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COMPARISON OF MEMBRANE IMMUNOFLUORESCENCE AND CYTOTOXICITY TESTS FOR DETECTION OF CELL SURFACE ANTIGENS OF MAREK'S DISEASE LYMPHOMA-DERIVED CELL LINE

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The reactivity of an anti-MDCC-MSB 1 rabbit serum to Marek's disease tumor-associated surface antigen (MATSA) of a Marek's disease lymphoma-derived cell line (MDCC-MSB 1) was compared by membrane immunofluorescence and cytotoxicity tests. Following results were obtained by both tests: 1) the similar reactivity of the antiserum in titrations, 2) the similar blocking activity patterns of the soluble antigens from MDCC-MSB 1 cells to the antiserum, and 3) the parallisms between the cytotoxic index and the frequency of positive cells for MATSA of the MDCC-MSB 1 cells treated with proteolytic enzymes. From these results, both tests appeared to be detecting a similar, if not identical, antigen. The cytotoxicity test was slightly more sensitive than the membrane immunofluorescence test.

INTRODUCTION

Marek's disease (MD) is a lymphoproliferative disease of chickens caused by a herpesvirus (CHURCHILL & BIGGS, '67; NAZERIAN et al., '68), and is characterized by lymphoma formation in the visceral organs and peripheral nerves. Several tumor transplants and cell lines have been established from MD lymphoma cells (AKIYAMA et al., '73; CALNEK et al., '78; NAZERIAN et al., '77; POWELL et al., '74; SEVOIAN et al., '64). The cells of MD tumor transplants, MD lymphoma, and cell lines carry the T-cell surface determinants and express the Marek's disease tumor-associated surface antigens (MATSA) (HUDSON & PAYNE, '73; MATSUDA et al.,[7,8] '76; NAZERIAN & SHARMA, '75; NAZERIAN et al., '77; NAZERIAN & WITTER, '75; PAYNE et al., '74; POWELL et al., '74; ROUSE et al., '73; WITTER et al., '75).

The T-cell surface determinants and the MATSA of these cells were detected by the membrane immunofluorescence test as well as by the cytotoxicity test (MATSUDA et al.,[7,8] '76; MIKAMI et al., '80; NAZERIAN et al., '77; POWELL et al., '74; SHARMA et al., '78; SUGIMOTO et al.,[21,22] '79; WITTER et al., '75). We have reported similar reactivities of anti-MDCC-MSB 1 and anti-MDCC-RP 1 sera against the corresponding

MDCC-MSB 1 and MDCC-RP 1 cells shown by both tests (MIKAMI et al., '80; SUGIMOTO et al.,[21] '79); however, no detailed comparative study on whether or not both tests were detecting the same antigen has been made. The purpose of this study was to compare both tests using an anti-MDCC-MSB 1 rabbit serum against MDCC-MSB 1 cells. The comparison was made by examination of the reactivity of the antiserum in titrations, the blocking activities of the soluble antigens from the MDCC-MSB 1 cells to the antiserum, and the parallisms between the cytotoxic index and the frequency of MATSA-positive cells of MDCC-MSB 1 cells treated with proteolytic enzymes.

MATERIALS AND METHODS

Cell and cell cultures

The MDCC-MSB 1 cell lines (AKIYAMA & KATO, '74; AKIYAMA et al., '73) were kindly provided by Dr. S. KATO (Research Institute for Microbial Diseases, Osaka University, Osaka, Japan) and were cultured as previously described (SUGIMOTO et al.,[22] '79). The nomenclature for the cell lines used herein is the same as that recently proposed by WITTER et al. ('79).

Antiserum

Antiserum against the MDCC-MSB 1 cells was prepared in a rabbit and absorbed with normal chicken erythrocytes and lymphocytes before use, as previously described (SUGIMOTO et al.,[21] '79).

The absorbed serum was found to be negative against the spleen, thymus and bursa cells of normal chickens (PDL-1 line) (MIKAMI et al., '80; SUGIMOTO et al.,[21] '79) and the 1104-X-5 cell line cells derived from a bursal lymphoma induced by avian leukosis virus (HIHARA et al., '74) in the membrane immunofluorescence and the cytotoxicity tests. Furthermore, the antiserum did not stain the membrane and intracellular antigens of the chick kidney cells infected with the Marek's disease herpesvirus.

