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IMMUNOLOGIC CHANGES IN EPITHELIUM OF MOUSE THYMUS DURING ACCIDENTAL INVOLUTION

L. V. Beletskaya, D. L. Belyaev, I. Yu. Mudrenko, V. P. Bukhova, and N. V. Kartashova

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The epithelial tissue of the thymus plays a determinant role in the formation and function of the immune system of the body. Its cells synthesize many hormones and biologically active substances [5, 9, 10, 11] and also secrete antigens of various tissues, including tissues of highly specialized organs, into the internal medium of the gland [4, 12]. By means of the ultrastructures of the cytoplasmic membranes of the epithelial cells of the thymus, lymphocytes are "familiarized" with the individual's histocompatibility antigens [13, 15]. It was shown previously that cells of the epithelial reticulum contain in their cytoplasm and processes antigens common with those of cells of the basal layer of stratified epithelium [1, 2, 7, 8]. With the aid of serum containing antibodies against basal-cell antigens, the epithelial reticulum of the thymus, unscreened by lymphocytes [3], can be electively demonstrated, so that the degree of its integrity and unmasking can be estimated. The complexity and the uninformativeness of the ordinary methods of assessing the action of immunodepressive preparations motivated an immunomorphologic study, which this paper describes, of the response of the epithelial tissue of the mouse thymus to prednisolone and azathioprine — drugs widely used in medical practice.

EXPERIMENTAL METHOD

Noninbred SHK albino mice and BALB/c mice weighing 17-20 g were used. Prednisolone, after suitable dilution, was injected intramuscularly into 28 mice in doses from 10 to 40 mg/kg over a period of 3-12 days. Azathioprine, in doses of 100 to 200 mg/kg, also was injected daily from one to nine times and with an interval of 3-7 days. When both drugs were given, a single dose of azathioprine of 100 to 250 mg/kg was given to 16 mice at the beginning of the course, and this was followed by injections of 10-40 mg/kg of prednisolone three times a day. The animals were killed by cervical dislocation 1-16 days after the last injection. The control consisted of 18 mice which received no treatment of any kind. Rabbit serum (B-1) containing high titers (1:128) of natural antibodies against basal-cell antigens, were used as the source of these antibodies. Pure antibodies against rabbit immunoglobulins, labeled with fluorescein isothiocyanate, or commercial luminescent serum against rabbit IgG, prepared by the Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, were used in the indirect immunofluorescence test. Pieces of thymus tissue were frozen to −85°C (with a mixture of dry ice and acetone). Sections 5 μ thick were cut in a cryostat (−20°C) and used unfixed. The sections were treated by the method described previously [6] and mounted in 60% neutral glycerin under a coverslip. They were examined under the LYUMAM-2 luminescence microscope. The intensity of the reaction was assessed in crosses (from 1 to 4). A very weak reaction was assessed as negative. Sections fixed in ethanol were stained with hematoxylin and eosin for use as the control.

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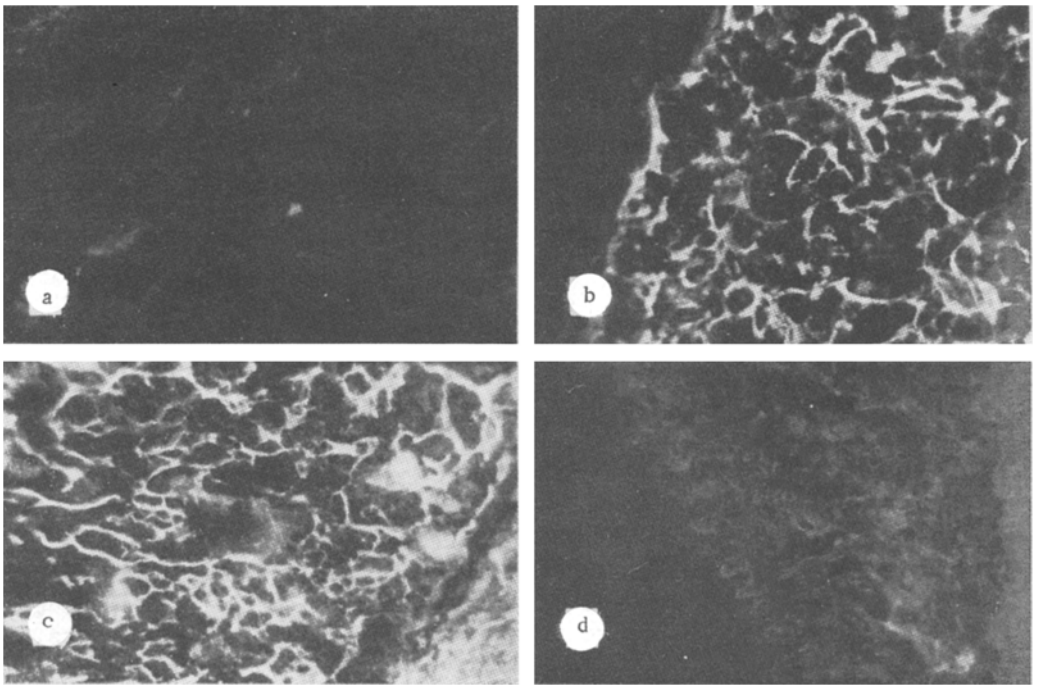

Fig. 1. Section through mouse thymus treated with serum containing antibodies against antigens of cells of epithelial reticulum. a) Section through thymus of control animal, reaction virtually absent; b) reaction with antigens of cytoplasmic processes of cells of epithelial reticulum; unmasking of network as a result of migration of thymic lymphocytes after injection of 10-20 mg/kg prednisolone, loops of network are of average size; c) the same: unmasking of network as a result of more active lymphocyte migration after injection of a combination of azathioprine and prednisolone, network of small loops; d) reaction with antigens of epithelial reticulum in thymus disappears after course of injections of azathioprine and prednisolone, evidently due to degenerative changes in cells of parenchyma of gland. Indirect immunogluorescence method. 120 ×.

