Acta virol. 34 : 19—26, 1990

ANTIBODIES TO RETROVIRUSES OF TYPES C AND D IN FEMALE PATIENTS WITH BENIGN AND MALIGNANT MAMMARY TUMOURS

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Received September 27, 1988

Summary. — The sera of women with benign and malignant mammary tumours were investigated for the presence of antibodies to structural proteins of retroviruses types C and D by indirect immunoenzyme assay (ELISA) and immunoblotting. Among 89 sera tested 5 reacted with the RD-114 protein, 2 with SSV, M-7, and bovine leukaemia virus proteins. None serum reacted with mouse mammary tumour virus proteins. On the basis of these results we conclude that the expression of antibodies to structural retrovirus proteins is not specific for mammary cancer.

Key words: *mammary gland cancer*; *antibodies*; *retroviruses of C and D type*

Introduction

It is known that antibodies to proteins immunologically related to structural proteins of mouse mammary tumour virus (MMTV) have been found in the sera of mammary tumour patients in more than 50% of cases (Tomana *et al.*, 1980; Day *et al.*, 1981; Zotter *et al.*, 1981; Litvinov *et al.*, 1984). However, it is not clear whether mammary tumour express antigens related to retrovirus type B only or whether retrovirus type C and D related antigens are expressed as well. Such antigens and corresponding antibodies have been reported (Kim, 1975; Ilyin and Morozov, 1980; Suni *et al.*, 1981; Schetters *et al.*, 1983; Jerabek *et al.*, 1984; Wahlstrom *et al.*, 1984; Anh-Tuan *et al.*, 1984; Schetters *et al.*, 1985). For example, simultaneous expression of antibodies to retroviruses types B and C was found in leukosis and haematological patients (Segal-Eiras *et al.*, 1983; Atkis *et al.*, in press).

The purpose of our investigation was to test the presence of antibodies to different retrovirus types C and D in malignant and benign mammary tumour patients by indirect immunoenzyme method, and to determine their specific reaction with individual retroviral proteins.

Materials and Methods

Patients' sera were obtained from the Mammary Cancer Department of All-Union Oncological Scientific Centre of the Academy of Medical Sciences and preserved under −20 °C.

The sera were tested in ELISA for the presence of antibodies reacting with proteins of the following retroviruses: simian sarcoma virus (SSV), M-7, bovine leukaemia virus (BLV), Mason-Pfizer monkey virus (MPMV), RD-114; tobacco mosaic virus (TMV) was used as control (the virus preparations were obtained from the National Cancer Institute, Bethesda, U.S.A.). The reaction was carried out as previously described (Litvinov *et al.*, 1984). The antigen was applied in 20 μg/ml concentration (2 μg virus protein per well). The patients' sera were used in 1/200 dilution. For competition binding test, the sera were incubated for 2 hr at 37 °C with viral specimens ar a ratio of 5 μg of virus protein per 100 μl of the diluted 1 : 300. Then the sera were used in ELISA as described above. To control of possible interaction of the sera with carbohydrate components of viral glycoproteins the reaction was carried out in the presence of 0.1 mol/l mono- and oligosaccharides, N-acetyl-D-galactosamin, L-ramnose, D-fucose, L-arabinose, D-fructose, D-lactose, D-glucose, maltose, D-arabinose.

The specificity of positive ELISA results was checked against individual viral proteins by immunoblotting. The proteins were separated by electrophoresis in 15% polyacrylamide gel according to Laemmli (1970) (5 μg of protein per sample at 10 V/cm) and transferred to a nitrocellulose filter (Du Dois and Rossen, 1983). All retroviruses were present on each filter, so that they served as a marker of antibody interaction with heterologous proteins. The filters were incubated overnight at 8 °C in 3% ovalbumin in phosphate buffer for binding of nitrocellulose free-bonds, and then they were incubated for 2 hr at room temperature with tested sera diluted 1/300 in phosphate buffer saline containing 0.1% Tween-20 and 1% ovalbumin. After washing with 0.1% PBS-Tween-20 the filters were incubated for 2 hr at room temperature with peroxidase labelled goat IgG against human immunoglobulins using the same buffer. After washing the blots were reacted with 3,3-diaminobenzidintetrahydrochloride as substrate (0.5 μg/ml in 0.05 mol/l Tris-HCl buffer pH 7.5) containing 2 μg/ml of 3% H_2O_2 solution.

