Peculiarities of Thymus Pathology in Regional and Generalized Herpetic Infections

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The use of methods for evaluation of the immune status of patients with different forms of herpetic infection has revealed certain immunological disorders, which, in turn, have made it possible to consider infection caused by the herpes simplex virus (HSV) as a candidate for the group of virus-induced immunodeficiencies [1,2,5]. This concept is confirmed by data attesting to a long-term persistence of HSV, accompanied in many cases by productive infection of practically all cells of the immune system, leading to their functional incompetence and promoting the development of immunodeficiency. In such cases a decrease in the total number of T lymphocytes, reduction of their functional activity, imbalance of regulatory subpopulations, and some other disturbances occur [7-11]. The developing functional insufficiency of the immune system is one of the main reasons underlying chronic recurrence of herpetic infection. On the basis of an analysis of serum activity of the thymus in patients with ophthalmoherpes, a number of researchers and clinicists point to the existence of a pathogenetic connection between an aggravated recurrent course of infection, on the one hand, and the state of the endocrine function of the thymus, on the other [3].

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The present study was undertaken to analyze the possibility and peculiarities of thymus involvement in the pathological process in different clinico-pathogenic forms of herpetic infection.

MATERIALS AND METHODS

The experiments were performed on 18 chinchilla rabbits weighing 1.5 kg. Generalized herpetic infection was modeled by retrosternal administration of 10% virus-containing brain suspension from a mouse which had died of herpetic encephalitis (HSV type I, strain C1, titer 10^{-5-7} LD_{50}/0.03 ml); ophthalmoherpes was modeled by rubbing virus-containing suspension onto scarified cornea preliminarily treated with tetracain. Control uninfected animals were divided into three groups (3 animals in each). In the first and second control groups inoculation was omitted and the animals were killed, respectively, on the first and the last (30th) day of the experiment. The animals of the third control group were subjected to retrosternal inoculation of 10 % brain suspension from a healthy mouse (1.0 ml). The experimental animals that survived and all control animals were killed one month after the beginning of the experiment and their thymus, spleen, lymph nodes and liver were taken for examination and analysis. Analogous organs for investigation were removed from the dead animals of the experimental group.

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Detection of HSV antigens in cells of different organs and morphological analysis were performed as described earlier [2]. The relative and absolute number of lymphocytes, and B and T lymphocytes in the peripheral blood was determined after Petrov et al. [4].

RESULTS

As established earlier, retrosternal infection of rabbits weighing 1.5 kg leads to the development of a generalized herpetic infection characterized by a high lethality (up to 80%, death occurred within 15-55 days) and involvement of the visceral organs and central nervous system. When virus-containing suspension is rubbed into rabbit cornea, a typical picture of herpetic keratoconjunctivitis develops, which ends in spontaneous recovery within 27-30 days. Mortality in young rabbits reaches 25-35%; as a rule, animals die with clinical signs of acute encephalitis within 20-35 days.

In our experiments one rabbit in the group of retrosternally infected animals died on the 26th day, while the other animals were killed 30 days after infection. The data on the weight of the body, thymus, and spleen are presented in Table 1. As follows from the table, there were no significant differences in the weight of the thymus and spleen or the weight coefficients of these organs for three control groups (either at the beginning of the experiment or after one month, in intact rabbits and in those inoculated with placebo). The weight of the thymus and its weight coefficient in rabbits infected retrosternally were reliably lower one month after infection (2.5-3 times) in comparison with the control animals. At the same time, the analogous indexes for the spleen in rabbits infected retrosternally did not differ significantly from those in the control groups of animals.

Immunohistochemical analysis of the thymus, spleen and lymph nodes in infected rabbits by indirect fluorescence revealed HSV antigens only in thymic cells (thymocytes and epithelial cells).

