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SHORT COMMUNICATION

# ISOLATION OF MONOCLONAL ANTIBODIES REACTIVE WITH MAREK'S DISEASE TUMOR-ASSOCIATED SURFACE ANTIGEN (MATSA)

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Two hybridoma clones producing monoclonal antibodies were obtained from mice immunized with the Marek's disease (MD)-lymphoblastoid cell line MSB1. These monoclonal antibodies reacted with the surface of MD-lymphoblastoid cell lines at higher titers than with avian lymphoid leukosis cell lines or with normal chicken thymus, bursa or peripheral blood lymphocytes. The serological specificity of these monoclonal antibodies seemed to correspond with that of rabbit antiserum reactive with MD tumor-associated surface antigen (MATSA).

Marek's disease (MD), caused by Marek's disease virus (MDV), is a highly contagious malignant lymphoma disease in chickens. A herpesvirus of turkeys (HVT) has been successfully used as vaccine against MD. Since the first MD lymphoma-derived lymphoblas-

toid cell line was established by Akiyama et al. (1973), many other cell lines have been established (Witter et al., 1979). All these cell lines have been shown to carry a T-cell marker (Nazerian and Sharma, 1975; Matsuda et al., 1976a; Calnek et al., 1978) and MD tumor-associated surface antigen (MATSA) (Powell et al., 1974; Witter et al., 1975; Matsuda et al., 1976b). On the other hand, cell lines derived from lymphomas of lymphoid leukosis (LL) caused by avian leukosis virus have been shown to carry a B-cell marker (Matsuda et al., 1978a) and have been used as negative controls of MATSA. MATSA-positive cells were transiently detected in chickens vaccinated with HVT (Powell and Rennie, 1978; Schat and Calnek, 1978; Kitamoto et al., 1979). In addition to MATSA, heterophile Hanganutziu-Deicher antigen was identified as MD tumor-associated surface antigen (Ikuta et al., 1981a). Heterophile Forssman antigen was identified in LL cell lines, but not in MD cell line (Ikuta et al., 1981b), whereas chicken fetal antigen was expressed on both MD and LL cell lines (Murthy et al., 1979). However, all these antigens are distinct from MDV-specific antigens normally present in productively infected fibroblasts (Witter et al., 1975). Recently, we isolated a series of mouse monoclonal antibodies against MDV and HVT antigens (Ikuta et al., 1982). Two types of cell surface antigens, gA and gB, which were expressed on cells productively infected with MDV or HVT, were identified with these monoclonal antibodies (Ikuta et al., 1983, 1984). However, these monoclonal antibodies did not react with MD-lymphoblastoid cell lines (unpublished data). Therefore, isolation of monoclonal antibodies specific to MD cell lines is required for clear-cut distinction of MD from LL in the field. Among the antigens expressed on the MD cell lines described above, MATSA and Hanganutziu-Deicher antigen seem to be useful for this purpose. However, mouse hybridoma cells producing monoclonal antibody against Hanganutziu-Deicher antigen are difficult to isolate, because the antigen is a self-antigen in mice. At present, therefore, MATSA is the only known antigen that is useful for preparation of monoclonal antibody. Rabbit or chicken antiserum against the MD-lymphoblastoid cell line shows specificity for MATSA antibody only after extensively repeated absorption with normal chicken cells and/or LL line cells. In addition, dissimilarity or partial qualitative differences of MATSA among various MD cell lines have been reported (Witter et al., 1975; Sharma et al., 1977; Nazerian et al., 1977; Sugimoto et al., 1979; Rennie and Powell, 1979). These results suggest that MATSA might consist of two or more different antigens. In order to isolate monoclonal antibody reactive with MATSA common to all MD cell lines, we tried to isolate hydridomas from mice immunized with the MD cell line MSB1.

