COMPARATIVE ANTILEUKEMIC ACTION OF RNA AND HISTONES FROM INTACT AND REGENERATING HOMOLOGOUS TISSUE

A. I. Svirnovskii, V. I. Levin, and V. V. Nikolaichik

UDC 616.155.392-092.9-085.272.6/7.

The effect of unfractionated preparations of RNA and histones isolated from intact and regenerating lymphoid tissue on the survival and growth of leukemic lymphocytes in syngeneic recipients was studied. The inhibitory action of histones on leukemic growth was well-marked but was independent of the morphological and functional state of the tissue at the time of their extraction from it. Electrophoresis in polyacrylamide gel showed no appreciable differences in the fractional composition of histones from intact and regenerating tissues. Preliminary treatment of the leukemic cells with RNA from regenerating tissue reduced the survival rate and increased the life span of the mice compared with animals receiving cells incubated with RNA from intact tissue. A spectrophotometric study of the melting profile of the RNA preparations revealed differences in the ratio between the thermostable and unstable components of RNA from intact and regenerating tissues.

KEY WORDS: RNA; histones – antileukemic action.

Many investigations have shown that tumor growth is inhibited by preparations of RNA and histones obtained from various tissues, including hematopoietic tissues [4]. Some factors appearing in the body during regeneration have also been shown to have a stronger biological action than the analogous agents from intact tissues on the course of several processes in differentiating cells [1, 2, 7, 14]. It has also been postulated that processes of regeneration and of tumor growth are antagonistic [12, 13]. The comparison of these data led to the conclusion that it would be worthwhile studying the action of factors isolated from homologous regenerating tissue on tumor cells [11].

This paper describes the results of a comparative study of the effect of unfractionated preparations of RNA and histones from intact and regenerating lymphoid tissue on the survival of the cells of lymphatic leukemia and its subsequent development in mice of line AKR.

EXPERIMENTAL METHOD

Preparations of RNA and histones were isolated from the spleen of rabbits in some of which regeneration of the lymphoid tissue has been induced in response to its preliminary destruction. RNA was isolated by phenol-temperature fractionation [6] with subsequent pooling of all the fractions. Histones were isolated from DNP [17] of cell nuclei [15]. In all the experiments preparations were isolated in parallel series from intact and regenerating tissue. To assay the RNA preparations, besides determining the content of DNA and protein, the degree of contamination with phenol, the spectrophotometric profile, and the 260/280 and 260/230 nm ratios, the character of their appearance was studied in solutions with an ionic strength of 0.14 and 0.014, using the constant-temperature cell of the SF-4a spectrophotometer. The characteristics of the histone preparations were determined by electrophoresis in polyacrylamide gel [18]

Leukemia Laboratory, Belorussian Scientific-Research Institute of Hematology and Blood Transfusion, Minsk. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 78, No. 9, pp. 87-90, September, 1974. Original article submitted January 2, 1974.

TABLE 1. Effect of Preliminary Incubation of Leukemic Cells with Preparations of RNA and Histones from Intact and Regenerating Rabbit Spleen on the Development of Transplanted Leukemia in AKR Mice

No. of series of expts.	Preparations incubated with leukemic cells	Intact spleen		Regenerating spleen		Significance of differences between experimental groups, P
		percent of successful takes	survival period of mice dying from leukemia	percent of successful takes	survival period of mice drying from leukemia	
I	Histones	43,8*	31,6±2,2	50,0*	26,6±2,2	>0,05
II	"	0*	—	20,0*	33,0±0,0	>0,05
III	"	10,7*	36,7±6,9	13,6*	47,0±11,6	>0,05
IV	RNA	100,0	37,1±1,2	80,0	62,8±4,9*	<0,01
V	RNA	100,0	42,7±1,5	100,0	48,3±1,8*	<0,02
VI	RNA	100,0	54,6±1,6*	42,3	86,1±10,2*	<0,01
VII	RNA	100,0	35,0±0,9*	100,0	35,2±0,8*	>0,05

*Changes significant compared with animals grafted with leukemic cells incubated under the same conditions but without preparations of RNA and histones.