Cytotoxicity test

The complement-dependent antibody cytotoxicity (CDAC) was examined by the trypan-blue dye-exclusion test, and the viability of the cells was established by counting a minimum of 200 cells according to the method described previously (SUGIMOTO et al.,[21] '79). The cytotoxicity index (CI) was calculated by the following formula:

$$CI = \frac{\%\ \text{viable cells in cell suspension alone} - \%\ \text{viable cells in test sample}}{\%\ \text{viable cells in cell suspension alone}} \times 100$$

Membrane immunofluorescence test

The membrane immunofluorescence test was performed by an indirect method (SUGIMOTO et al.,[21] '79) using fluorescein-isothiocyanate-conjugated anti-rabbit IgG goat serum. The proportion of MATSA-positive cells was established by counting a minimum of 200 cells.

Blocking test

The ability of the soluble antigens prepared from MDCC-MSB 1 to block the membrane reactivity or the cytotoxicity of the antiserum was examined by both tests. One volume of antiserum (diluted to 1 : 8 with phosphate-buffered saline, PBS) was first treated with equal amounts of varying dilutions of soluble antigens in the well of a microplate for 60 min at 4°C, and then one half of the mixture was used for test samples, and the other half was used as the antigen control in both tests. As a positive control of the antibody, the antiserum was mixed with an equal volume of PBS instead of the soluble antigens. These mixures showed MATSA-reactivity stain to 75% of the MDCC-MSB 1 cells when determined by the membrane immunofluorescence test, or showed a cytotoxic index of 75 when determined by the cytotoxicity test.

Suitable controls for the antiserum or complements were employed in each test. The blocking index (BI) for the cytotoxicity was calculated using the following formula :

$$\text{BI (cytotoxicity)} = \frac{\text{CI of positive control} - \text{CI of test sample}}{\text{CI of positive control}} \times 100$$

The BI of the membrane immunofluorescence (MI) was calculated using the following formula :

$$\text{BI (MI)} = \frac{\text{\% MI-positive cells with positive control serum} - \text{\% MI-positive cells with test samples}}{\text{\% MI-positive cells with positive control serum}} \times 100$$

Soluble antigens

Soluble antigens were prepared from MDCC-MSB 1 cells either by freezing and thawing (F-S) three times or by treatment with sodium deoxycholate (SD, 16 mg/ml) and gentle stirring for 16 hours at 4°C. These soluble antigens will be referred to hereafter as F-S Ag and SD Ag. The details of their preparation will be described elsewhere (SUZUKI et al., manuscript in preparation).

Enzyme treatment

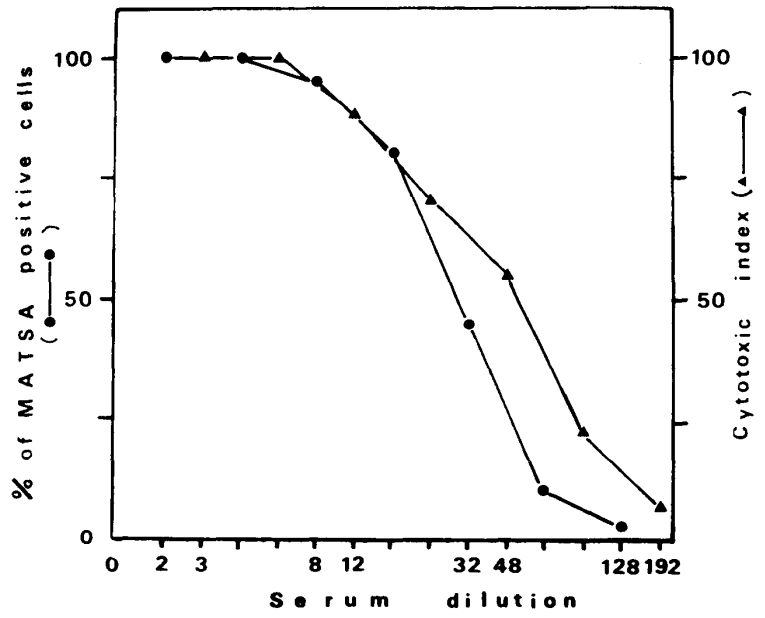
Pronase (Sigma Chemical Co., St. Louis, U. S. A. 17 U/mg solid, crystallized twice), trypsin (DIFCO Lab., Detroit, U. S. A. 1 : 250) and papain (Sigma Chemical Co., 20 U/mg, crystallized twice) used in the experiments. The MDCC-MSB 1 cells (5×10^6/ml) were washed three times with PBS, and the resultant pellets were treated with one ml of each of the proteolytic enzymes, which were serially diluted with PBS. The mixtures were incubated for 30 min at 37°C and then washed three times with PBS at 4°C by centrifugation. After centrifugation the cells were examined for the residual surface antigens by the membrane immunofluorescence and cytotoxicity tests.