Results

The sera of 89 patients were tested for the presence of antibodies against different retroviruses. Simultaneously all viral proteins, including TMV, were stained to monitor the nonspecific reaction of serum components. The medium square deviation of the estimated nonspecific reaction was determined from the reaction of each serum with TMV. Assay for antibodies to MPMV was performed without the TMV samples so that in latter case the statistical data were based on this estimation only. When the reaction level exceeded $\bar{x} + 3.5$, the sera were estimated as probably positive. The results are presented in Fig. 1. When determining the background reaction, one serum reacted positively with TMV in ELISA, but this was not confirmed by immunoblotting.

The specificity of positive sera to individual proteins of retroviral preparations was determined by immunoblotting. The lack of obvious reaction with other viral proteins on the same filter, even under analogous condition of treatment, served as internal control of the reaction specificity.

Table 1 shows that among sera reacting with RD-114 three types of reactions were apparent. In the first case the serum activity was directed against p23 minor protein, whereas the reaction with p30, a major viral protein, was not detected. This serum did not interact with any other retrovirus. Another type of the reaction is represented by 2 sera. Their major

activity was directed against p30 of RD-114; in addition, in one case against p25 of BLV, in the other against major internal proteins of BLV, M-7, and SSV. The third type reaction was a faint with both proteins of RD-114 (p23 and p30). Furthermore, one serum contained individual specificity for p15 of M-7, and the other for p30 of SSV. In none of the sera a detectable reaction with the internal proteins of MPMV was found. This may reflect the absence of antibodies to MPMV, because in the same conditions the structural proteins of this retrovirus had been revealed by specific antiserum and human blood sera (Morozov *et al.*, in press) containing antibodies to MPMV. The other sera reacted either with retroviral glycoproteins only or failed to yield any reaction (Fig. 2).

Taking into account the possibility that several viral proteins might have reacted simultaneously by different cross determinants, competitive ELISA was performed to bind patients' antibodies to one retrovirus by another

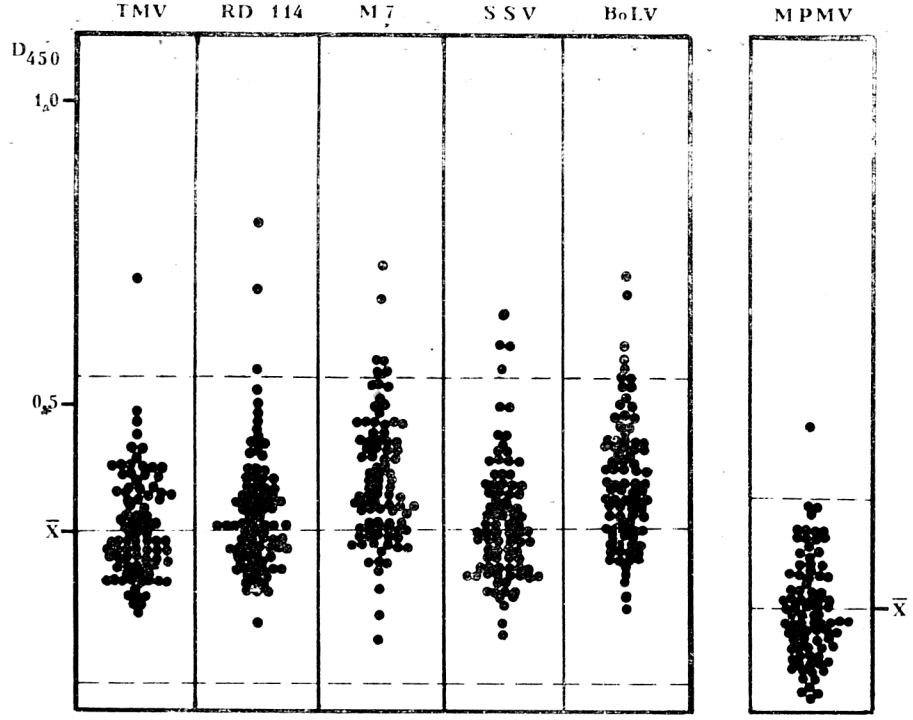

Fig. 1.

Immunoenzyme analysis

Reaction of serum of female patients with benign and malignant mammary gland tumours with preparations of TMV and retroviruses. The dotted line — the limit of variation of unspecific reaction ($\bar{x} + 3$).

