DIFFERENTIATION OF TYPE D VIRUS FROM TRANSPLANTABLE HUMAN CELLS (IL'IN–BYKOVSKII VIRUS) AND MASON–PFIZER MONKEY VIRUS ON THE BASIS OF VIRUS ENVELOPE ANTIGENS

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To differentiate between Il'in–Bykovskii virus (IBV) and Mason–Pfizer monkey virus (MPMV) the method of neutralization of the viruses by antisera against virus envelope antigens was used. The viruses were cultured on the same human embryonic cells. The results of virus neutralization were determined by the presence or absence of gs antigen in the affected cells. Antiserum against IBV envelope antigens neutralized IBV but not MPMV. Antiserum against MPMV did not neutralize IBV. It is concluded that IBV and MPMV differ in their virus envelope antigens and must be regarded as different viruses.

KEY WORDS: Il'in–Bykovskii virus; Mason–Pfizer virus; virus envelope antigens; neutralization reaction.

Type D oncornavirus, or Il'in–Bykovskii virus (see Supplement No. 6 to the Protocol of the Second Joint Soviet–American Conference on Malignant Neoplasms, Moscow, USSR, June 2-4, 1975), was isolated from transplantable human carcinoma cells [6, 7]. Association between Il'in–Bykovskii virus (IBV) and certain types of human tumors has been established [1, 2, 14]. The IBV genome has been found to be incorporated into the genome of human breast fibroadenoma cells, and the virus genome is expressed in the cells of these tumors up to the level of gs-antigen synthesis [3-5, 8]. IBV has a common gs antigen (p27) or a common determinant of this protein with Mason–Pfizer monkey virus (MPMV) [9, 13], isolated from a spontaneous monkey breast carcinoma [11]. No investigations have yet been undertaken to compare the antigens of the envelopes of these viruses, which belong to the group of type D oncornaviruses of primates. Such a study could be undertaken by the method of neutralization of viruses by antisera against virus envelope antigens. This is an important matter, because a solution to the problem could help to explain whether IBV is the monkey virus contaminating transplantable human cells or a different virus. This is particularly interesting in connection with the discovery of the gs antigen of this group of viruses in fibroadenomas of the human breast [3-5, 8] and the nucleotide sequences of their genome in the genome of cells of carcinomas of the human breast [1, 2, 12].

In a previous paper the writers described having obtained antisera against purified IBV, treated with Tween-20, and against "ghosts" of HEp-2 cells, in which this virus is produced [5, 8]. It was shown by an immunofluorescence method that these antisera, absorbed with IBV gs antigen, test the surface antigens of human embryonic cells infected with IBV but do not test this antigen in cells infected with MPMV [5]. Antiserum against IBV (Tween-20), containing antibodies against IBV envelope antigens, after absorption by gs antigen, precipitated IBV (Tween-20) with one precipitation band but did not precipitate MPMV (Tween-20) [8].

The object of this investigation was to compare IBV and MPMV by the method of neutralization of viruses by antisera containing virus envelope antigens.

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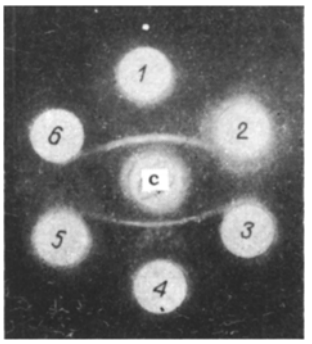

Fig. 1. Immunoautoradiography test of antiserum against IBV envelope antigens with IBV and MPMV antigens treated with Tween-20: 1) IBV; 2) MPMV; 3) buffer; 4) IBV; 5) MPMV; 6) buffer. Central well (c) contains antiserum against IBV envelope antigens. Antiserum against IBV envelope antigens precipitates antigen of IBV but does not precipitate antigen of MPMV.

EXPERIMENTAL METHOD

Viruses. MPMV (obtained from the USA) and IBV were cultured on human embryonic cells from tissue obtained from the same embryo. The cells were infected with cell-free material containing the virus. Production of the viruses was demonstrated by the presence of gs antigen in the immunodiffusion test using a test system for IBV described previously [13], which is a test system for the inner core protein of the virus with a molecular weight of 27,000 daltons (p27).

Virus-Neutralizing Sera. Production of antiserum against IBV envelope antigens was described previously [8]. The antiserum was obtained by immunization of a rabbit by injection of purified IBV, treated with Tween-20 in a final concentration of 0.2%, into a lymph node.

Immunodiffusion Test Using Immunoautoradiography. Antiserum against IBV (Tween-20), absorbed with homogenate of human embryonic tissue, bovine serum, and gs antigen of IBV was used in the reaction. The antigens were purified IBV and MPMV, treated with Tween-20. The IBV and MBMV gs antigens were found by a test system for IBV gs antigen described previously [13]. The gs antigen was obtained from the infected cells by repeated freezing and thawing of the cells in a concentration of 40 million cells/ml.

