INVOLUTION AND REGENERATION OF THE MOUSE THYMUS AFTER LIPOPOLYSACCHARIDE INJECTION

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It is a well-known fact that lipopolysaccharide (LPS) isolated from the outer membrane of Gram-negative bacteria possesses high immunoregulatory activity, which cannot be reduced simply to interaction with B cells [9, 14]. Bacterial endotoxins bind with numerous lymphoid cells in the body, including with T lymphocytes [5]. The various systems of the body and organs, including the thymus, respond to injection of LPS. In particular, we know that injection of LPS leads to a marked decrease in weight of the thymus and to a change in its immunologic activity [2, 3, 13]. However, processes taking place in the thymus after injection of LPS have not been adequately studied. The aim of this investigation was to study the dynamics of the morphological and proliferative changes induced in the thymus by LPS in a dose causing polyclonal activation of splenic B cells.

EXPERIMENTAL METHOD

Experiments were carried out on male (CBA $\times$C57BL/6)F_1 mice weighing 17.0-19.0 g, obtained from the "Stolbovaya" nursery, Academy of Medical Sciences of the USSR. LPS isolated from *Shigella sonnei* by Boivin's method (strain 9090, series 568) was injected intraperitoneally in a single dose of 50 μg per mouse. The polyclonal immune response was assessed as the number of AFC in the spleen by a modified method of Jerne and Nordin [1], against trinitrophenyl (TNP), using a conjugate of trinitrobenzenesulfonic acid ("Serva," West Germany) with sheep's erythrocytes. Vinblastine sulfate ("Gedeon Richter,") was injected intraperitoneally in a single dose of 1.5 mg/kg 5 h before sacrifice. The mice were killed at the same time of day, 1, 2, 3, 5, 7, and 13 days after injection of LPS (group 1). Some of the animals receiving LPS also received vinblastine (group 2). Control animals received neither preparation (group 3) or vinblastine alone (group 4). Every day five animals were selected from groups 1 and 2 and eight animals in groups 3 and 4. After weighing, the thymus was fixed in Bouin's fluid and morphometric analysis carried out with the aid of Avtandilov's grid in 4-μ paraffin sections stained with azure II-eosin. The relative areas of the cortex and medulla and of nonparenchymatous tissue (connective and adipose tissue, blood vessels, cysts) were calculated under magnification of 20$\times$ (objective) and 10$\times$ (ocular). The density of the thymocytes (Tc), and the number of mitoses (Mi), blast cells (Bl), pycnotic nuclei (Py), and plasma cells (PlC) was determined under magnification of 90$\times$ (objective) and 10$\times$ (ocular) in 40 small squares of the grid in the subcapsular zone and the cortex and medulla (absolute and relative (percentage) numbers). The numerical data were subjected to statistical analysis by the Wilcoxin—Mann—Whitney nonparametric U test.

EXPERIMENTAL RESULTS

LPS in a dose of 50 μg/mouse induced a polyclonal immune response in the spleen, revealed by the presence of AFC against TNP (Fig. 1) with a peak on the 1st-2nd days. Even on the 13th day the level of anti-TNP AFC was 17 times greater than the background value. This was accompanied by a thymolytic effect, manifested clearly as a decrease in weight of the thymus and its cell density (Fig. 2a). The maximal decrease in weight of the thymus (by 2.5-3 times) was observed on the 3rd day after

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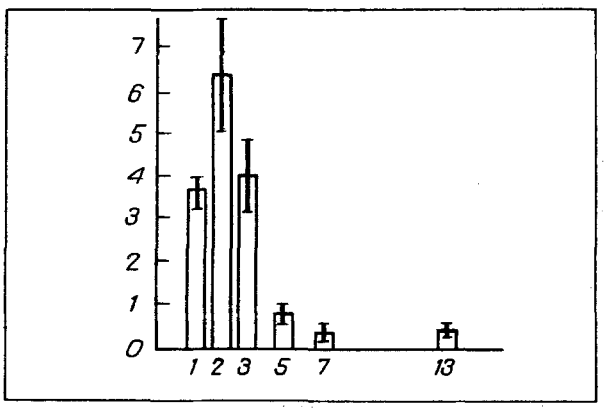

Fig. 1. Time course of number of anti-TNP AFC in mouse spleen after injection of LPS. Abscissa, time after injection of LPS (days); ordinate, number of anti-TNP AFC ($\times 10^{-4}$) per spleen.

injection of LPS. The maximal fall of cell density was observed on the 1st day – down to 60-70% of normal. Starting with the 5th-7th day these parameters began to recover, and recovery was complete by the 13th day. In this case restoration of cell density preceded restoration of the weight of the organ.

