TRANSITION FROM THE G_2 PERIOD INTO THE RESTING STATE AT DIFFERENT STAGES OF DEVELOPMENT OF MOUSE ASCITES HEPATOMA 22a

I. D. Balyaeva and V. M. Rodionov

UDC 616.36-006-092.9-018.15

KEY WORDS: ascites hepatoma 22a of mice.

The study of the particular features of separate periods of the mitotic cycle in tumor cells and the possibility of transition from them into the resting state, and the reversibility or irreversibility of these transitions are of undoubted interest. Data have recently been published on the transition of ascites tumor cells from the postsynthetic G_2 period to the resting state (R_2 or Q_2) and the possibility that such cells may return to the mitotic cycle in response to stimulation of division [6-11]. However, the available data on this problem are by no means complete, and in some details they are also contradictory with regard to ascites tumors of different origin [5, 12].

The object of this investigation was to study these matters so far as mouse ascites hepatoma 22a (AH22A) cells are concerned. Attention was concentrated on determination of the size of the subpopulation of cells in the reversible resting state R_2 and their role in proliferation of AH22A at different stages of development.

EXPERIMENTAL METHOD

The Gel'shtein AH22A was used. The tumor was maintained by inoculations of 0.25 cm^3 of ascites fluid (about $40 \cdot 10^6$ cells) at intervals of 2 weeks in C3HA mice. ^{3}H-thymidine was injected in a dose of 0.2-0.3 μCi/g and colchicine in a dose of 0.5-0.75 μg/g. The preparations were covered with liquid type M emulsion and exposed at 4°C for 2-4 weeks. The number of mitoses was determined by counting 1000 cells in each animal and was expressed in promille.

EXPERIMENTAL RESULTS

It was shown previously that during development of AH22A the total duration of the mitotic cycle is increased on account of an increase in the duration of all its periods, including the postsynthetic G_2 period. The mean duration of this period increases from 2.6 h in a tumor on the 2nd day of development to 16 h in the terminal tumor [1, 3]. In the present investigation the size of the G_2 population (cells in the G_2 period of the mitotic cycle) was determined at different stages of development of the AH22A. For this purpose, after injection of ^{3}H-thymidine mitoses during 4-6-hourly intervals were collected with colchicine until the 100% (or about 100%) level of labeled mitoses was reached. Naturally, as the tumor aged and the duration of the G_2 period lengthened, the collection time was increased. In tumors on the 2nd day of development it was 6 h, on the 5th day 18 h, the 8th day 24 h, the 11th day 32 h, and in the terminal tumor (14th day) it was 48 h. The total of all unlabeled mitoses collected in such experiments gave the size of the G_2 subpopulation. The results are given in Table 1. The size of the G_2 subpopulation varied relatively little during development of the tumor, but was rather smaller in the later stages. The sizes of any of the subpopulations of cyclic (i.e., in the mitotic cycle) cells at each stage of development of the tumor were determined by the relative duration of that period in the mitotic cycle and by the size of the proliferative pool (a reduction in the pool due to an increase in the number of noncyclic cells leads to a decrease in each subpopulation of cyclic cells). As will be clear from Table 1, interaction between these factors for the G_2 subpopulation leads to maintenance of the relative stability of its size.

Laboratory of Chemical Factors of Regulation of Cell Growth and Division, Institute of Biological and Medical Chemistry, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 90, No. 10, pp. 474-476, October, 1980. Original article submitted February 8, 1980.