EXPERIMENTAL RESULTS

Histologic study of sections through the thymus of animals of the control groups revealed the usual structure of the gland: a wide cortical zone, packed with many lymphocytes, and a small medullary zone, containing few lymphoid cells. Epithelial corpuscles were observed only in BALB/c mice. Treatment of sections through the thymus of noninbred animals with B-1 serum led to a reaction with the outer zone of cytoplasm of cells with few processes in the medullary zone, whereas in BALB/c mice, in addition, cells of the surface layer of thymic corpuscles responded. In the cortical zone of most control animals fluorescence of only single short think processes was observed (Fig. 1a). In many parts of the cortical zone there was no reaction (Fig. 1a). After injection of 10-20 mg/kg of prednisolone into the mice (daily for 3-12 days) a tendency was observed for the weight of the thymus to decrease, although the values exhibited considerable scatter — from 40 to 80 mg, compared with a mean weight in the control of 62 ± 3.4 mg. The gland tissue also lost its normal turgor and became flabby. Some decreases in the number of lymphocytes in the cortical zone and consequent widening of the medullary zone were observed morphologically. The immunomorphological study revealed a reaction in the cortical zone in the form of a network of processes of the epithelial cells. No unmasking of whole cells was observed. After a course of three injections of prednisolone in a dose of 10 mg/kg the network appeared in only certain areas of the cortex (a total area of about 30% of the area of the cortical zone). If the course was lengthened to 7-12 injections or the dose increased to 20 mg/kg, the territory on which the reaction was observed became continuous and accounted for 50-70% of the total (Fig. 1b). The pattern revealed could be described as a network with average-sized loops, consisting of thin or moderately thick processes. The intensity of fluorescence was assessed as 2-3 crosses. With a further increase in the dose of prednisolone to 40 mg/kg (3-9 injections) the network still occupied the same percentage of the total area (up to 70%), but it consisted now of small loops, due to the more active migration of lymphocytes from the gland, which was already

clearly distinguishable in histological sections also. The intensity of the immunofluorescence reaction remained high. The weight of the thymus fell progressively with an increase in the number of injections. After a single injection of azathioprine (100 mg/kg), despite different times of sacrifice of the animals after injection (from 3 to 16 days) the immunomorphologic picture after injection of the drug was mainly similar. The area occupied by the network of large or medium-sized loops was 10-50% in the cortical zone, the intensity of fluorescence corresponded to 2-3 crosses, and the processes were of average thickness. An increase in the dose to 200 mg/kg and a large number of injections were accompanied by a progressive decrease in weight of the thymus (in some cases to 23 mg), but the intensity of the reaction remained the same as before. The area on which unmasking of the epithelium could be detected in this case was close to 50%. Against the background of a network of medium-sized loops, areas with small loops could already be distinguished, with shortening and thickening of the processes of the epithelial cells. Injection of a combination of the drugs (10 or 40 mg/kg of prednisolone on 3 consecutive days and a single injection of 100-250 mg/kg of azathioprine on the 1st day of the course) was accompanied by considerable widening of the medullary zone on account of contraction of the cortex. In some cases a uniform distribution of lymphocytes was observed in the cortical and medullary zones. The principal distinguishing features of the immunofluorescence picture following combined injection of the drugs were a high percentage of the total area occupied by the reaction (50-70) and the small-looped character of the network (Fig. 1c). However, a completely new feature of the disturbances in the gland was the dramatic weakening of the intensity of the immunofluorescence reaction observed in some animals, so that it could be assessed only as + or ± (Fig. 1d). The results of the morphological investigation revealed a considerable reduction in the number of lymphocytes in the gland.

The results agree with those obtained by other workers [14] who showed, in particular, that involution of the thymus in mice during administration of corticosteroids begins with disappearance of lymphocytes of the Lyt 2^+ phenotype from the outer zone of the cortex of the gland. The results of the present investigation are evidence that the use of serum containing antibodies against antigens of cells of the epithelial reticulum can reveal the early stage of accidental involution of the thymus, in the form of unmasking of processes of the epithelial cells and can give an indication of the duration of the aftereffect of the drugs used. It is difficult to do this histologically because of the absence of methods of elective demonstration of the epithelial tissue of the thymus. The first signs of degenerative changes in the parenchyma of the thymus likewise are difficult to detect histologically. In the present investigation, disappearance of the basal-cell antigen from cells of the epithelial reticulum was noted in the thymus of mice receiving a combination of the drugs. This phenomenon indicates profound changes in the parenchyma of this central lymphoid organ, which must be reflected in its function.

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