Metastasizing of Human Melanoma on Immunodeficient Mice. Tumor Cells in the Circulation

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The subline Bro of human melanoma xenograft with a high metastasizing activity is studied. The data obtained by flow fluorometry after staining with tumor-specific monoclonal antibodies indicate that free melanoma cells are present in the peripheral blood of animals; the largest number of these cells is found in mice with combined immunodeficiencies (beige/nude).

Key Words: *metastasizing; melanoma; blood; nude mice; beige/nude mice*

The development of new chemotherapeutic agents is impossible without an adequate *in vivo* model for testing their pharmacological activity. Although animal models of spontaneous, induced, and passaged tumors are helpful at the screening stage, they are not completely adequate, because drugs that exhibit good therapeutic activity in these models often turn out to be ineffective during clinical treatment. The use of thymus-free animals allows for the investigation of human tumors; however, even highly malignant tumors rarely produce metastases in these models [7], which, of course, once again divorces the model from the clinical reality. In addition, metastasizing activity may vary in different models [2]. The reason for this phenomenon is unclear; several variants of metastasis blocking are possible at different stages [5].

In this study we attempted to find out how metastasizing of human melanoma cells depends on the dissemination of tumor cells in the circulation, using mice with different immunodeficiencies (nude and beige/nude).

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MATERIALS AND METHODS

A human melanoma cell line (MEL-7) was obtained in the Laboratory of Experimental Models (Oncological Research Center, Russian Academy of Medical Sciences) from the Bro strain [6] and was serially passaged 27 times (lung metastases → subcutaneous injection) on beige/nude mice. Mice with congenital immunodeficiencies (nude and beige/nude) were bred in the laboratory. The cells were injected in healthy animals in the 7th week subcutaneously in the back. Blood (0.5 ml, 2-4 days before death from the tumor) was collected aseptically in a sterile solution (10 ml) containing 0.14 M NaCl, 0.01 M NaH_2PO_4, 0.07 M $Na_2HC_6H_5O_9$, and 0.5% Glu at pH 7.4, 2°C. After being shipped (less than 2 h), the specimens were twice washed with phosphate-saline solution (0.14 M NaCl, 0.01 M NaH_2PO_4, pH 7.4, and 1% bovine serum albumin, 2°C). FITC-conjugated monoclonal antibodies to tumor-associated surface antigen of melanoma were used for visualization of human melanoma cells in the circulation. After intraperitoneal culturing of the producer hybridoma, ascitic fluid was centrifuged, and K_2SO_4 was added to it to a final concentration of 0.5 M. After a second centrifugation, the solution was applied onto a T-gel column (1.6×40) equili-

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TABLE 1. Mean Logarithm of Fluorescence Intensity ($p \leq 0.05$)

Autofluorescence	Mice			
	without tumor		with tumor	
	nude	beige/nude	nude	beige/nude
57.87	63.35	61.54	66.39	72.77
	64.00	64.06	65.87	73.04
	62.85	63.30	66.06	72.57
		63.77	67.21	73.21
			65.29	74.35
			66.58	72.97
Mean	63.40	63.17	66.23	73.15

brated with 0.5 M K_2SO_4 and 0.01 M KH_2PO_4, pH 8.0 [4]. The column was washed with 10 volumes of starting buffer, and immunoglobulins were eluted with K_2SO_4-free buffer. After being dialyzed and concentrated, the immunoglobulins were conjugated with FITC as described [3]. Analysis for the presence of tumor cells was performed by flow cytofluorimetry. Cells were stained with the conjugate (1:10, 30 min, 4°C), washed three times with phosphate-buffered saline, suspended in 1 ml of buffer solution, and analyzed in an EPICS-V cytofluorimeter (Coultronics) equipped with an argon laser (Spectra Physics, excitation wavelength 488 nm, power 200 mW, and cut-off filter 515 nm). At least 100,000 cells were analyzed in each experiment. Histograms were mathematically processed using MDADS software (Coultronics). The background fluorescence was measured in control cultures not treated with the fluorochrome.