Atrophy of the thymus induced by LPS and its subsequent recovery were accompanied by the following morphological changes in the organ. The structures of the thymus was disturbed 24 h after injection of LPS: in some cases the distinct division into cortex and medulla disappeared, with an unequal cell density throughout the organ, whereas in other cases typical "inversion" took place, with a decrease in area of the cortex and of the cell density in it, accompanied by an increase in area of the medulla and of the cell density in it. The incidence of B1 and Mi in the cortex and, in particular, in the subcapsular zone fell sharply by 7 and 20 times, respectively. Meanwhile, the number of Py showed a sharp increase (about sixfold pCTL-2 throughout the cortex. Over the whole thymus, but more especially in the cortex, cavities filled with solitary Py or clusters of them, and also with neutrophils, appeared, together with laminar structures from large epithelial cells. The vessels were dilated and congested and surrounded by clusters of B1. Profusely scattered PlC appeared and their number in the cortex reached 0.5%.

On the 2nd day after injection of LPS the modified structure of the thymus just described was preserved, but the number of B1 and Mi was increased, especially in the subcapcular zone.

On the 3rd day after injection of LPS the structure of the thymus was changed: it was divided into regions with a large number of activated Tc and B1, corresponding to the cortex, and regions virtually without B1. The number of B1 in the subcapsular region and cortex amounted to 50 and 43% of the total number of cells respectively compared with 27 and 8% in the control. The number of Py continued to decrease under these circumstances (Py fell from 2.5% on the 1st day to 0.3% in the cortex) and the number of Mi increased (Mi reached 2% in the subcapsular zone compared with the virtual absence of Mi 24 h after injection of LPS). Single cysts lined with ciliated epithelium and laminated epithelial structures were seen, blood vessels were dilated and congested, and clusters of B1 were seen around them. In the subcapsular zone, the number of PlC did not exceed 0.8%.

Starting with the 5th day after injection of LPS the structure of the thymus gradually returned to normal: division into cortex and medulla was restored together with the ratio of their areas and the cell density in them. The number of B1 in the subcapsular zone and cortex fell (23.7 and 15.3%, respectively), but B1 in the cortex was higher than the corresponding value in the control. Mi reached a maximum at this time in the cortex, but gradually returned to normal in the later stages. The number of cysts and laminar epithelial structures gradually decreased and by the 30th day after injection of LPS the structure of the thymus was very similar to normal, except the activated Tc and B1, the numbers of which in the subcapsular zone and cortex were higher than in the control.

The kinetics of the quantitative parameters studied is illustrated in Fig. 2. As Fig. 2c shows, the number of B1 fell sharply 24 h after injection of LPS, as shown by a four-fivefold reduction of B1 (3.2% on average for the thymus in the experiment compared with 13.2% in the control). Starting with the 2nd day the relative number of B1 rose sharply to reach a maximum, which was much higher (twice or more) than the control for the thymus as a whole (B1 in the cortex was increased by 5.5 times compared with the control), but later B1 was only a little higher than in the control.