followed by densitometry on the ERI-65 apparatus. A cell suspension was prepared from the organs of AKR mice, affected with lymphatic leukemia but otherwise untreated, in Hanks' medium or physiological saline, and incubated at 37°C with shaking in the presence of the RNA preparations in a concentration of 0.8-3.4 mg/ml for 1 h or the histones in a concentration of 1 mg/ml for 2 h. Meanwhile some samples were incubated without RNA and histones under the same conditions. The state of the cells after incubation was assessed on the basis of their morphological changes and their resistance to staining with eosin. The cells were then injected in a dose of 0.5 million intraperitoneally into mice of the same AKR line. The number of animals successfully grafted with leukemia and the duration of their survival were determined. Three months after transplantation the mice that still survived were killed and their spleen, thymus, lymph glands, liver, and kidneys, together with organs of the animals that had died previously, were subjected to morphological investigation. Altogether 7 series of experiments were performed on 441 mice.

EXPERIMENTAL RESULTS

In the control experiments, in which the incubation medium contained neither RNA nor histones, all the mice developed leukemia after inoculation. As Table 1 shows, the unfractionated histones had a well-marked inhibitory action on the successful grafting of cells incubated with them, and this was accompanied by an increase in the survival period of the animals that developed leukemia, amounting to 8-27 days in different series. However, differences were not significant between groups of mice injected with cells treated with histones from intact and regenerating tissues. On electrophoresis in polyacrylamide gel no marked qualitative or quantitative changes were found in the composition of the principal histone fractions isolated from the tissue at different periods of its morphological and functional state.

By contrast, in the experiments with RNA preparations differences in the life span of the mice were found depending on the state of the tissues at the time of extraction of the RNA preparations used for treating the grafted cells from them. In the 3 series of experiments in which RNA was used in concentrations of between 1.6 and 3.4 ml/mg, a marked increase was observed in the life span of the leukemic animals grafted with cells incubated with RNA from regenerating spleen compared, not only with the controls, but also with mice injected with leukemic cells treated with RNA from the intact spleen. These differences between the two experimental groups disappeared (series VII) when the RNA concentration in the incubation medium was reduced to 0.8 mg/ml. The percentage of successful inoculations in some series was reduced only by the use of RNA preparations from the regenerating organ. The course of the melting curves of RNA preparations from the intact and regenerating spleen (Fig. 1) indicated that these preparations differed in the relative proportions of their components having different degrees of stability of their secondary structure. RNA preparations from the regenerating spleen were characterized by an increase in the content of more thermostable fractions. This conclusion regarding differences in the composition of the heterogeneic RNA preparations in the intact and regenerating organs is in agreement with the facts indicating depression of part of the genome during regeneration [8] and differences in the complement of RNA from intact and regenrating tissues discovered in experiments with hybridization of DNA and the corresponding RNA preparations [16].

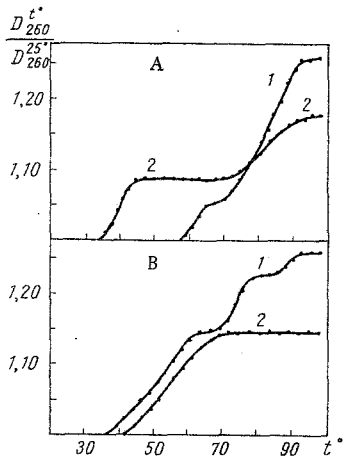

Fig. 1. Spectrophotometric melting profile of RNA preparations: A) RNA from regenerating rabbit spleen; B) RNA from intact rabbit spleen; 1) ionic strength 0.14, 2) ionic strength 0.014. Abscissa, temperature (in °C); ordinate, ratio $D^{t°}_{260}/D^{25°}_{260}$, where $D^{25°}_{260}$ is the optical density at a wavelength of 260 nm and a temperature of 25°, and $D^{t°}_{260}$ is the same at temperature t°C.