Morphological analysis of the thymus, spleen and lymph nodes in the control animals did not reveal any pathological changes or significant differences. In thymic sections of rabbits infected retrosternally the regions with marked destruction and those where the organ structure was preserved could be clearly differentiated. In the regions with preserved structure the thymocytes become fewer in the cortical zone, while in the medullar zone this thinning is even more expressed, and the number of Hassal's corpuscles is significantly reduced. In the affected regions it is possible to detect Clarke's bodies varying in shape and size. In thymic cells vacuolar dystrophy, lysis of thymocytes, formation of necrotic regions, and necrobiosis were observed. Both epithelial and lymphoid cells were affected. In the lymph nodes of these animals we observed depletion of the paracortical zone, and expansion of the germinal centers and medullary threads. Thinning of lymphoid cells and an increase in the number of replication centers were also observed in the spleen, T zones were almost unexpressed.

Thus, histological analysis shows that retrosternal infection of rabbits affects both the cortical and medullary thymic zones with the formation of regions of necrosis and necrobiosis; HSV antigens can be detected in these cells. In the peripheral lymphoid organs only the T zones are affected and the presence of abundant germinal centers indicates a pronounced humoral response.

Analysis of sera from infected animals by indirect immunofluorescence has shown that on the 8th day after infection the titer of anti-HSV antibodies constituted 1:16 and rose to 1:32-1:64 by the end of the experiment. It is noteworthy that in rabbits with ophthalmoherpes or those infected internasally the antibody titer after 30 days reached, as a rule, a higher level: 1:64-1:128.

The possible involvement of the thymus in the development of immunosuppression in cytomegaly, measles and AIDS has been discussed elsewhere [12,13].

TABLE 1. Changes in the Weight of the Body, Spleen, and Thymus and their Weight Coefficients in Rabbits after Retrosternal Infection with HSV

Index	Group I: control animals killed on the first day	Group II: control animals killed after 1 month	Group III: control animals killed 1 month after injection of brain suspension from a healthy mouse	Experimental animals killed after 1 month
Body weight, kg	1.5±0.3	2.2±0.2	2.0±0.1	2.0±0.2
Weight of thymus, g	2.8±0.2	3.2±0.1	3.0±0.2	1.2±0.1*
Weight of spleen, g	1.4±0.1	1.6±0.1	1.5±0.1	1.4±0.1
Weight coefficient of thymus, ‰	1.87±0.12	1.45±0.14	1.50±0.17	0.60±0.08*
Weight coefficient of spleen, ‰	0.93±0.11	0.73±0.07	0.75±0.09	0.70±0.05

Note. * Changes reliable with respect to indexes of control animals of all three groups (p...0.05).

TABLE 2. Dynamics of Immunological Indexes during the Experiment

Animal group	Period of analysis	Lymphoc	B-lymphocytes		T-lymphocytes				Theophylline-resistant theophylline-sensitive
			%	Abs. number, $\times 10^9/l$	%	Abs. number, $\times 10^9/l$	Theophylline-resistant, %	Theophylline-sensitive, %	
Experimental animals infected with HSV retrosternally	before infection (1)	5.7±0.1	9±0.3	0.8±0.1	53±1.7	5.0±0.5	45±3.0	10±0.7	4.5±0.5
	5th day (2)	5.2±0.2	12±0.1*	0.7±0.1	30±2.0*	3.2±0.2*	32±1.3*	8±0.7	4.0±0.5
	12th day (3)	5.0±0.2*	12±0.7*	0.9±0.1	40±2.3*	2.3±0.2*	21±1.3*	8±0.3	2.6±0.3*
Control animals (group III) inoculated retrosternally with placebo	before infection (4)	5.6±0.1	10±1.7	0.9±0.1	59±2.7	4.7±0.1	44±0.7	9±1.0	4.9±0.5
	5th day (5)	5.3±0.2	10±1.0	1.0±0.1	58±1.0	4.8±0.1	45±2.0	11±1.3	4.3±0.3
	12th day (6)	5.6±0.1	11±0.3	0.9±0.2	60±2.7	5.2±0.2	45±1.3	10±1.0	4.5±0.5
		$p_{1-3}\leqslant 0.05$ $p_{3-6}\leqslant 0.02$	$p_{1-2}\leqslant 0.05$ $p_{1-3}\leqslant 0.02$		$p_{1-2}\leqslant 0.001$ $p_{1-3}\leqslant 0.02$	$p_{1-2}\leqslant 0.05$ $p_{1-3}\leqslant 0.05$ $p_{2-5}\leqslant 0.001$ $p_{3-6}\leqslant 0.001$	$p_{1-2}\leqslant 0.05$ $p_{1-3}\leqslant 0.01$ $p_{2-5}\leqslant 0.01$ $p_{3-6}\leqslant 0.001$	$p_{1-3}\leqslant 0.05$ $p_{3-6}\leqslant 0.05$ $p_{2-5}\leqslant 0.01$ $p_{3-6}\leqslant 0.001$	

