the thymus after HS [14]. Thus, both heat exposure and immune reaction to pathogenic microorganisms can cause HS. Both exo- and endogenous HS suppress the immune system, specifically, via activation of apoptosis and disorders in thymocyte maturation and selection in the thymus.

A central role in apoptosis is played by caspases, proteases activated by the cascade principle: first initiator caspases are activated and they activate effector caspases. The receptor-mediated and mitochondrial pathways of caspase activation are now well studied. The pathway mediated by the so-called "death receptors" is triggered by external stimuli, death ligands (tumor necrosis factor-α or Fas) [7]. This pathway triggers activation of initiator caspase 8. Activation of caspases can be triggered by the release of proapoptotic cytochrome *c* signal molecule from mitochondria. This pathway triggers activation of initiator caspase 9 [11]. Initiator caspases activated by this or that pathway proteolytically activate effector caspases 3, 6, and 7. Effector caspases cleave target proteins by asparaginic acid residues triggering irreversible apoptosis [8].

Recent experimental and clinical data indicate that diseases associated with apoptosis disorders, such as diabetes, rheumatoid arthritis, neurodegenerative and cardiovascular diseases can be effectively prevented and treated by adaptation to various environmental factors [13]. This suggests that adaptation of the organism can prevent excessive intensification of apoptosis. At the same time, the effect of adaptation on the key mechanism of apoptosis, cascade activation of caspases, was never investigated and therefore the possibility of using adaptation for preventing and correcting stress-induced immunological and endocrine dysfunctions of the thymus was not evaluated.

Here we studied the patterns of activities of the initiator and effector caspases in the thymus during HS and the effects of adaptation on different stages of HS-induced cascade activation of caspases.

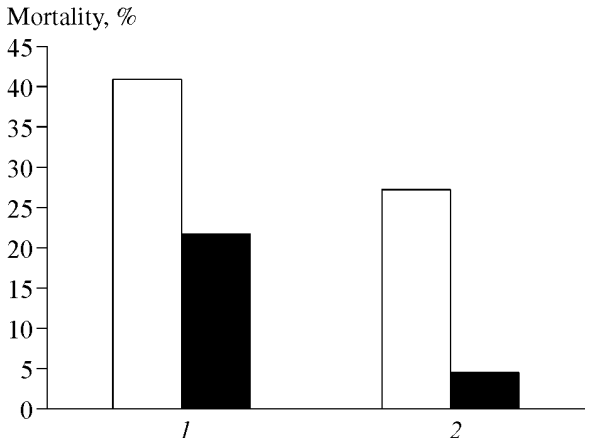

Fig. 1. Mortality of controls (light bars) and adapted rats (dark bars) during 25-min heat shock (*1*) and over subsequent 24 h (*2*). Here and in Fig. 2: *$p<0.01$ compared to the control.

MATERIALS AND METHODS

Experiments were carried out on male Wistar rats (220-240 g). Adaptation to heat was carried out for 6 days. The animals were daily placed in a thermostat at 41±1°C for 25 min.

For evaluation of the efficiency of adaptation, the controls and adapted animals were exposed to HS (25 min at 52±1°C, HS_{25}). The number of animals died during HS_{25} and over 24 h after HS_{25} was determined.

HS not leading to animal death (20 min 52±1°C, HS_{20}) at was used as activator of caspases in the thymus. The thymus was isolated 3 h after HS_{20} and frozen in liquid nitrogen.

For evaluation of caspase activities, the thymus was homogenized on ice in a glass homogenizer in a lysing buffer containing 10 mM HEPES (pH 7.2), 5 mM EGTA, 0.1% CHAPS, 5 mM dithiotreitol, 1.5 mM MgCl, and were protease inhibitor cocktail (all reagents from Sigma). The homogenate was centrifuged for 20 min at 20,000 rpm. Total protein in the supernatant was measured by the method of Lowry. Supernatant containing 650 μg protein was transferred into cuvette, and 10 μl substrate (Ac-DEVD-AFC for caspase 3, Ac-LETD-AFC for caspase 8, and Ac-LEHD-AFC for caspase 9), 40 μl buffer (250 mM HEPES, pH 7.4, 50 mM EDTA, 2.5% CHAPS, 125 mM dithiotreitol; BioRad) were added. The mixture was incubated at 25°C for 3 h and fluorescence of 7-amino-4-trifluromethylcoumarine (AFC) released during substrate cleavage was measured every 30 min. The release of AFC was calculated (in nmol/min) by a calibration curve constructed using free AFC [5].