 0007-4888/80/9010-1430$07.50

TABLE 1. Changes in Size (in $^{o}/_{oo}$) of G_2 Subpopulation and of Cell Subpopulation in Reversible Resting State R_2 at Different Stages of Development of AH22A (M ± m)

Index studied	Age of tumor, days					
	2	5	8	11	14 (terminal)	18 (delayed)
Size of G_2 subpopulation (determined in the absence of transplantation)	74±12	65±11	50±5	46	42	
Size of G_2 subpopulation + subpopulation of cells in reversible resting R_2 state (determined during stimulation of division)		67±6	66	78±11	89±17	28±9
Δ		2	16	32	47	

Note: Results of 3-5 experiments in each of which from six to 30 animals were used are given; where errors of means are not shown, mean results of two experiments are given. Size of G_2 subpopulation shown with allowance for increase in total size of population of tumor cells during collection of unlabeled mitoses.

During transplantation of AH22A of different ages into a new host, sharp stimulation of division takes place. During the first few hours cells moved into mitosis on account of shortening of the duration of the G_2 period. In addition during stimulation of division cells could also proceed from the reversible resting R_2 state into mitosis. If simultaneously with transplantation ^{3}H-thymidine was injected and the mitoses subsequently collected with colchicine, the number of unlabeled mitoses collected (their overwhelming majority made the transition in the first 6 h after transplantation) and consisted of the sum of the G_2 subpopulation and the subpopulation of cells in the reversible resting R_2 state. The relative number of these cells was determined during transplantation of AH22A of different ages (Table 1). At all stages of development of the tumor the number of unlabeled mitoses collected with colchicine during stimulation of division was greater than their number in the G_2 subpopulation; this difference (Δ) increased somewhat during aging of the tumor. The presence of a difference in the sizes of the subpopulations of the cells in question evidently indicates the existence of cells in the reversible resting R_2 state, and also that the size of this subpopulation is increased toward the later stages of tumor development.

After transplantation of a delayed (18 days) tumor [2] the number of unlabeled mitoses collected by colchicine was reduced (to $28^{o}/_{oo}$ compared with $89^{o}/_{oo}$ in the terminal tumor). It was shown previously that during 4 days of delay the number of cells in irreversible resting states in AH22A increases considerably [4]. The transition to the irreversible resting state evidently also took place in the case of cells from the G_2 period and from the irreversible resting R_2 state.

The conclusion that cells in the irreversible resting R_2 state were present and that their number gradually increased can also be drawn from the results of experiments conducted by a different scheme. In them, mice with AH22A at the 8th and 11th day of development were injected with ^{3}H-thymidine at intervals of 8-12 h for 24 and 32 h and mitoses were collected (at 4-hourly intervals) during the 12 h after the last injection of isotope, either without transplantation or with stimulation of division by transplantation (in the latter case the last injection of ^{3}H-thymidine was given at the moment of transplantation). In the experiments without transplantation, only $1^{o}/_{oo}$ of unlabeled cells achieved the transition into mitosis in 8-day AH22A (and, moreover, only in the first 8 h), compared with $3.5^{o}/_{oo}$ in the 11-day tumors. In this case unlabeled mitoses corresponded to division of cells with the maximal duration of the G_2 period. In the experiments with stimulation of division, in the case of transplantation of an 8-day tumor $15.5^{o}/_{oo}$ of cells made the transition to mitosis in the early stages after transplantation, compared with $27^{o}/_{oo}$ of unlabeled mitoses in the case of transplantation of the 11-day tumor. Most of the latter were evidently cells brought out of a reversible resting R_2 state under conditions of stimulation of division. In each of the experiments six animals were used and numbers of unlabeled mitoses were aggregated.

To answer the question how long cells can remain in the reversible resting R_2 state in the terminal stage of development of AH22A, the following experiments were conducted. ^{3}H-thymidine was injected at 12-hourly intervals for 36 or 48 h into mice with a tumor at 13-14 days of development, after which the tumor was transplanted into intact mice and mitoses were collected by colchicine in the early stages after transplantation. If the label was injected

for a period of 36 h, 7.8 ± 2.1‰ of unlabeled mitoses were collected after transplantation, If, however, the label was injected for 48 h, hardly any unlabeled mitoses were found (only 0.25 ± 0.25‰; five animals were used in each experiment). The facts are evidence that in the terminal stage of development a few cells may be in the G_2 period and then in the reversible restingR_2 state for over 36 h, but this time cannot be increased to 48 h. With an increase in the length of stay in the reversible resting R_2 state the cells evidently proceed into the irreversible resting state.