BALB/c mice of 6–8 weeks old were immunized 4 to 6 times intraperitoneally at weekly intervals with about $5\times10^6$ cells of MD cell line MDCC-MSB1 (Akiyama and Kato, 1974). Spleen cells removed from immunized mice 3 days after the last immunization were used for isolation of hybridoma cells with mouse myeloma cells (SP2/O-Ag14; Shulman et al., 1978). The methods for production and culture of hybridomas were described in detail elsewhere (Ikuta et al., 1982). Screening of monoclonal antibodies against MATSA was carried out by the indirect membrane immunofluorescence (MIF) test with fluorescein isothiocyanate-conjugated sheep anti-mouse IgG (Cappel Laboratories) as described previously (Ikuta et al., 1982). Hybridoma cells was cloned by end-point dilution in 96-well microplates. Clones producing antibodies were injected intraperitoneally into BALB/c mice. The resulting ascites fluids were harvested and clarified, and their titers of antibody against the surface of line cells or normal chicken cells were assayed by the indirect MIF test with serial 10-fold dilutions of the ascites fluid.

TABLE 1. *Antibody titers of monoclonal antibodies obtained from mice immunized with MSB1 cells against the surfaces of MSB1 cells, 1104B1 cells and normal chicken thymus cells*

| Hybridoma clone[a] | MIF titer against | | |
|---|---|---|---|
| | MSB1 | 1104B1 | Thymus[b] |
| MT1 | 5[c] | 2 | 2 |
| MT2 | 5 | 2 | 5 |
| MT3 | 5 | 3 | 5 |
| MT4 | 4 | 1 | 2 |
| MT5 | 6 | 6 | 4 |
| MT6 | 5 | 2 | 5 |
| MT7 | 5 | 5 | 5 |
| MT8 | 3 | 2 | 3 |
| MT9 | 4 | 4 | 3 |
| MT10 | 4 | 4 | 4 |
| MT11 | 4 | 3 | 5 |
| MT12 | 4 | 4 | 3 |
| MT13 | 5 | 1 | 5 |
| MT14 | 2 | 1 | 2 |
| MT15 | 5 | 5 | 5 |
| MT16 | 3 | 3 | 3 |

[a] Serial 10-fold dilutions of ascites fluids containing each monoclonal antibody.
[b] Thymus cells of SPF chickens.
[c] $Log_{10}$ of the highest antibody dilution showing positive fluorescence.

Sixteen hybridomas (MT1 to MT16) produced high titers of monoclonal antibodies reacting with the surface of MSB1 cells, as shown in Table 1. However, the monoclonal antibodies from all these hybridoma clones except MT1 and MT4 were not specific for MSB1 cells, since their titers for reaction with LSCC-1104B1 cells (Hihara et al., 1974) and thymus cells of specific pathogen-free (SPF) chickens were almost the same as those for reaction with MSB1 cells. Next, we examined the reactivities of the MT1 and MT4 antibodies with several MD cell lines by the MIF test, and the results are shown in Table 2. The titers of these two antibodies against MSB1 cells were similar to those against the other MD cell lines, MDCC-MSB1-41C (Yamada et al., 1983), MDCC-HP1 (Powell et al., 1974) and MDCC-LS1 (Tanaka et al., 1978). In addition, the titers of these antibodies against the LL cell line LSCC-1104X5 (Hihara et al., 1974) and peripheral blood lymphocytes of SPF chickens were as low as those against 1104B1 cells and thymus cells of SPF chickens.