RESULTS AND DISCUSSION

Using the indirect immunofluorescence test, MATSA was first demonstrated on MD tumor cells, JMV lymphoblastic leukemia cells, and the cultured cells of lymphoblastoid cell lines derived from MD lymphomas (POWELL et al., '74; WITTER et al., '75). Recently, the surface antigen of these lymphoblastoid cell lines was also detected by cytotoxicity tests using the ^{51}Cr release method (SHARMA et al., '78) or the trypan-blue dye-exclusion method (MIKAMI et al., '80; SUGIMOTO et al.,[21,22] '79). Although it is reasonable to believe that a similar tumor-associated surface antigen is detectable by the cytotoxicity test because of the specificity of the test, no detailed study has been made to compare whether or not both the cytotoxicity and the immunofluoresecence tests detect the same antigen. Through the present experiments we have provided direct or indriect evidence that both tests are detecting a similar, if not identical, antigen.

An anti-MDCC-MSB 1 serum, which had been absorbed with normal chicken cells, was titrated by both cytotoxicity and membrane immunofluorescence tests to determine whether or not there were qualitative differences between the cell surface antigens detected by the two tests, and to determine which of the tests was more sensitive (fig. 1). The reactivities of the two-fold, serial dilutions of the serum against the MDCC-MSB 1 cells in both tests were similar. The serum titer in the cytotoxicity test, however,

FIGURE 1 *The reactivity of an anti-MDCC-MBS 1 serum against MDCC-MSB 1 cells*

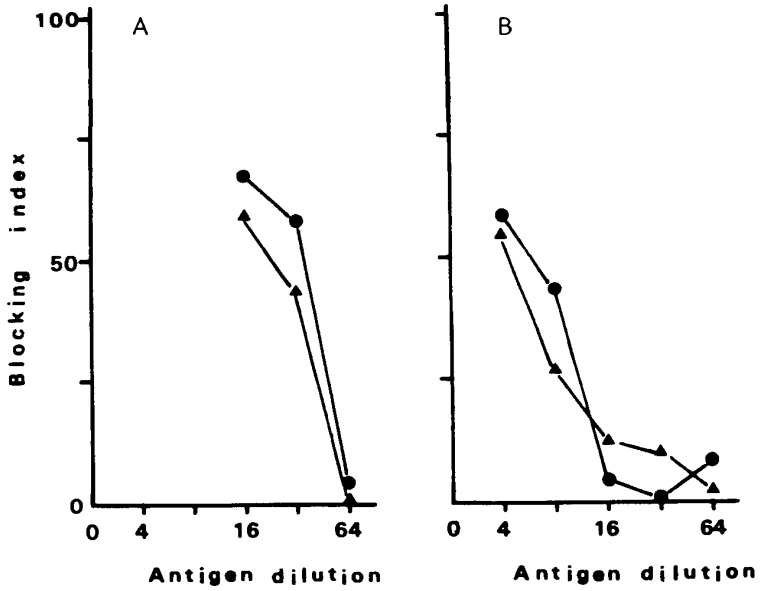


The reactivity was examined by the cytotoxicity (▲——▲) and membrane immunofluorescence (●——●) tests.

was slightly higher than that obtained in the membrane immunofluorescence test. Similar results were observed consistently in experiments using other lots of antisera (MIKAMI et al., '80; SUGIMOTO et al.,[21] '79).

