

MORPHOLOGICAL CHANGES IN THE THYMUS AFTER THYMALIN ADMINISTRATION
IN EXPERIMENTAL OSTEOMYELITIS

M. M. Solov'ev, T. K. Supiev,
I. I. Grintsevich, G. M. Alekhova,
V. G. Morozov, and V. Kh. Khavinson

UDC 616.71-018.46-002-092.9-07:616.438-091

KEY WORDS: thymus; thymalin; experimental osteomyelitis.

The study of the hormone-like factors of the thymus is of great practical importance because its aim is to promote their wider use both experimentally and in clinical practice to correct disturbances of the immune system [1, 4, 5].

The aim of this investigation was to study the effect of thymalin [2] on the morphological and functional state of the thymus in rats of different ages in experimental osteomyelitis of the lower jaw.

EXPERIMENTAL METHOD

Experiments were carried out on 100 noninbred male albino rats of two age groups: young animals weighing 80-100 g and aged 4-6 weeks (n = 65) and old rats weighing 350-400 g and over 18 months old (n = 35). Osteomyelitis was produced by subperiosteal injection of a 24-h culture of *Staphylococcus aureus* [3]. All the animals after this injection were divided into two groups: experimental and control. The experimental rats were given thymalin in a dose of 0.2 mg/kg body weight intramuscularly once daily in the form of prophylactic and therapeutic courses. The prophylactic course of thymalin given to 18 old and 20 young rats began 2 days before injection of the microbial culture and continued 5 days after infection. Another 24 young rats were given thymalin as a therapeutic course, i.e., daily for 7 days from the 5th day after injection of the microbial culture. Animals of the control group received physiological saline at the corresponding times. Signs of a local inflammatory process and general changes in the body were recorded in all the rats. On the 14th and 28th days the animals were decapitated for morphological investigations. Tissue of the thymus was fixed in 10% neutral formalin solution. Paraffin sections were stained with hematoxylin and eosin, azure II-eosin, with methyl green and pyronine by Unna's method and picrofuchsin by Van Gieson's method. The mean index of destruction of bone tissue was determined macroscopically in specimens of the lower jaw. The microscopic description of the thymus tissue was based on the results of morphometry. The experimental results were subjected to statistical analysis by parametric and nonparametric methods.

EXPERIMENTAL RESULTS

Prophylactic injection of thymalin in old rats had a beneficial effect on the course of the inflammatory process and caused a statistically significant decrease in the area of destruction of bone tissue ($12.89 \pm 1.56 \text{ mm}^2$ in the experiment, $27.93 \pm 5.22 \text{ mm}^2$ in the control, $P < 0.05$). A tendency toward absorption of the inflammatory infiltrate was noted 2 weeks after infection in soft tissues adjacent to the inflammatory focus. Microscopic examination of the thymus 2 weeks after infection revealed signs of restoration of its structure. The relative weight of the gland increased (0.94 ± 0.12 in the experiment, 0.48 ± 0.11 in the control, $P < 0.05$), and larger lobules with clear subdivision into layers began to predominate. The thickness of the cortex of the lobules increased and the ratio between cortex and medulla became much greater than in animals of the control group (0.97 ± 0.04 in the experiment, 0.56 ± 0.02 in the control, $P < 0.05$). The number of cells per conventional unit of area of

Department of Pediatric Stomatology and Maxillofacial Surgery, Academician I. P. Pavlov First Medical Institute, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.) Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 95, No. 6, pp. 123-124, June, 1983. Original article submitted November 19, 1982.

the cortex increased (141.80 ± 7.88 in the experiments, 114.60 ± 7.11 in the control, $P < 0.05$) mainly on account of small lymphocytes and lymphoblasts. After 4 weeks the relative weight of the thymus showed some decrease but still remained higher than in the control. As before large lobules with distinct subdivision into layers predominated. The nuclei of the cortical reticuloepithelial cells of the lobules of the thymus 2 weeks after infection were larger in the experimental animals than in the control rats (experiments 0.1171 ± 0.0046 conventional unit - c.u. - 0.1039 ± 0.0016 c.u. in the control, $P < 0.05$); after 4 weeks they were almost identical in size. The nuclei of the reticuloepithelial cells of the medulla were larger in the experimental animals at both times of observation. After 2 weeks, in animals, receiving thymalin they measured 0.1530 ± 0.0044 c.u. compared with 0.1388 ± 0.0028 c.u. in the controls ($P < 0.05$). The mean area of cross section of nuclei of the cortical reticuloepithelial cells of the lobules 4 weeks after infection was 0.1622 ± 0.0044 c.u. in the experimental animals and 0.1532 ± 0.0062 c.u. in the controls. The number of cells per unit area was the same in both experiment and control in the 4th week. In most animals of both groups plasma cells were equally numerous in the 2nd week, but after 4 weeks very few plasma cells were found in the control rats, and none whatever in animals receiving thymalin.