The immunodiffusion test was set up in 2% agar. Some tests were carried out by the immunoautoradiography method using donkey antirabbit γ-globulin labeled with ^{125}I [10, 15].

Virus Neutralization Test. IBV and MPMV were treated with antiserum against IBV envelope antigens. IBV was treated with antiserum against MPMV. Neutralization of the viruses with the antisera was carried out by the following method: 0.5 ml of whole antiserum was added to the virus precipitated from 1 liter of the liquid phase of the culture. The same volume of serum was added to 1/10 of the initial amount of virus. After exposure for 40 min at 37°C the embryonic tissue cells were infected. Cells infected with virus after exposure with normal sera served as the control. The results of the virus neutralization tests were assessed from the presence or absence of gs antigen in the immunodiffusion test in the infected cells.

EXPERIMENTAL RESULTS

Antiserum against IBV envelope antigens, after absorption with IBV gs antigen, precipitated IBV (Tween-20) antigens in the immunodiffusion test but did not precipitate MPMV (Tween-20) antigens (Fig. 1). The same serum, before absorption with IBV gs antigen precipitated the gs antigens of IBV and MPMV in preparations of IBV and MPMV treated with Tween-20, to give an identical precipitation band. Consequently, the viruses contained a common gs antigen, but IBV contained an antigen which was not detected in MPMV even by immunoautoradiography. It can be postulated that this antigen was an IBV envelope antigen.

In human embryonic tissue cells infected with different doses of IBV, after exposure to antiserum against IBV envelope antigens no gs antigens could be found during tests for 1.5 months. The gs antigen was found in control cells from the 7th-10th day after infection (Fig. 2A). In cells infected with different doses of MPMV, gs antigen was found after exposure to antiserum against IBV envelope antigens and also in control cells from

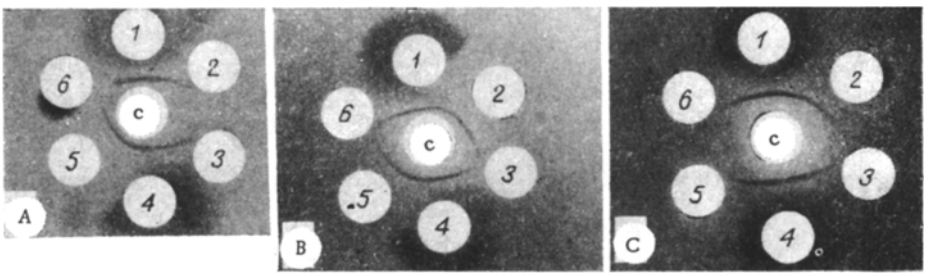

Fig. 2. Result of test of neutralization of IBV and MPMV for antigen of viruses in infected cells. A: 1) gs antigen; 2) IBV + antiserum against IBV envelope antigens; 3) buffer; 4) gs antigen; 5) IBV + normal serum; 6) buffer. Central well (c) contains antiserum against gs antigen. B: 1) gs antigen; 2) MPMV + antiserum against IBV envelope antigen; 3) buffer; 4) gs antigen; 5) MPMV + normal serum; 6) buffer. Central well contains antiserum against gs antigen. C: 1) gs antigen; 2) IBV + antiserum against MPMV; 3) buffer; 4) gs antigen; 5) IBV + antiserum against IBV envelope antigens; 6) buffer. Central well contains antiserum against gs antigen. Antiserum against IBV envelope antigens neutralizes IBV but does not neutralize MPMV. Antiserum against MPMV does not neutralize IBV.

the 7th to 10th day after infection (Fig. 2B). In cells infected with different doses of IBV, gs antigen was found after exposure to antiserum against MPMV from the 7th to 10th day after infection (Fig. 2C), just as in the control cells.

The experiments thus showed that antiserum against IBV envelope antigens reveals an IBV antigen which is not the gs antigen, but does not reveal this antigen in MPMV. This is evidently an envelope antigen. Antiserum against IBV envelope antigens neutralizes IBV but does not neutralize MPMV. Antiserum against whole MPMV has no neutralizing action on IBV even if the dose of virus is reduced. Both viruses were cultured on the same cells before neutralization and the difference in the envelope antigens cannot therefore be explained by the incorporation of components of the cells.

It can be concluded from these results that the envelope antigens of IBV and MPMV are different and, consequently, the viruses must also be regarded as different. Differentiation of these viruses by the virus-neutralization test provides a method of studying association of a concrete virus with human tumors and, in particular, with carcinomas and fibroadenomas of the breast.

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