STATE OF THE ERYTHROCYTE SURFACE (ECHINOCYTOSIS) IN EXPERIMENTAL CARCINOGENESIS

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A comparative investigation of the erythrocytes of mice differing in their original susceptibility to spontaneous carcinogenesis of the mammary gland revealed characteristic deformation of the cells (echinocytosis) in C3H mice with a well-marked predisposition to cancer. The number of erythrocytes with spinous projections was 58.6% in the early pretumor period (age 3-4 months) and reached 91.1% in the late pretumor period (11-12 months). In intact C57BL/6 mice resistance to mammary gland carcinogenesis the number of echinocytes at the same age periods varied from 16 to 18.7%. Growth of tumors (spontaneous in C3H mice and transplanted Ehrlich's adenocarcinoma in C57-BL/6 mice) was accompanied by an increase in the number of echinocytes and in the degree of severity of the echinocytosis (96.6 and 65.3% respectively). The possible mechanisms of the pathogenesis of echinocytosis during carcinogenesis are discussed.

KEY WORDS: erythrocytes; echinocytosis; carcinogenesis; mammary gland.

Recent investigations have shown that with respect to their surface structure the erythrocytes of the healthy organism constitute a heterogeneous cell population, the composition of which can vary dynamically under the influence of physiological and pathological factors [1, 2].

One type of erythrocyte associated with the development of pathological states is the echinocyte — a cell covered with spinous projections. In certain diseases (pathology of the liver, various types of muscular dystrophies), the number of echinocytes in the blood rises and accordingly attempts have been made to make use of this sign for diagnostic purposes [4, 5].

The object of the present investigation was to determine whether this type of deformation of erythrocytes occurs in experimental carcinogenesis.

EXPERIMENTAL METHOD

Experiments were carried out on 92 female C57BL/6 and C3H mice. The state of the erythrocytes was studied in the following groups of animals: 1) C3H mice aged 3-4 months (the early pretumor period); 2) C3H mice aged 11-12 months (the late pretumor period); 3) C3H mice aged 12-14 months with developed spontaneous mammary gland tumors; 4) C57BL/6 mice aged 3-4 months (control 1); 5) C57BL/6 mice aged 12-14 months (control 2); 6) C57BL/6 mice aged 3-4 months with transplanted Erhlich's adenocarcinomas growing subcutaneously.

Fresh blood taken from the animals' tails was fixed in 3.5% glutaraldehyde solution in phosphate buffer, pH 7.4. "Crushed drop" preparations were then made and unstained erythrocytes examined in the light microscope under a magnification of 280×. On the basis of Bessis' classification [1, 2] the following cell forms were differentiated: discocytes — cells with a smooth surface; echinocytes I — cells with a slightly wavy surface; echinocytes II — cells with well-marked indentation of their surface, and echinocytes III — cells with spinous projections. The nomenclature of erythrocytes with respect to their shape, including more than 20 names, was compiled by Bessis on the basis of the results of scanning electron microscopy. However, subsequent investigations [5, 6] showed that echinocytes can be detected under the light microscope, such as was used in certain experiments. The number of these various forms in 500 cells was counted in each preparation and the results expressed as percentages.

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TABLE 1. Relative Percentages of Various Forms of Erythrocytes during Carcinogenesis

Line of mice	Group of animals	No. of animals	Discocytes		Echinocytes I		Echinocytes II		Echinocytes III	
			$M \pm m$	P	$M \pm m$	P	$M \pm m$	P	$M \pm m$	P
C57Bl/6	Control 1 (aged 3-4 months)	15	84,0±0,9		14,9±0,4		1,05±0,6		0,013±0,07	
	Control 2 (aged 12-14 months)	25	81,3±0,6		17,0±1,5		1,3±0,5		0,31±0,2	
	Animals with tumors (Erhlich's adenocarcinoma)	7	34,7±4,5	<0,001*	32,0±2,5	<0,001*	27,1±4,4	<0,001*	6,17±2,1	<0,001*
C3H	Early pretumor period (aged 3-4 months)	15	41,4±2,2	<0,001*	33,1±1,0	<0,001*	22,8±1,3	<0,001*	2,7±0,6	<0,001*
	Late pretumor period (aged 11-12 months)	9	8,9±1,4	<0,001†	34,8±1,6	<0,001†	40,8±1,6	<0,001†	15,3±1,8	<0,001†
	Animals with tumors (spontaneous mammary gland carcinoma, age 12-14 months)	21	3,4±1,27	<0,001†	39,7±2,6	<0,001†	43,7±2;1	<0,001†	13,2±2,3	<0,001†

Legend. Values of P marked by one asterisk refer to data compared with control 1, those marked by a dagger data compared with control 2.

