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ANTIBODIES INTERACTING WITH MOUSE MAMMARY TUMOR VIRUS PROTEINS IN SERA
OF HEALTHY WOMEN

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It is stated in the literature that humoral antibodies reacting with structural proteins of mouse mammary tumor virus (MMTV) are found in man [1, 2, 4, 6, 8]. The prevalence of these antibodies in patients with breast carcinoma is 10-20 times higher than in healthy women, and accordingly the question of their importance for the diagnosis of breast cancer has been raised [2, 9]. According to data published by various workers the percentage of healthy women who produce antibodies capable of reacting with MMTV proteins varies from 3 to 5 [1, 2, 4, 6, 9]. These fluctuations are determined not only by differences in the populations studied, but also by the methods used to test the serum. However, virtually nowhere is there any exact definition of the term "healthy women," except that it implies the absence of breast cancer. Since data of different workers may be based on the investigation of groups that differ in age, or the presence of benign changes in the breast, and so on, it is important to know how all these factors may affect expression of antibodies reacting with MMTV proteins in man. With information of this kind it will be possible to make a more detailed analysis of the data for patients with breast cancer, and ultimately to judge the applicability of the MMTV antibody test in diagnosis.

In the investigation described below, in order to study expression of antibodies to MMTV in women without breast cancer, it was decided to study the age, inheritance, endocrine status, and benign changes in the breast in conjunction with analysis of the prevalence of antibodies to MMTV in different groups. The presence of antibodies was determined by enzyme-linked immunosorbent assay (ELISA).

EXPERIMENTAL METHOD

Sera were obtained from women during out-patient attendance at a Moscow factory medical clinic, kept at -20°C without overfreezing until required for the reaction. The presence of antibodies to MMTV in the sera was determined by ELISA on polystyrene plates [7] in the modification used by the writers previously [4]. A preparation of MMTV from C3H mice was used as the antigen: this consisted of virus precipitated from the culture medium of cells of mouse mammary tumor MM5/mt. Blood donors' sera were used in dilutions of 1:100-1:300, and incubation with the test sera was carried out overnight at $6-8^{\circ}\text{C}$. Rabbit antibodies against human immunoglobulins (produced by the N. F. Gamaleya Research Institute of Microbiology and Epidemiology, Academy of Medical Sciences of the USSR) were conjugated with peroxidase (1000 units/mg, from Serva, West Germany) by the method in [5]. Dilutions of sera and conjugate were made up in PBS buffer with 0.01% Triton X-100 and 5% normal rabbit serum. After each incubation the samples were washed with the same buffer without addition of the serum. As the chromogen, 5-aminosalicylic acid (0.8 mg/ml, pH 6.0) was used, with the addition of hydrogen peroxide. The marked difference between the positive and negative reactions made it possible to assess the reaction visibly.

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TABLE 1. Prevalence of Antibodies to MMTV in Healthy Women (investigation of various groups of blood donors, M ± m)

Features used to distinguish groups of donors	Number of sera tested		Number of positive donors, per cent
	number of positive sera	total number	
Whole group	16	404	3,96±0,97
With mastopathy or breast cancer	4	112	3,57±1,79
With no breast diseases	12	281	4,2±1,9
With diffuse or fibrocystic mastopathy	4	84	4,76±2,3
Women in the reproduction period and premenopause	14	336	4,16±1,09
Women in the postmenopause	2	57	3,5±2,43
Age:			
20—29 years	4	102	3,92±1,92
30—39 »	6	128	4,69±1,86
40—49 »	4	98	4,08±2,00
50—59 »	1	42	2,38±2,35
Women having given birth	14	302	4,64±1,47
Women not having given birth	2	91	2,2±1,5
Having given birth twice or more	7	110	6,00±2,2
Having given birth once or not at all	9	283	3,18±1,04
Women whose mothers had breast cancer	1	13	—
Number of them without mastopathy	1	7	—

Legend. Information collected about 393 donors, all with positive questionnaires.

EXPERIMENTAL RESULTS

Altogether 405 sera were tested, of which 16 specifically interacted with the MMTV preparation. The preparation used contained all the main virus proteins and their ratio in the preparation could be estimated from the densitogram of the electrophoretically fractionated MMTV preparation (by the method in [3]). The criterion of specificity of the reaction was the absence of binding of antibodies of the test sera when MMTV was replaced by preparations of primate retroviruses of the C-type (M7) or D-type (MPMV), and also blocking of the reaction by previous exhaustion of the test serum with the MMTV preparation. None of the sera with a positive reaction with MMTV interacted with proteins of the control viruses, but among women with diffuse mastopathy two donors were found whose sera interacted with the MPMV preparation.

During investigation of women by the specialist in breast diseases the diagnosis was based on palpation. Altogether in this group the following benign changes were found in the breast: diffuse mastopathy, diffuse with a tendency toward nodular, nodular mastopathy, fibrocystic disease, fibroadenoma. In one woman carcinoma of the breast was found (the diagnosis was later confirmed histologically). Questionnaire data were collected by oral questioning of the subjects. They included the age when menstruation began and the menopause occurred, childbirth and pregnancies, cancer in near relatives. Groups were identified on the basis of a number of features and the prevalence of antibodies to MMTV determined for each of them; the

significant range was calculated by the equation $m = \sqrt{\frac{x(1-x)}{n}}$, where x denotes the prevalence of antibodies and n the size of the sample.

The results of the investigation of correlation between the presence of antibodies to MMTV and a particular feature or group of features showed that statistically significant differences were not present for all the groups distinguished (Table 1); later, therefore, we shall discuss only certain tendencies revealed by this analysis.

The presence of diffuse mastopathy evidently does not affect expression of antibodies to MMTV in women, and we consider this to be important, for it means that this factor can be disregarded during analysis of the prevalence of antibodies to MMTV in women with breast cancer and with other forms of cancer. It is difficult to estimate whether other benign changes in the breast can influence the appearance of antibodies to MMTV, but in the groups of subjects

tested we did not find any such antibodies in the serum of women with either nodular or fibrocystic mastopathy or with fibroadenoma. The only woman in the group in whom breast cancer was found likewise did not have antibodies to MMTV in her serum at a testable level.

The age of the donors, as well as the age of the menopause, did not significantly affect the prevalence of antibodies to MMTV in healthy women.

In the writers' view the rather higher prevalence of antibodies to MMTV in women who had given birth to children compared with those who had not is interesting. The more frequent discovery of antibodies interacting with MMTV proteins in pregnant women was demonstrated previously. Since the appearance of humoral antibodies reflects the expression of MMTV-related antigens in man, these antigens are evidently induced by pregnancy. From this point of view, the decreased prevalence of antibodies to MMTV in women who had not given birth may reflect the absence of expression of MMTV-related antigens other than in association with pregnancy and childbirth (at least in part).

A somewhat higher percentage of positive donors was found in the group of women whose mothers had had breast carcinoma, but because of the small size of this group these differences cannot be regarded as statistically significant.

On the whole these results differ from those of other investigations [8] in which, among elderly women and women with benign mastopathy, the percentage of positive donors was much higher than among young and healthy women. We do not know whether this was caused by differences in the methods used, in the size of the sample, or the composition of the population, but in the group of women whom we studied (in the present and the previous investigation [4]), the presence of antibodies to MMTV in the serum was considerably increased only in the presence of breast cancer.

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