PROLIFERATION AND DIFFERENTIATION OF LYMPHOCYTES IN ORGAN CULTURES OF THE THYMUS OF NEWBORN MICE

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Proliferation and differentiation of lymphocytes were studied by means of thymidine-H^3 in organ cultures of the thymus of newborn mice. Large and small lymphocytes composing the proliferative pool proliferated intensively in vitro. The minimal time of differentiation of a DNA-synthesizing lymphocyte into a small lymphocyte not synthesizing DNA was about 7 h. A change in the cellular composition of the lymphoid tissue was observed in cultures; the proportion of large and medium lymphocytes was between 80 and 95%.

As was shown previously, after explantation of the thymus of newborn mice, lymphocytes persist for a long time in organ culture as compact groups in close association with cells of the stroma [1]. The object of this investigation was to determine whether proliferation and differentiation of the lymphocytes take place under these conditions.

EXPERIMENTAL METHOD

The thymus of newborn mice was cultivated as a whole (or as its separate lobes) in organ cultures in AUFS Millipore filters (pore diameter 0.6-0.9 μ) by the method suggested earlier [2]. The nutrient medium consisted of 70% medium No. 199, 20% bovine serum, 10% chick embryonic extract, vitamin C, L-glutamine, glucose, and antibiotics [3]. The medium was renewed every 2-3 days as it became acidified.

On the 10th day thymidine-H^3 was added to the medium in a concentration of 1 μCi/ml nutrient medium. The incubation time with thymidine-H^3 was 1-72 h. Fresh thymidine was added once a day.

Impressions were taken from 6-12 cultures after 1, 3, 7, 24, 48, and 72 h, fixed with methanol, treated with perchloric acid, coated with type M photographic emulsion, exposed and developed in the usual way, and then stained by the Romanowsky method.

In each impression taken from the cultures 1000 lymphocytes were counted, and the labeling index was determined for the categories of large, medium and small lymphocytes.

EXPERIMENTAL RESULTS

Large and medium lymphocytes were predominant in the impressions from the cultures at all times of the investigation.

After incubation for 1 h with thymidine-H^3 label was found in 3.5-6% of large and medium lymphocytes (Table 1). Mainly large cells with a delicate structure of the nucleus and, judging from the morphological features, lymphoblasts were labeled. No small lymphocytes containing the label could be found. With an increase in the period of incubation with thymidine-H^3, there was an increase in the percentage of labeled large and medium lymphocytes (Fig. 1), and the labeling index of the lymphocytes differed negligibly in the cultures obtained from two different experiments. Labeled small lymphocytes were first found

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TABLE 1. Changes in Labeling Index (after 1, 3, 7, 24, 48, and 72 h among large, medium and small lymphocytes following addition of thymidine-H^3 to a 10-day culture of the thymus of newborn mice)

Lymphocytes	Percentage of lymphocytes	Labeling index (in percent) time in h					
		1	3	7	24	48	72
Large and medium	88	3,5 6*	6	7	16,5 27*	42*	53*
Small	12	—	—	0,4	7, 7*	11*	18*

*Counts carried out on impressions from the second experiment.

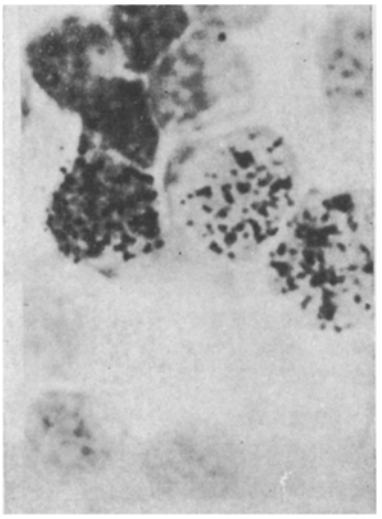

Fig. 1. Labeled lymphocytes in impressions of 11-day cultures from the thymus of newborn mice. Time of incubation with thymidine-H^3 24 h.

in one of the experiments after incubation for 7 h with thymidine-H^3. The labeling index was 0.4%. After cultivation for 24 h the number of labeled small lymphocytes reached 7%, rising to 18% after 72 h. The number of labeled large and medium lymphocytes continued to increase throughout the period of observation. The labeling index of the large and medium lymphocytes after 72 h was 53%.

In organ cultures of the thymus of newborn mice intensive proliferation of lymphocytes thus takes place. The proliferative pool in vitro contained large and medium lymphocytes, i.e., the same categories as are capable of proliferation in vivo also. The proliferative pool in the population of large and medium lymphocytes in culture was high, more than 50%. During cultivation for 72 h in the presence of thymidine-H^3, it was impossible to determine the extent of the proliferative pool.

Although large and medium lymphocytes predominated over small during growth in organ cultures, differentiation of large and medium lymphocytes into small nevertheless took place in vitro. The minimum time required for conversion of a lymphocyte synthesizing DNA into a small lymphocyte not synthesizing DNA was evidently about 7 h, for it was after this time that the first labeled small lymphocytes appeared.

It is interesting to note that the composition of the lymphoid tissue in the organ cultures showed a marked change from its composition in vivo. Whereas in the intact thymus there were about 8.5% of young lymphoid cells (large and medium lymphocytes), in the organ cultures these cells predominated (from 80 to 95%). The change in the cellular composition of the lymphoid tissue of the thymus in vitro was probably due to the fact that under the conditions prevailing in the cultures repopulation could not take place. Small lymphocytes are known to migrate intensively from the thymus and to spread throughout the lymphoid system, settling in the cortical zone of the lymph glands and in the periarterial white pulp of the spleen. In organ cultures the pathways along which migration of the small lymphocytes takes place are probably disturbed and the signals regulating this process are absent. Presumably if differentiation of large and medium lymphocytes into small is regulated in vivo by the rate of migration of small lymphocytes from the thymus, in the absence of migration of small lymphocytes from the thymus in organ cultures the intensity of differentiation will be sharply reduced.

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