Table 1. Antibodies to structural proteins types C and D retroviruses in the sera of patients with mammary gland cancer and benign tumours

Serum	Retroviruses and their proteins						
	RD-114		M-7		SSV	BLV	MPMV
	p30	p23	p30	p15	p30	p25	
A	−	++	−	−	−	−	−
B	+	−	−	−	−	+	−
C*	+	+	+	−	+	+	−
D	HT		HT	+	+	−	−
E*	+	−	−	+	−	−	−
F−G*	+	+	−	−	−	−	−

* — the sera reacting with retroviral glycoproteins

virus preparation. Neither RD-114 nor MPMV blocked the reaction of serum with BLV, M-7, and SSV, while the latter 3 may have had cross determinants (Fig. 3). In general, the results correlated with the data of Barbacid *et al.*, (1980) and Schetters *et al.* (1983) who discussed the existence of interspecies-specific antigenic determinants in C and B retrovirus proteins, though

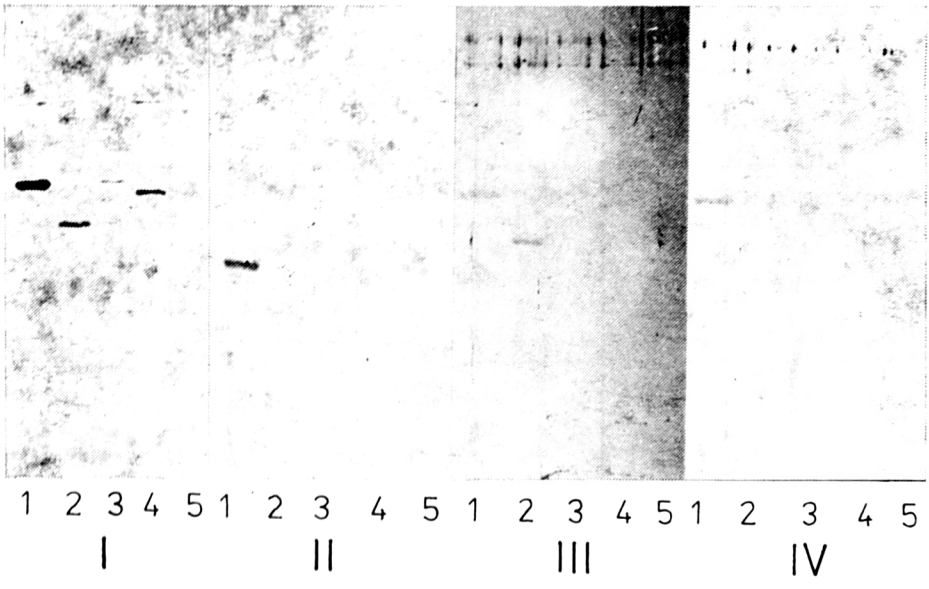

Fig. 2.

Immunoblotting of different donor sera positively reacting with viral preparations (II-IV) I — electrophoregram of retrovirus preparations; lanes: (1 — RD-114, 2 — BLV, 3 — M-7, 4 — SSV, 5 — MPMV).

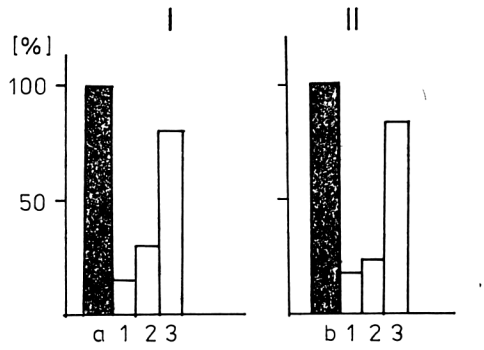

Fig. 3.
The exhaustion of the reaction of binding of the antibodies with the proteins of M-7 (I) and BLV (II) preparations
Preliminary incubation with preparations of M-7 (1), BLV (2) and RD-114 (3). Black columns — the reaction of the sera with viral preparations without exhaustion.

Barbacid especially selected BLV, which did not exhibit cross-immune reactions with other retroviruses. Another explanation for decreasing the extent of reaction with M-7 or SSV by preliminary exhaustion of sera with BLV or vice versa may be binding of antibodies with the carbohydrate portion of viral glycoproteins (Snyder *et al.*, 1980). Since several sera reacted with these glycoproteins in immunoblotting, they were tested in ELISA for the ability of binding with viral preparations in the presence of different carbohydrates. Fig. 4 presents an example of exhaustion of a serum with carbohydrates leading to almost twofold decrease of the reaction intensity