EXPERIMENTAL RESULTS

The results are given in Table 1. They show that in intact C3H mice in the pretumor period (aged 3-4 months) most (58.6%) erythrocytes were echinocytes at various stages, the number of which amounted to almost 90% at the time of appearance of neoplasm (11-12 months). In animals with developed spontaneous mammary gland carcinoma nearly all the erythrocytes were echinocytes, and only 3.4% of discocytes still remained.

During the period of onset and growth of the tumors, many erythrocytes (56-57%) belonged to stages II and III of echinocytosis.

For intact C57BL/6 mice, regardless of age, most cells in the blood were discocytes (over 80%). The only age change noted was an increase in the number of echinocytes III (from 0.013 to 0.31%). Meanwhile the development of transplanted Erhlich's adenocarcinoma in young C57BL/6 mice was accompanied by a marked shift toward an increase in the number of echinocytes, which constituted two-thirds of the total number of cells studied.

The echinocytosis in intact C3H animals, which are characterized by a natural predisposition to spontaneous tumors, discovered in this investigation suggests a possible role of the genetic factor in the modification of the surface structure of the erythrocytes. The deformation observed may perhaps be a reflection of a systemic change in the cell membranes connected with structural changes in their lipid layer, which must influence the distribution of cations and lead ultimately to specific changes in the structure of the cell surface [6]. These changes could also be due to genetically determined metabolic changes in the medium surrounding the erythrocytes, i.e., in the blood plasma (its protein, lipid, carbohydrate, and mineral composition, the level of circulating hormones, mediators, immunologically active substances, and so on).

The possible role of a virus in the phenomenon of echinocytosis observed in intact animals susceptible to mammary gland carcinogenesis may also be indicated. Bittner [3] demonstrated some years ago the ability of erythrocytes to adsorb viruses on their surface, and this may be one of the pathogenetic mechanisms of the deformation observed in erythrocytes.

Both during development of spontaneous mammary gland tumors in C3H mice predisposed to the development of such tumors and during growth of transplanted neoplasm in C57BL/6 mice resistant to cancer in this situation, the number of modified forms of erythrocytes was increased. This is evidence of a direct echinocytogenic influence of the growing tumor, independent of the strain of mice used or of the experimental model chosen.

Among the possible pathogenetic mechanisms of the transforming effect of the tumor on

erythrocytes one which cannot be overlooked is the autoimmune processes that accompany malignant growth, and which take place invariably with the participation of erythrocytes. The role of hormonal and neurotrophic disturbances connected with the development of neoplasms in metabolic changes leading to echinocytosis must also be studied. Investigation of the problems listed above will help not only to discover the causes of the change in configuration of the erythrocytes, but also to shed light on some other aspects of the pathogenesis of tumor growth.

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EFFECT OF AGE, CASTRATION, AND PREGNANCY ON CARCINOGENESIS INDUCED IN CBA MICE BY 1,2-DIMETHYLHYDRAZINE

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When injected subcutaneously in a dose of 8 mg/kg weekly into female CBA mice, 1,2-dimethylhydrazine (DMH) induced the development of tumors of the intestine, anal region, uterus, and liver. When DMH was injected into mice aged 12-13 months the appearance of sarcomas of the uterus was observed earlier (at 8 weeks) and the incidence of tumors of the anal region rose more rapidly than in mice aged 3 months. In mice receiving DMH against the background of repeated pregnancies, a statistically significant decrease in the frequency of sarcomas of the uterus was observed (10.3% compared with 48.3% in nonpregnant mice); pregnancy did not affect the frequency of tumors of other organs. Castration had no significant effect on the time of appearance or the frequency of tumors in all situations.

KEY WORDS: 1,2-dimethylhydrazine; tumors; aging; castration; pregnancy.

A definite role in the carcinogenic effect of 1,2-dimethylhydrazine (DMH) is ascribed to the effect of this carcinogen on the neuroendocrine system and the associated hormonal-metabolic disturbances [2, 3]. In rats, DMH induces predominantly intestinal tumors, and castration changes their localization in different segments of the large intestine [6]. In CBA mice, besides intestinal tumors, in a high percentage of cases DMH induces epithelial tumors in the anal region, sarcomas of the uterus, and hemangioendotheliomas of the liver [7].

The object of the present investigation was to study the effect of factors such as age, castration, and pregnancy on carcinogenesis induced by DMH in CBA mice.

EXPERIMENTAL METHOD

Female CBA mice obtained from the Stolbovaya nursery, Academy of Medical Sciences of the USSR, received subcutaneous injections of DMH dissolved in distilled water in a dose of 8 mg/kg once a week for 30 weeks. The experimental mice were divided into four groups: young virgin, castrated, pregnant, and old virgin. The first three groups consisted of 30 mice aged 3 months and weighing 19-23 g. The group of old mice consisted of 50 animals aged 12-13 months and weighing 30-45 g. Bilateral castration was carried out 2 weeks before the begin-

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