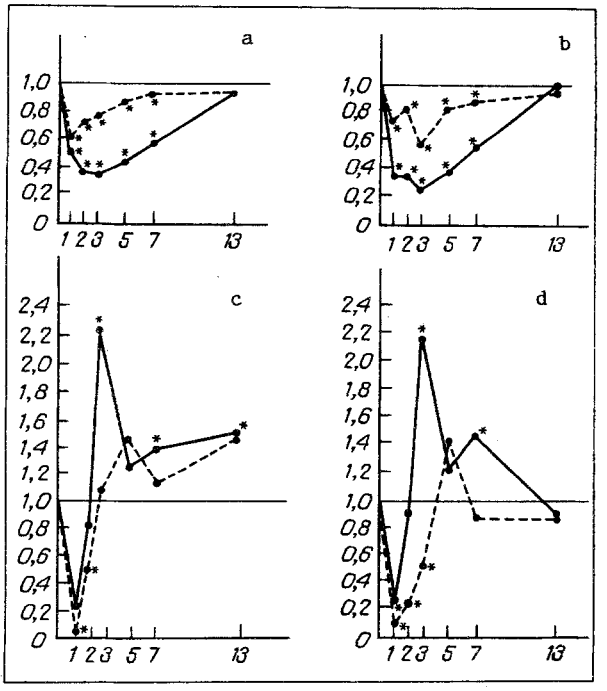

Fig. 2. Dynamics of weight and morphometric parameters of thymus after injection of LPS into mice. Abscissa: time after injection of LPS (days); ordinate: parameters normalized against control. a) Dynamics of weight of thymus (continuous line) and cell density in it (broken line); b) the same as in a, but with injection of vinblastine, c) change in number of blast cells (continuous line) and Mi (broken line), d) the same as in c, but with injection of vinblastine. $^*p \leq 0.05$ Compared with control.

The number of Mi in the thymus fell even more sharply under the influence of LPS: on the 1st day, for instance, they virtually disappeared and Mi fell 30-fold (0.03% compared 0.9% in the control), and on the next 2 days Mi quickly returned to normal and continued to rise, to reach a maximum on the 5th day. Later the number of Mi decreased but remained a little higher than in the control.

Similar changes in B1 and Mi were found after injection of LPS and vinblastine (Fig. 2d).

The number of Mi in the thymus increased during the first 2 days after injection of LPS (0.8% compared with 0.4%), mainly in the subcapsular zone. Mi was restored to normal on the 3rd-5th day.

Similar morphological and morphometric changes (Fig. 2b, d) were discovered in the thymus of the two groups of mice, but during the first 2 days after injection of LPS vinblastine did not "accumulate" Mi, and the vinblastine index returned to normal more slowly than Mi.

The results show that the peak polyclonal immune response to LPS is realized under conditions of marked atrophy of the thymus in the first 3 days. The morphological picture in the thymus as a whole is similar to the typical reaction of the thymus to various kinds of stress [8]. The action of LPS on the thymus may be effected by adrenal hormones [2, 3, 13], although there is evidence of the direct effect of LPS on T cells [14]. Irrespective of the mechanism, LPS has an immunotropic action on the thymus: potentiates the graft versus host reaction and helper and killer activity through selection of the appropriate Tc of the organ, increases the sensitivity of Tc to phytohemagglutinin and concanavalin A [2, 3, 13]; all these facts are evidence of enrichment of the thymus with more mature Tc [2]. Our own results agree with these data and demonstrate removal mainly of dividing Tc, but not even vinblastine can "accumulate" Mi. However, generation of the thymus begins as early as on the 2nd day, as is shown by the increase in B1 and Mi; the wave of blast cells with its peak on the 3rd day, moreover, was replaced by a wave of Mi with its peak on the 5th day. T-committed stem cells [2], in the resting state, which probably accounts for their resistance to LPS, may act as the source of regeneration. Analysis of the dynamics of B1 and Mi after injection of LPS suggests that the stage of activation of stem cells, together with the subsequent passage through the cell cycle, takes about 48 h. Although activa-

tion of cell division in the thymus has virtually not been studied, an important role in these processes has been ascribed to the interleukin-2 (IL-2) system, which is utilized independently in the process of proliferation and differentiation of Tc [4, 6, 12]. Early regeneration of the thymus in the present experiments can also take place with the participation of LPS itself, for in the mitogenic reactions of Tc it can play the role of a cofactor [10] or, by activating macrophages, it can lead to the release of IL-1, which is an inducer of IL-2 production by T cells [9].

It can be concluded from the facts described above that despite the marked involution of the thymus induced by LPS, it retains its high proliferative potential, partly, perhaps, due to LPS itself.

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