The blocking activities of both soluble antigens prepared from the MDCC-MSB 1 cells against an anti-MDCC-MSB 1 serum were examined in the membrane immunofluorescence and cytotoxicity tests. As shown in figure 2, activities were observed in both tests, and they decreased with the increasing dilution of the antigens. Furthermore, the results of the membrane immunofluorescence test were very similar to those of the cytotoxicity test. Because of the nonspecific cytotoxic activity of the antigens in high concentrations, it was difficult to obtain the BI of both antigens in the cytotoxicity test (below 1 : 8 for F-S Ag, and 1 : 2 for SD Ag). It was also observed that the anti-MATSA-titers determined by the cytotoxicity test correlated well with those determined by the membrane immunofluorescence test (MIKAMI et al., '80; SUGIMOTO et al.,[21] '79).

FIGURE 2 *Blocking activity of soluble antigens from MDCC-MSB 1 cells to an antiserum*

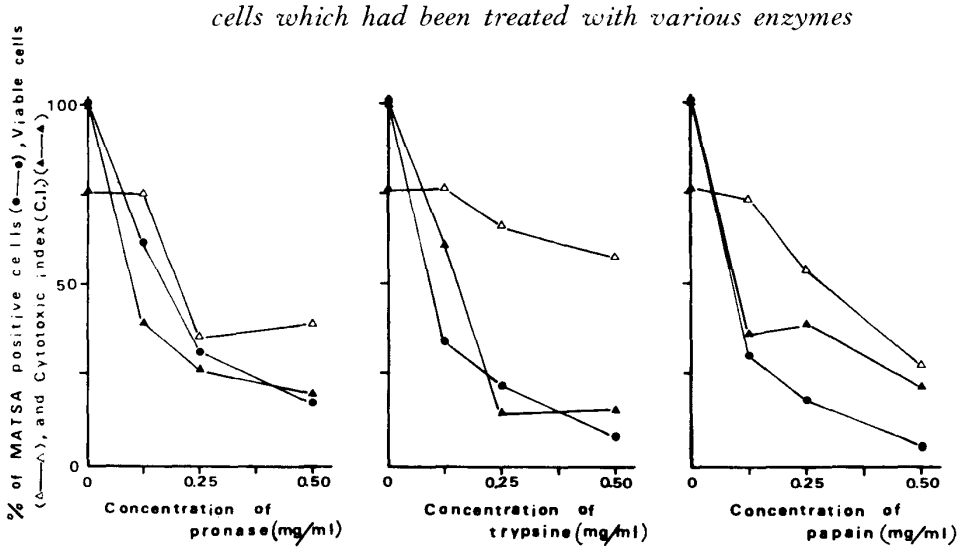


The antigens were solubilized by either freezing and thawing three times (A) or by sodium deoxycholate (B). The blocking activity was examined by the inhibition of the membrane immunofluorescence test (FA, ●——●) or by the cytotoxicity test (CDAC, ▲——▲).

Experiments were designed to establish whether the reactivity of the antiserum against the MDCC-MSB 1 cells treated with various enzymes was identical in both tests. As shown in figure 3, the frequency of MATSA-positive cells and the CI of the treated cells decreased with increasing concentrations of pronase, trypsin and papain.

In addition, the reactive patterns of both tests were very similar. The results from the present experiments indicated that both tests were detecting a similar, if not identical, antigen present on the surface of MDCC-MSB 1 cells.

FIGURE 3 *The reactivity of an antiserum against MDCC-MSB 1 cells which had been treated with various enzymes*



The reactivity was examined by the membrane immunofluorescence (●——●) and cytotoxicity (▲——▲) tests. The viable MDCC-MSB 1 cells (△——△) were also examined.

Recently, success in solubilizing the membrane antigens from cells infected with Herpesvirus saimiri or Epstein-Barr virus in an immunologically active state by use of papain digestion has been reported (PEARSON & QUALTIERE, '78 ; QUALTIERE & PEARSON, '78). In other investigations MATSA was found to be sensitive to treatment with either trypsin or pronase, but resistant to treatment with either papain or pepsin (SUZUKI & MIKAMI, unpublished data). Although there is no information on the purification and biochemical characterization of MATSA, the inhibition of the cytotoxicity test may be an efficient method for monitoring the purity of the antigen during the process of purification and characterization. Enzymes such as papain and pepsin may also be used to solubilize MATSA in an immunologically active form from the MDCC-MSB 1 cells or other line cells derived from MD lymphomas.

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