During development of AH22A the size of the G_2 subpopulation thus undergoes relatively little change, probably as a result of interaction between the factors determining its size, Between the 5th day of development and the terminal stage, a subpopulation of cells in AH22A can be found in the reversible resting R_2 state, and which return to the mitotic cycle in the early period after stimulation of division. The size of this subpopulation increases somewhat as the tumor ages. However, in the terminal stage its size is small (47‰), and on that account there can be no question of any significant accumulation of cells in the reversible resting R_2 state. This state of affairs and the fact that the size of the G_2 subpopulation + the subpopulation of cells in the reversible resting R_2 state is reduced in a delayed tumor are evidence that the R_2 state is a relatively short-term transient state during emergence of the cells from the G_2 period of the mitotic cycle.

LITERATURE CITED

1. I. D. Belyaeva and T. S. Ivleva, Byull. Eksp. Biol. Med., No. 8, 97 (1974).
2. I. D. Belyaeva and T. S. Ivleva, Byull. Eksp. Biol. Med., No. 2, 218 (1977).
3. T. S. Ivleva and I. D. Belyaeva, Byull. Eksp. Biol. Med., No. 10, 479 (1978).
4. T. S. Ivlevaand I. D. Belyaeva, Byull. Eksp. Biol. Med., No. 10, 445 (1979),
5. G. Andersson, J. Cell Physiol., 90, 329 (1977).
6. G. Andersonand P. Kjellstrand, Arch. Pathol. Anat. Abt. Zellpath., 16, 311 (1974),
7. G. Anderson,S. Österberg, and O. Heby, Int. J. Biochem., 4, 269 (1978),
8. P. Bichel, Eur. J. Cancer, 7, 349 (1971).
9. P. Bichel and P. Dombernowsky, Cell Tissue Kinet., 6, 359 (1973).
10. P. Bichel and P. Dombernowsky, Eur. J. Cancer, 11, 425 (1975).
11. P. Dombernowsky, P. Bichel, and N. Hartmann, Cell Tissue Kinet., 6, 347 (1973),
12. P. Dombernowsky, P. Bichel, and N. Hartmann,Cell Tissue Kinet., 7, 47 (1974),
13. P. Dombernowsky and P. Bichel, Acta Pathol. Microbiol. Scand., 83, 222 (1975),
14. P. Dombernowsky and P. Bichel, Cell Tissue Kinet., 9, 9 (1976),
15. N. Hartmann and P. Dombernowsky, Cancer Res., 34, 3296 (1974),

CORRELATION ANALYSIS OF FREE MAMMARY GLAND STROMAL CELLS DURING AGE CHANGES AND DEVELOPMENT OF SPONTANEOUS TUMORS

T. B. Zhuravleva and E. L. Abrakova

UDC 618.19-053.9+618.19-006.018.1]

KEY WORDS: free cells; correlation analysis; dyshormonal hyperplasia,

The fact that communication between cells taking part in immunologic reactions takes place is not nowadays in dispute. However, the nature of this communication and the extent to which changes in it determine the character of the pathological process has not yet been investigated. Many workers [1, 4] consider that the development of the immunologic response is determined by cooperative relations between cells belonging to the systems of specific and nonspecific immunity.

We know that the development of a pathological process is based on insufficiency or disturbance of certain components of coordinating systems. However, despite much research in im-

Department of Pathological Anatomy, I. P. Pavlov First Leningrad Medical Institute.

(Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Chernigovskii.) Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 90, No. 10, pp. 476-478, October, 1980. Original article submitted November 16, 1979,

0007-4888/80/9010-1432$07.50