When the mechanism of the effect of the various RNA and histone preparations on leukemic cells is discussed the possibility of their action on the genetic apparatus after penetration inside the cell must be considered. At the same time, the fact must not be forgotten that RNA and histones can alter the course of intracellular metabolic processes [5, 10] by acting on the cytoplasmic structures. In addition, the presence of RNA and histones on the surface of the cells must itself affect the character of the relations between the tumor and normal cells, and this may be expressed as delay of tumor growth or even the complete prevention of proliferation of the tumor cells.

There are thus good grounds for attributing the quantitative differences in the action of RNA preparations isolated from intact and regenerating tissue on the transplantability of leukemic cells to the greater sensitivity of leukemic cells to RNA fractions that appear or are present in larger quantities in regenerating tissue. In the experiments with histones the degree of the inhibitory effect of these RNA fractions in the concentration used may have been very high, for we know that if the concentration of histones is increased to more than 0.1 mg/ml, their toxic effect may be strengthened and this, in turn, would not allow the quantitative differences in the action of the preparations from intact and regenerating tissue (if they exist), to be detected. Observed differences in the quantitative effect of total histones from chick embryos at different periods of development on the rate of successful transplantation of tumor cells [3] can be regarded as indirect evidence in support of this view. Certain changes in the DNA/histone ratio and in the ratio between the histone fractions have also been demonstrated during differentiation of cells [9], although no sharp changes in the composition of the main histone fractions with a change in the rate of growth must be expected [19].

In view of the findings described above, further and more detailed comparative studies of the fractional composition of preparations of RNA and histones) and, possibly, other components also from intact and regenerating tissue) and a study of the effect of the individual fractions on tumor growth must be regarded as important.

LITERATURE CITED

1. A. G. Babaeva, N. A. Kraskina, and A. D. Liozner, Tsitologiya, No. 12, 1511 (1969).
2. A. M. Belous, Byull. Éksperim. Biol. i Med., No. 2, 111 (1967).
3. A. Yu. Bronovitskii and I. G. Dad'kov, in: Proceedings of the 2nd Scientific Conference of Pathophysiologists of the Baltic Republics and White Russia [in Russian], Vilnius–Kaunas (1972), p. 24.
4. Z. A. Butenko, Vestn. Akad. Med. Nauk SSSR, No. 3, 30 (1968).
5. L. Sh. Ganelina and A. D. Braun, Tsitologiya, No. 4, 507 (1971).
6. G. P. Georgiev and V. L. Mant'eva, Biokhimiya, No. 5, 949 (1962).
7. M. A. Guberniev and E. M. Leikina, Zh. Obshch. Biol., 30, No. 4, 442 (1969).
8. S. S. Debov, Vestn. Akad. Med. Nauk SSSR, No. 12, 32 (1971).
9. A. I. Klimenko, in: Molecular and Functional Bases of Ontogeny [in Russian], Moscow (1970), p. 89.
10. A. I. Svirnovskii, V. I. Levin, and T. V. Shimanskaya, Abstracts of Proceedings of the 12th International Congress on Blood Transfusion [in Russian], Moscow (1969), p. 361.
11. A. I. Svirnovskii, in: Proceedings of the 2nd Scientific Conference of Pathophysiologists of the Baltic Republics and White Russia [in Russian], Vilnius–Kaunas (1972), p. 114.
12. A. N. Studitskii, in: The Experimental Histology of Tumor Growth [in Russian], Moscow (1966), p. 215.
13. B. P. Tokin, Regeneration and Somatic Embryogenesis [in Russian], Leningrad (1959).
14. N. A. Fedorov, V. P. Reshchikov, M. P. Khokhlova, et al., in: The Pathogenesis, Treatment, and Epidemiology of Leukemia [in Russian], Riga (1971), p. 5.
15. J. Chauveau, I. Moule, and C. A. Ronniller, Exp. Cell Res., 11, 317 (1956).
16. R. Church and B. McCarthy, Proc. Nat. Acad. Sci. (Washington), 58, 1548 (1967).

17. E. W. Johns, D. M. P. Philips, P. Simson, et al., Biochem. J., 80, 189 (1961).
18. E. W. Johns, Biochem. J., 92, 55 (1964).
19. E. W. Johns, A. J. S. Davies, and M. E. Barton, Biochim. Biophys. Acta, 213, 537 (1970).