Rabbit or chicken anti-MSB1 serum has usually been used as MATSA antibody only after repeated absorption with normal chicken cells and/or LL cell lines. For comparison of the specificities of MT1 and MT4 antibodies with those of antiserum reactive with MATSA, antiserum against MSB1 cells was prepared from rabbits immunized with MSB1 cells as described previously (Matsuda et al., 1976b). This antiserum was absorbed more than ten times with cells from peripheral blood, thymus, spleen and bursa of SPF chickens, which were fixed with 1% glutaraldehyde. The reactivities of this antiserum with MSB1, 1104B1 and SPF chicken thymus cells in the MIF test are shown in Table 3. The antiserum showed a slightly higher titer with MSB1 cells than with 1104B1 or normal chicken lymphocytes. It seems that the serological specificities of MT1 and MT4 monoclonal antibodies are similar to that of the repeatedly absorbed anti-MSB1 serum, although the titer of anti-MSB1 serum was lower than those of the monoclonal antibodies shown in Table 2.

Next, we made a preliminary examination of whether MT1 and MT4 antibodies are useful for clear-cut distinction of MD from LL in the field using two MD chickens and one LL chicken. The IF-positive cells were observed in cells prepared from ovary tumors of the MD chickens by the MIF test with $10^4$-fold and $10^3$-fold dilutions of ascites fluids of MT1 and MT4, respectively. The percentage of IF-positive cells reactive with MT1 or MT4 antibody was less than 1% in both tumors, but cells prepared from the liver tumor of the LL chicken did not react with

TABLE 2. *Reactivities of MT1 and MT4 antibodies with the surfaces of several MD cells lines, LL cell lines and normal chicken cells*

| Hybridoma clone[a] | MIF titer against | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | MD cell line | | | | LL cell line | | SPF chicken | |
| | MSB1 | MSB1-41C | HP1 | LS1 | 1104B1 | 1104X5 | Thymus | PBL[c] |
| MT1 | 5[b] | 5 | 4 | 5 | 2 | 1 | 2 | 2 |
| MT4 | 4 | 5 | 3 | 4 | 1 | 2 | 2 | 2 |

[a] See footnote of Table 1.
[b] See footnote of Table 1.
[c] Peripheral blood lymphocytes.

these antibodies at all.

Recently, several monoclonal antibodies with MATSA antibody specificity were prepared by an other group (Lee et al., 1983; Liu and Lee, 1983). They determined the specificity of the antibody mainly by the MIF test with culture fluid of the hybridoma, and found that the antibody reacted at higher titers with MD cell lines than with an LL cell line or normal thymus cells. In this work, we also used the MIF test for determination of the titers and serological specificities of monoclonal antibodies. Our results showed that MT1 and MT4 antibodies reacted with the antigens like MATSA reactive with rabbit anti-MSB1 serum after repeated absorption. Further studies are required to determine whether MT1 and MT4 antibodies recognize different antigens. The monoclonal antibodies related to MATSA prepared in another laboratory (Lee et al., 1983) as well as in this laboratory also reacted with normal cells, suggesting the existence of antigenic determinants common to MD cells and normal cells, but the reactivities with normal cells are not well known at present. The proportion of IF-positive cells in MD tumor cell preparations that reacted with MT1 or MT4 antibody in our study, appeared to be lower than that observed by Lee et al. (1983). This difference could be due to a difference in the antibody titers used or the tumor tissues examined or both. Qualitative analysis of the antigenic components recognized with MT1 and MT4 antibodies will be important for clarifying the nature of MATSA. After submitting this manuscript, we learned that Higashihara et al. (1984) had reported experiments on monoclonal antibodies against MATSA. Their results were essentially similar to ours reported here.

TABLE 3. *Reactivities of rabbit anti-MSB1 serum with the surface of MSB1, 1104B1 and normal chicken cell after extensive absorption with normal chicken cells*

| Absorption[a] | MIF titer against | | |
|---|---|---|---|
| | MSB1 cells | 1104B1 cells | Thymus cells[b] |
| − | 2,560[c] | 640 | 2,560 |
| + | 640 | 160 | 160 |

[a] Absorbed with cells from SPF chicken thymus, bursa, spleen and peripheral blood, which were fixed with 1% glutaraldehyde.
[b] Thymus cells of SPF chickens.
[c] Reciprocal of the highest dilution showing positive fluorescence.

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