COMPARATIVE INVESTIGATION OF ADENOSINE DEAMINASE ACTIVITY IN SPLEEN, THYMUS, AND BONE MARROW OF AKR AND C57BL MICE

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In C57BL mice, with a low incidence of spontaneous leukemia, there is a progressive decrease with age in adenosine deaminase activity in the thymus and an increase in its activity in the spleen, whereas in AKR mice, with a high incidence of spontaneous leukemia, the changes in activity of this emzyme follow a parallel course and activity in the thymus is always higher than in the spleen. The adenine deaminase activity in the bone marrow of animals of both lines changed in the same direction with age.

By the use of inbred mice in biochemical research it is possible to detect genetically determined quantitative differences in the course of metabolism in animals, and hence to obtain an approach to the study of the biochemical substrate for the pathogenesis of diseases found only in animals of particular lines, notably tumors of specific localization and leukemias.

The object of the present investigation was to study the content of an intermediate enzyme of nucleic acid metabolism, adenosine deaminase (3.5.4.4), in the spleen, thymus, and bone marrow of mice belonging to two lines, AKR and C57BL. The first of these lines has a high incidence of spontaneous leukemia.

Adenosine deaminase participates in the conversions of adenosine and deoxyadenosine, two important anabolic and catabolic components of nucleic acid metabolism. This test was chosen because, as this writer's investigations [1] showed, the content of this enzyme (which is widely distributed in various tissues [3]) may be connected with the character and intensity of nucleic acid metabolism in a cell population.

EXPERIMENTAL METHOD

Three hundred male AKR and C57BL mice aged 1, 2, and 6-10 months were used in the experiments. The AKR mice, some of which have leukemia at the age of 6-10 months, were selected for the experiment at that age only when morphological examination of the organs most commonly affected by leukemia had excluded the disease.

Adenosine deaminase activity was determined in tissue extracts. To prepare the extracts, the spleens, thymus glands and bone marrow taken from three to seven mice were pooled and minced, and then homogenized in phosphate buffer, pH 7.4. Total disintegration of the cells was achieved by freezing and thawing the homogenates and then by triturating them with powdered glass. The supernatant obtained after centrifugation of the homogenates was added to an incubation medium containing a buffered solution of adenosine in a final concentration of 0.2%. The enzymic activity was then determined by Straub's method [6] and expressed as the amount of nitrogen (in micrograms) of ammonia liberated from the adenosine under standard conditions per milligram dry weight of tissue.

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TABLE 1. Adenosine Deaminase Activity in Bone Marrow, Spleen, and Thymus of AKR and C57BL Mice (in μg N$-NH_3$/mg dry weight/h)

Tissue studied	Line of mice	Age of mice (in months)		
		1	2	6–10
Spleen	AKR	17,3±2,5	30,2±2,8	19,2±6,0
	C57BL	18,7±1,2	22,2±3,0	38,8±3,1
P		>0,1	>0,05	<0,01
Thymus	AKR	53,9±4,6	58,2±5,2	32,1±2,9
	C57BL	47,9±2,2	39,5±2,0	12,9±2,3
P		>0,1	<0,02	<0,001
Bone marrow	AKR	19,6±1,4	27,1±3,1	34,9±6,4
	C57BL	17,0±1,8	22,8±3,4	26,3±6,4
P		>0,1	>0,1	>0,1

EXPERIMENTAL RESULTS

The adenosine deaminase activity in all the tissues studied showed no significant difference in mice of the two lines at the age of 1 month (Table 1). The highest activity of the enzyme was found in the thymus, in good agreement with observations showing that the rate of synthesis of nucleic acids is higher in this gland than in other lymphoid organs [2]. The lymphoid cells of the thymus, which are morphologically indistinguishable from the analogous cells in the spleen, evidently differ, quantitatively at least, in their biochemical properties.

By the age of 2 months some increase in the activity of the enzyme was observed in the thymus of the AKR mice, and a decrease in the thymus of the C57BL mice, leading to statistically significant differences in the content of adenosine deaminase in the gland in the two lines. This difference was even greater in the animals aged 6-10 months, in which the activity of the enzyme was reduced in both lines, although to a greater degree in the C57BL mice (reduced by 67% by comparison with the age of 2 months, but by only 44% in the AKR mice of corresponding ages). The opposite phenomenon was observed with respect to the dynamics of adenosine deamiase activity in the spleen of the two lines of mice. The adenosine deaminase activity of splenic extracts from C57BL mice increased with age, and by the age of 6-10 months it was twice that in AKR mice.

In the mice of these two lines the changes taking place in adenosine deaminase activity in the bone marrow with aging were in the same direction. The increase in the quantitative differences between the activities of the two lines was not significant.

It is difficult to compare the adenosine deaminase activity in the bone marrow, on the one hand, and the spleen and thymus on the other, among the mice of the same line, for nucleic acid metabolism in myeloid and lymphoid tissue exhibits certain specific features [4]. The distribution of enzyme activity in the bone marrow and lymphoid organs thus provides no grounds for the conclusion that the intensity of nucleic acid metabolism is higher or lower in these organs than in the bone marrow.

Comparison of the content of adenosine deaminase in the spleen and thymus of mice of these two lines showed that in AKR mice at all the age periods studied the enzyme activity of the thymus is higher than that of the spleen, whereas in young C57BL mice the differences between the activity of the enzyme in the thymus and spleen were not so marked, and at the age of 6-10 months the adenosine deaminase activity of the thymus of these animals was only 33% of that in the spleen. The relative and absolute weights of the lymphoid organs of AKR and C57BL mice in adult life indicate that the biochemical differences are associated with the morphological individuality of the inbred animals.

The dynamics of the changes in the content of the enzyme in the thymus and spleen, which evidently differs in mice belonging to lines with a high and low incidence of spontaneous leukemia, reflects certain interlinear differences in nucleic acid metabolism in the organs studied. Further work is necessary to explain how the general principles revealed by this investigation are related to the pathogenesis of leukemia in AKR mice, for at present not enough information is available on the possible link between changes in adenosine deaminase activity (and activity of certain other enzymes of nucleic acid metabolism) and the state of cell proliferation and differentiation, or on the replication of the Gross leukemogenic virus in the thymus in the preleukemic period. It must be remembered that in AKR mice it is the thymus whose cells are the first to undergo neoplastic transformation at the age of 6-10 months [5].

Consequently, the preservation of higher activity of adenosine deaminase and, perhaps, of other enzymes of nucleic acid metabolism in the thymus than in the spleen, during aging, can be regarded as a characteristic feature of pure-line mice subsequently developing spontaneous leukemia.

LITERATURE CITED

1. A. I. Svirnovskii, Vopr. Med. Khimii, No. 1, 36 (1970).
2. E. Andreasen and J. Ottesen, Acta Path. Microbiol. Scand., 21, 25 (1944).

3. E. J. Convey and R. Cooke, Biochem. J., 33, 479 (1939).
4. L. D. Hamilton, J. Clin. Invest., 34, 988 (1955).
5. D. Metcalf, in: Ciba Foundation Symposium on Tumour Viruses of Murine Origin, London (1962), p. 233.
6. F. B. Straub, O. Stephaneck, and G. Acs, Biokhimiya, 22, 118 (1957).