In young rats receiving a prophylactic course of thymalin an improvement in the course of the inflammation was observed in the 2nd week of the experiment. A small increase was observed in the relative weight of their thymus (1.53 ± 0.24 compared with 1.45 ± 0.36 in the control) with an increase in size of the lobules with distinct subdivision into layers, and an increase in size of the nuclei, but only of the medulla in the thymus lobules (0.1494 ± 0.0075 c.u. in the experiment, 0.1328 ± 0.0068 , $P < 0.05$) in the control. However, the number of cells per unit area of the cortex was less than in the control (experiment 115.00 ± 5.0 , control 132.25 ± 0.87 , $P < 0.05$). Starting from the 3rd week after discontinuing thymalin, the course of the inflammatory process deteriorated sharply in nearly all the rats. The areas of infiltration in adjacent soft tissues began to increase in size, but sinuses were formed in only 33% of the animals. The area of bone destruction was almost identical in the animals of both groups (13.23 ± 3.12 mm² in the experiment, 11.88 ± 1.67 mm² in the control). Spreading of the inflammatory process into adjacent muscle tissues was frequently observed. Signs of accidental involution were observed, on the one hand, in the thymus at the 4th week: a low relative weight, a decrease in size of the lobules, foci of depopulation of lymphocytes; but on the other hand the number of cells per unit area of the cortex was increased in places where depopulation was not found, mainly on account of small lymphocytes. The nuclei of the reticuloepithelial cells in the experimental rats in the 4th week were the same size as in the 2nd week.

In the young rats receiving a therapeutic course of thymalin the mean index of destruction of bone tissue was reduced almost by half (5.10 ± 1.31 mm² in the experiment, 10.73 ± 1.71 mm² in the control, $P < 0.05$). Small foci of infiltration in the soft tissues of the lower jaw had a tendency to be absorbed from the very beginning. In the thymus a clear picture of accidental involution was observed after 2 weeks. The relative weight fell to 0.92 ± 0.23 (1.45 ± 0.36 in the control, $P < 0.05$) and the lobules decreased in size; the reduction in the number of cells per unit area of cortex to 89.75 ± 9.81 (132.25 ± 0.87 , $P < 0.05$) was replaced by the 4th week by restoration of the structure of the thymus, with an increase in its relative weight to 1.71 ± 0.29 and by restoration of the cell population mainly on account of small lymphocytes and lymphoblasts.

Administration of thymalin as a prophylactic course thus had a beneficial effect on the course of the disease in old rats, whereas in young animals cessation of administration of thymalin led to the appearance of signs of further progression of the inflammatory infectious process. However, a therapeutic course of thymalin given to young rats was followed by a lasting beneficial effect. Thymalin helped to restore the structure of the thymus in old animals. In young rats receiving thymalin as a prophylactic course, the severity of the clinical manifestations of the disease did not correspond to the functional state of the thymus.

When the results of the morphological study of the thymus are interpreted it must be recalled that in the old animals signs of age involution were present in the thymus when thymalin administration began, and the activity of the immune system was depressed. In young animals receiving thymalin as a therapeutic course the thymus also underwent acute involutional changes on account of the developing inflammatory process. In both cases thymalin administration gave good results. Preliminary administration of thymalin to young intact rats perhaps depressed the existing activity of the immune system to some degree. The results confirm the well known view that reactivity of an organism changes with age, and this must be taken into account when measures aimed at correcting the immune system are planned.

LITERATURE CITED

1. V. Ya. Arion, in: Progress in Science and Technology. Immunology Series [in Russian], Moscow (1981), pp. 10-50
2. V. G. Morozov and V. Kh. Khavinson, Dokl. Akad. Nauk SSSR, 240, No. 4, 1004 (1978).
3. M. M. Solov'ev, Stomatologiya, No. 6, 40 (1969).
4. A. K. Goldstein, A. Guha, M. M. Zatz, et al., Proc. Natl. Acad. Sci. USA, 69, 1800 (1972).
5. N. Trainin, Physiol. Rev., 54, 272 (1974).