The results were statistically processed using Student's *t* test and presented as $M \pm m$.

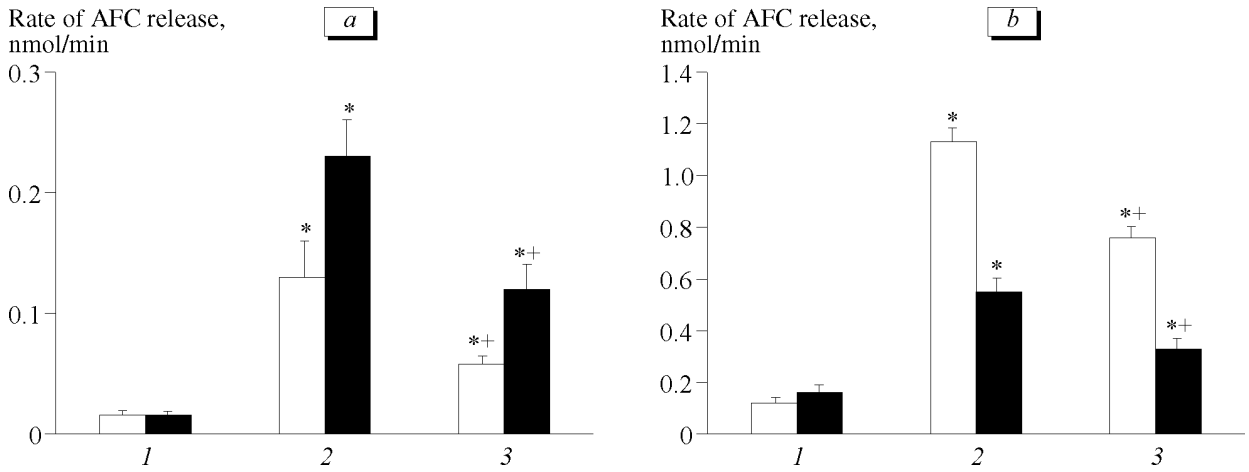

Fig. 2. Effect of adaptation to heat on activation of the initiator (*a*) and effector (*b*) caspases in the thymus induced by 20-min heat shock (HS_{20}). *1*) intact controls; *2*) HS_{20}; *3*) adaptation+HS_{20}. Light bars: caspases 8 (*a*) and 3 (*b*); dark bars: caspases 9 (*a*) and 6 (*b*). $^{+}p<0.05$ compared to HS_{20}. AFC: 7-amino-4-trifluoromethylcoumarine.

RESULTS

Adaptation decreased animal mortality during HS_{25} and over the subsequent 24 h (Fig. 1). During HS_{25} the mortality rates on the control and adapted groups were 41and 21%, respectively. During the subsequent 24 h the protective effect of adaptation was more pronounced: the mortality rate decreased almost 6-fold.

Hence, adaptation to heat considerably improved animal resistance to HS_{25}.

In intact controls activities of caspases 8 and 9 were 0.016±0.003 and 0.016±0.002 nmol/min, respectively. HS_{20} increased activities of caspases 8 and 9 to 0.13±0.03 and 0.23±0.04 nmol/min, respectively. Hence, activation of caspase 9 during HS_{20} was almost 2-fold more pronounced (Fig. 2, *a*).

Preadaptation decreased HS-induced activation of both initiator caspases by a half (Fig. 2, *a*).

HS_{20} led to activation of both effector caspases: activity of caspase 3 increased 9.4-fold and activity of caspase 6 increased 3.4-fold (Fig. 2, *b*). Similarly as for the initiator caspases, preadaptation 1.5-fold decreased HS_{20}-induced activation of both effector caspases.