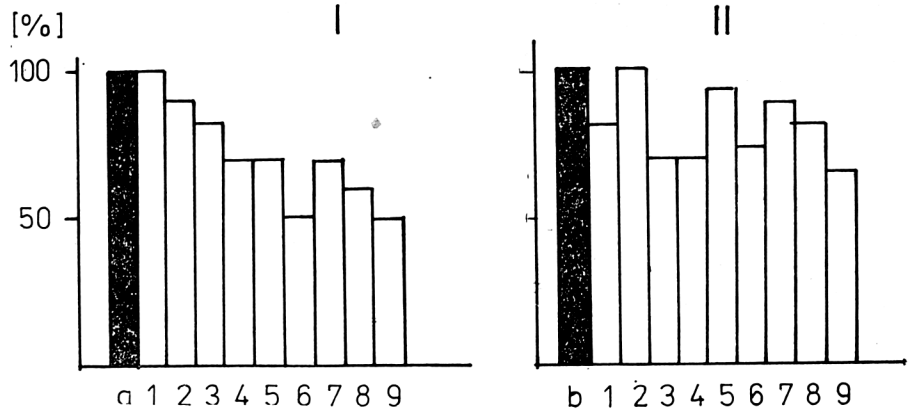

Fig. 4.
The exhaustion of the positive in ELISA serum reaction by their preincubation with oligosaccharides
I — the reaction with M-7 preparation, II — the reaction with BLV preparation. Black column — the reaction without exhaustion. Oligosaccharides: 1 — N-acetyl-D-galactosamin, 2 — L-ramnose, 3 — D-fucose, 4 — L-arabinose, 5 — D-fructose, 6 — D-lactose, 7 — D-glucose, 8 — maltose, 9 — D-arabinose.

Table 2. Frequency of antibodies to structural proteins of C and D viruses in patients with mammary gland cancer and benign tumours

Disease	Serum number	Antibodies to proteins				
		RD-114	M-7	SSV	BLV	MPMV
Mammary gland cancer	67	4	2	2	1	—
Benign tumours and displasias	27	1	—	—	1	—

against M-7 and BLV. The presence of antigens to carbohydrate residues of viral glycoproteins also enables to explain the fact that sera strongly positive with respect to antibodies against all retroviruses in the preliminary testing (in our selection there were 4 of them) reacted in immunoblotting only with glycoproteins but not with the core *gag* gene products.

Among 89 blood sera of patients with mammary tumour and from donors with benign tumours and displasia, sera specifically reacting with structural proteins of different type C retroviruses were selected. Among them, two sera contained antibodies to individual proteins of SSV, M-7, BLV (2.3% for both viruses), 5 sera to RD-114 (5.7%) but not a single to MPMV (Table 2). All sera containing antiviral antibodies were from the patients with mammary tumours, except of the patients with mastopathia cystica reacting with p25 of BLV and p3P of RD-114. The frequency of antibodies to SSV, M-7, and PLV in our sample group is small and may be estimated as common for the population. In addition, the cross antigen determinants in this virus group may increase the reaction frequency as the two sera mentioned above contained antibodies to structural proteins of several retroviruses and, accordingly, were several times listed in Table 2.

Discussion

The appearance in humans of humoral antibodies reacting with structural proteins of different retroviruses may result from the synthesis of antigens (in any tissues) containing common determinants with retrovirus proteins. In some cases such proteins are coded by retrovirus sequence in the human genome (Wahlström *et al.*, 1985; One *et al.*, 1986) and the appearance of antibodies reacting with retroviral proteins reflects the activation of the endogeneous human retrovirus, connected as a rule with some proliferative (often tumourous) processes in the tissues. Thus, expression of the antigen related to gp52 of MPMV is associated with mammary gland tumour, and the antigen related to p30 RD-114 with renal adenocarcinoma (Wahlström *et al.*, 1985). However, the antibodies expressed in processes such as leukaemia crossreact with determinants of individual epitopes of the majority of retroviral proteins (Segal-Eiras *et al.*, 1983; Atkin *et al.*, in press). The antibodies

against retroviruses are found in 50% of mammary tumour patients and, as supported by numerous researchers, specifically distinguish only epitopes of the MMTV proteins (Tomana *et al.*, 1980; Zotter *et al.*, 1981; Day *et al.*, 1981; Litvinov *et al.*, 1984). We, however, tried to find out whether antibodies to structural proteins of other retrovirus have been formed.

The data obtained confirm that though in some patients with mammary gland tumour antibodies were formed reacting with the proteins of retrovirus RD-114 or SSV. This is a rare phenomenon so that antibodies reacting with MMTV are the only ones specific for this tumour as previously reported (Litvinov *et al.*, 1984).

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