Possible Pathways of Circulation of Human Endogenous Retrovirus Similar to Mouse Mammary Tumor Virus

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It is shown that polypeptides which are immunologically related to gp52 mammary tumor virus are found in T and B peripheral blood lymphocytes in all breast cancer patients, in children with B-cell lymphosarcomas, and in B lymphocytes of some healthy donors. These proteins are not found in patients with tumors of other sites.

Key Words: *mouse mammary tumor virus; human endogenous retrovirus; lymphocytes*

During the 1970s it was discovered that the successful transport of mouse mammary tumor virus (MMTV), which is transmitted with the milk from the digestive tract to the mammary gland, depends on the functional integrity of the immune system: thymectomy in young mice of a highly carcinogenic strain resulted in a sharp decrease of breast cancer incidence [2]. The reason recently became clear after study of the MMTV superantigens, factors that are encoded on the open reading frame of the 3' long terminal repeat of the provirus sequence [5]. Being bound with $CD4^+$ lymphocytes which bear receptors with a specific V-β-chain, superantigens stimulate them to proliferate and initiate cell death in the same lymphocyte subpopulation during its maturation in the thymus in mice carrying only endogenous MMTV.

During the study of MMTV superantigens, the use of different mouse models established that the B lymphocyte is the primary target cell for this retrovirus [1]. An infected B cell which carries an encoded MMTV superanantigen interacting with the $CD4^+$ fraction of T lymphocytes activates them, and this in turn stimulates B-cell proliferation. It was shown [4] that co-expression of the *env* MMTV gene product, gp52, plays a significant role in effective superantigen presentation. In this manner, MMTV was found not only to determine the functional integrity of the immune system, but also to use the lymphocyte pathway of circulation in the mouse organism.

Human endogenous retrovirus, which has been studied in detail, is akin to MMTV according to a whole complex of morphological features, antigens to structural proteins, and homology of the nucleotide sequences [3]. Expression of a protein which is an immunological analog of gp52 correlates specifically with the presence of breast cancer in human beings.

However, neither the role which the endogenous MMTV-related human retrovirus plays in tumor incidence nor its other biological functions have been elucidated. The pathways of its circulation in the human organism are not yet clear. We speculated that the processes in man must be similar to the lymphocytic pathway of MMTV circulation in mice, and thus sought the presence of an antigen, which would be an immunological analog of the gp52 of MMTV in lysates of peripheral blood lymphocytes in patients with tumors of different location and in healthy donors.

MATERIALS AND METHODS

Heparin-treated blood was obtained from various departments of the Research Institute of Clinical

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Oncology at the Cancer Research Center of the Russian Academy of Medical Sciences. The lymphocytic fraction was isolated in a Ficoll-Paque (Serva) gradient or by the addition of an equal volume of polyglucin (Krasnoyarskmedpreparaty, Russia) followed by incubation at 37°C for 1.5 h and sedimentation of the lymphocytic fraction. After washing, the T and B lymphocytic fractions were enriched in the following manner: monospecific antiserum against human IgM (Gamaleya Research Institute of Epidemiology and Microbiology) was layered onto a plastic Petri dish. After a 45-minute incubation followed by thrice-repeated washing of the dish with phosphate-saline buffer, the lymphocytes resuspended in this buffer were introduced and incubated for 1 h at room temperature with slight shaking every 20 minutes. The nonadherent cells, which were mainly T lymphocytes, were carefully collected, and then the cell fraction with mainly B lymphocytes bound with antibodies against IgM was removed by pipetting. After sedimentation by centrifugation, both fractions were treated with lysing buffer (100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA, 0.5% Triton X-100, 0.1% sodium dodecyl sulfate, and 1 mM phenylmethylsulfonyl fluoride). The preparations were equalized in terms of protein content in dot-blotting on nitrocellulose filters with Ponceau solution (Sigma) staining and separated in polyacrylamide gel as described elsewhere [9]. Immunoblotting was performed using rabbit antiserum against MMTV gp52 and conjugate of goat IgG against rabbit IgG labeled with horseradish peroxidase in working dilutions (Sigma).