Hence, we first demonstrated selective activation initiator caspase 9 and effector caspase 3 in the thymus during HS. More pronounced activation of caspase 9 in comparison with caspase 8 during HS suggests that in animals exposed to HS apoptosis is triggered mainly by mitochondrial dysfunction, while the receptor-dependent pathway mediated by caspase 8 is less significant. This hypothesis is in line with the data that induction of apoptosis during HS is mediated by activation of stress-induced proteinkinase c-Jun (Janus) N-terminal kinase (JNK) [3], which, in turn, promoted the release of cytochrome *c* from mitochondria [15]. Dysfunction of mitochondria in thymocytes and other cells during HS was also proved [8].

We found that preadaptation to heat improved animal survival after HS and decreased HS-induced activation of both initiator and effector caspases to the same extent. This means that antiapoptotic effects of adaptation are not selective towards the receptor-dependent or mitochondrial pathway of the caspase cascade activation, i.e. adaptation activates mechanisms inhibiting both pathways of apoptosis induction. The most likely candidate for such a universal mechanism is adaptation-induced accumulation of heat shock proteins HSP70 in different organs [12]. HSP70 can limit both caspases 9 and 8 [2].

The possibility of preventing apoptosis by preliminary single HS was experimentally demonstrated on animals and cell cultures. A significant feature of this protection is that preliminary HS causes cell damage, which can result in activation of antiapoptotic mechanisms. For example, in the thymus preliminary HS protected thymocytes from apoptosis induced by dexamethasone or calcium ionophore, but at the same time it caused considerable fragmentation of cell DNA [14]. Here we demonstrated that antiapoptotic protection of the thymus can be attained through preliminary repeated mild thermal exposures. The mechanisms of this protection deserve further investigation. It is however obvious that adaptation can be effective in preventing and probably correction of disorders caused by pathological intensification of apoptosis in immune organs.

The study was supported by the Russian Foundation for Basic Research (grant No. 00-04-48808) and INTAS-OPEN (grant No. 524).

REFERENCES

1. I. Brenner, P. N. Shek, J. Zamecnik, and R. J. Shephard, *Int. J. Sports Med.*, **19**, 130-143 (1998).
2. C. Garrido, S. Gurbaxani, L. Ravagnan, and G. Kroemer, *Biochem. Biophys. Res. Commun.*, **286**, 433-442 (2001).

3. A. W. Caron, D. D. Mosser, L. Bourget, *et al.*, *Mol. Cell. Biol.*, **17**, 5317-5327 (1997).
4. J. J. Cohen, *Semin. Immunol.*, **4**, 363-369 (1992).
5. A. M. Gorman, U. A. Hirt, and B. Zhivotovsky, *J. Immun. Methods*, **226**, Nos. 1-2, 43-48 (1999).
6. D. R. Green, *Cell*, **94**, No. 6, 695-698 (1998).
7. T. S. Griffith and T. A. Fergusson, *Immunol. Today*, **18**, 240-244 (1997).
8. H. E. Kaiser and B. Bodey, *In Vivo*, **14**, No. 6, 789-803 (2000).
9. Y. Kinoshita and F. Hato, *Cell. Mol. Biol. (Noisy-le-Grand)*, **47**, No. 1, 103-117 (2001).
10. H. Kishimoto and J. Sprent, *Clin. Immunol.*, **95**, 3-7 (2000).
11. G. Kroemer, N. Zamzani, and S. A. Susin, *Immunol. Today*, **18**, 44-51 (1997).
12. A. Maloyan, A. Palmon, and M. Horowitz, *Am. J. Physiol.*, **276**, R1506-R1515 (1999).
13. F. Z. Meerson, *Essentials of Adaptive Medicine: Protective Effects of Adaptation*, Moscow (1994).
14. G. Migliorati, I. Nicoletti, F. Crocicchio, *et al.*, *Cell Immunol.*, **43**, No. 2, 348-356 (1992).
15. C. Tournier, P. Hess, D. D. Yang, *et al.*, *Science*, **288**, No. 5467, 870-874 (2000).