RESULTS

After staining with FITC-conjugated monoclonal antibodies the mean intensity of fluorescence of blood samples obtained from both nude and beige/nude mice carrying the tumor was significantly higher compared with that in the control animals (Table 1). The number of cells with positive staining (discriminating value of the fluorescence window >150) per 100,000 cells varied from 300 to more than 5000 (Table 2).

TABLE 2. Number of Melanoma-Associated Antigen-Carrying Cells Detected in the Mouse Circulation

Mice			
without tumor		with tumor	
nude	beige/nude	nude	beige/nude
0	0	315	4780
0	0	289	5064
0	0	308	4863
0	0	457	5114
0	0	264	5210
0	0	321	4930

Note. Calculations were performed relative to the histograms of the logarithm of the intensity of fluorescence of the cells obtained from tumor-free mice (IMMUNO software, Coultronics).

Such a great number of fluorogen-positive cells, that can be regarded as circulating melanoma cells from the primary focus (0.3-5% of the total amount, including erythrocytes and other blood cells), engaged our attention. This cannot be explained only by the terminal stage of the disease but probably reflects intense metastasizing of the melanoma.

There is known to be a difference in the metastasis-forming behavior of human tumor xenografts (including melanomas) on nude and scid mice (scid mice with several combined immunodeficiencies are similar to beige/nude mice used in our experiments), which manifests itself in the fact that the tumors are equally successfully transplanted on these animals, but produce no metastases in nude mice [8]. A considerable difference in the number of circulating tumor cells in different lines may be one factor (all other conditions being equal), although even the minimal numbers of detected cells are comparable to the total number of circulating lymphocytes.

The predominant tropism of metastases toward the lungs observed in beige/nude mice may result from the high number of circulating metastasizing cells. After entering the venous circulation, the bulk of large (relative to mouse blood cells) human melanoma cells are primarily retained in the capillaries of the lesser circulation. This is consistent with the results obtained in experimental metastasizing of melanoma cells inoculated in the greater or lesser circulation [1].

The presence of large numbers of melanoma cells in the circulation of immunodeficient mice makes this model suitable for the investigation of the antigenic profile of metastasizing cells *in vivo* without their preliminary deaggregation.

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MORPHOLOGY AND PATHOMORPHOLOGY

Ultrastructural Analysis and Autoradiography of Gastric Mucosa Bioptates in Chronic Active Hepatitis

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The results of gastrobiopsy are studied for a general pathological process in the liver. It is shown that the atrophic-sclerotic reactions predominating in the gastric mucosa are characterized by disturbances in the mucocyte ultrastructure attended by a reduction of cytoplasmic organelles, a marked decrease of biosynthesis in parenchymatous cells, and stroma collagenization, which is a systemic manifestation of regenerative-plastic insufficiency.

Key Words: *stomach; electron microscopy; regenerative-plastic insufficiency*

The seminal studies of Aruin *et al.* [1], Ugolev *et al.* [7], Uspenskii [8], and Yasinovskii [9] have shed much light on the structural and functional reactions of the digestive system in physiology and pathology. However, studies performed on clinical models, assessing the systemic reactions which reflect the interaction between organs of the digestive system in health and pathology, are few and far between [2,3]. The importance of such an approach is to be stressed, as it allows for assessment of cell and tissue heterogeneity, taking into account the integrity of structural and metabolic responses. The aim of the present study was to perform a complex morphological analysis of gastric mucosa specimens in chronic active hepatitis.

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MATERIALS AND METHODS

Seventy-two gastric mucosa (GM) specimens obtained during fibrogastroscopy were morphologically investigated. A larger part of each specimen was used for the preparation of paraffin sections (fixing in 10% neutral Formalin and hematoxylin-eosin staining in combination with Perls reaction, Van Gieson staining, and Schiff reagent). A

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