Monospecific rabbit antiserum to MMTV gp52 was obtained by the immunization technique on nitrocellulose filters [7]. The MMTV preparation (National Cancer Institute, USA) was separated electrophoretically in polyacrylamide gel and transferred to nitrocellulose filters, after which the zone of correspondent molecular weight was cut out and used for rabbit immunization.

RESULTS

A total of 54 blood samples were examined including those from a group of patients with breast cancer as well as groups of patients with tumors of other sites and a group of healthy donors. Antiserum against MMTV gp52 revealed specifically the proteins with molecular weights of 60 and 68 kD. The reaction was strongest in the T and B lymphocyte fraction from the breast cancer patients. These antigens were identified in 3 patients with B-cell childhood lymphosarcoma (an unseparated preparation of lymphocytes was tested in this case). The reaction was revealed only in B lymphocytes in 3 of the 14 healthy donors, and in one case it was represented only by one protein with a molecular weight of 68 kD.

The results of the analysis in immunoblotting are listed in Table 1. All samples were positive in the group of patients from the breast cancer department. The reaction was negative in the group from the gynecology department, which we used as a control according to the principle of tumor hormone dependence. It is interesting that 3 samples of the 4 B-cell childhood lymphosarcomas have 60 and 68 kD proteins, whereas a reliable positive reaction was not found among the hemoblastoses in adult persons, including 3 cases of lymphosarcomas. The group of healthy donors with a positive reac-

TABLE 1. Identification of Polypeptides with Molecular Weights of 60 and 68 kD with Rabbit Antiserum to gp52 of MMTV (Immunoblotting) in Enriched Preparations of T and B Lymphocytes from Donors of Various Groups

Group	Positive reaction/ total in group	Type of reaction in preparations of T and B lymphocytes	
Breast cancer	12/13	T-lc +++	B-lc +++
	1/13	+	+
Breast fibroadenoma	1/1	++	+++
Cervical carcinoma	0/8	T-lc -	B-lc -
Ovarian carcinoma	0/4	-	-
B-cell childhood lymphosarcoma	3/4	Total preparation lc ++	
Hemoblastoses of adults (acute and chronic myelo- and lymphoblastic leukoses, lymphosarcomas)	0/7	T-lc -	B-lc -
	0/3	67 kD -	-
Healthy donors	3/14:	T-lc	B-lc
	1/14	-	++
	1/14	-	+
	1/14	-	67 kD ++

Note. lc = lymphocytes. +, ++, and +++ denote the degree of reaction.

tion in enriched B-lymphocyte preparations is of interest as well.

It is known that the weak level of endogenous MMTV transcription is enhanced in lipopolysaccharide-induced differentiation both in normal B lymphocytes and in B-cell mouse lymphomas [6]. It is conceivable that the positive reaction in the B-lymphocytic fraction of the healthy donors is a result of normal proliferation stimulated by repeated blood donation.

The presence of a positive reaction in the lymphocytic preparations from the breast cancer patients and its absence in tumors of other locations is very demonstrative. The expression of proteins related to MMTV gp52 in both the B- and T-cell fraction (as contrasted to the healthy donors) merits attention. In mice lymphocytes of both types are involved in the life cycle of the virus [1,5]. In man the mechanism of MMTV interaction with immune cells appears to be preserved in phylogenesis and is evolutionarily conservative [8], although the sequence which encodes the MMTV-related superantigen has not been found.

The processes causing virus antigen expression in man are not known. However, we think that the development of this new line of investigation offers promise as a way of studying the MMTV life cycle in man and its lymphogenic circulation in connection with the expression of proteins with molecular weights of 60 and 68 kD which are immunologically akin to gp52 of MMTV and are revealed in immunoblotting in both the T- and B- lymphocytic fractions obtained from breast cancer patients.

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