Detection of viral antigens in thymic histosections attests to the possible replication of HSV in thymic cells. These results are in good agreement with the data on replication of measles virus, HIV and cytomegaly virus in epithelial cells of the thymus in humans and mice [12,13], as well as replication of HSV in transformed or stimulated, but not in intact lymphocytes [6]. In the latter case, the ability of HSV to replicate is usually connected with hyperexpression of receptors to this virus in stimulated lymphocytes.

It is known that in the cortical layer of the thymus there occurs an active propagation of thymocytes. However, cortical lymphocytes are immature and differentiate progressively into mature T lymphocytes. Afterwards, they migrate into the medullary layer and finally into the blood. The results of morphological investigations providing evidence of the destruction of all thymic elements correlate with the data on functional disorders of the thymus obtained in the study of the dynamics of some immunological indexes in infected and control rabbits (Table 2). In infected animals the total lymphocyte content was reduced and this reduction became reliable by the 12th day. However, the absolute number of B lymphocytes did not differ from the data for control animals, while that of T lymphocytes was reliably lower on the 5th and 12th days. In the T-lymphocyte population we registered a reliable decrease (more than 2-fold) in the share of theophylline-resistant lymphocytes, the share of theophylline-sensitive T lymphocytes was also slightly reduced.

When a regional herpetic infection was developed in the form of ophthalmoherpes, morphological changes in the thymus included a slight thinning of lymphoid tissue in the cortical zone, while in the medullary zone we failed to reveal any noticeable deviations in comparison with the control. Unlike in animals with a generalized form of infection, the cortico-medullary structure of the thymus in animals with a regional herpetic infection was preserved. The absence of HSV antigens in thymal cells in these animals attests that there still remains the blood-thymus barrier which protects this organ from the penetration of foreign antigens. In the spleen the proliferation of follicles and germinal centers is well expressed, marginal zones are reduced, and T-zones are well marked. In the lymph nodes we observed hyperplasia of follicles and formation of replicative centers. We did not detect HSV antigens on the 30th day of the experiment in the cells of the spleen and lymph nodes.

The results obtained correlate with the data on morphological changes in the organs of immunogenesis in newborns and babies who have died of generalized herpetic infection. In all cases the accidental transformation of the mature type was observed in the thymus (in most cases - of the 1st-2nd degree, in 3 cases - of the 3rd-5th degree), lymphoid follicles in the spleen were not numerous and small, and the B-zones were well preserved. In premature babies changes in the thymus and reduction of lymphoid follicles in the spleen were even more pronounced [2].

Thus, in animals with a regional herpetic infection (ophthalmoherpes) only insignificant signs of thymic pathology can be registered against the background of a strong immune response. A slight rarefaction in the cortical zone of the thymus evidences structural changes in the main organ of the immune

system which may lead to functional disorders. The most marked structural-functional changes in the thymus were revealed in animals with a generalized herpetic infection. These findings justify the includsion of immunomodulating preparations such as Thymogen, Tactivin, and Timalin, created on the basis of active peptides of the thymus, into complex etiopathogenetic therapy of herpetic infection, in particular, of its generalized form.

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ONCOLOGY

Suppressor Activity of Nonadhesive Bone Marrow Cells is Determined by the Cells Bearing Erythroblast Antigen

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Bone marrow (BM) of humans and animals is known to have so-called natural supressor cells (NSC), which are able to inhibit mitogen- and antigen-stimulated proliferation of lymphocytes, proliferation of T cells and formation of cytotoxic T lymphocytes in MLC, as well as proliferation of tumor cells *in vitro* [5,7-10]. NSC have been shown to have receptors to the wheat germ agglutinin; however, they have no markers of mature immunocompetent cells [9]. Elimination of the cells bearing erythroblast antigen from BM pro-

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