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THE CHARACTERIZATION OF MURINE MONOCLONAL ANTIBODIES AGAINST MYELIN-ASSOCIATED GLYCOPROTEIN (MAG) OF THE HUMAN BRAIN

II. THE DIFFERENCE OF THE ANTIGENIC DETERMINANT BETWEEN CLONE, MC-P2, REACTING WITH HUMAN NATURAL KILLER CELLS AND LEU-7

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SUMMARY

We examined the reactivity of five murine monoclonal antibodies against myelin-associated glycoprotein (MAG) with the measles virus. One of the three antibodies recognizing the carbohydrate moiety of MAG and human mononuclear cells (MC-P2) could react with the measles ELISA antigen but another two reacting with peptide moiety could not. Eleven murine monoclonal antibodies against human lymphocytes including anti-Leu-7, and anti-MAG monoclonal IgM from a patient with demyelinating polyneuropathy could not react with the measles ELISA antigen. However, in an immunoblotting study, two bands, 56 kDa and 63 kDa, were stained by MC-P2, which differed from those —H(78 kDa), P(69 kDa) and NP(60 kDa) - stained by the serum of a patient with subacute sclerosing panencephalitis. It may be possible that the antigens reacting with MC-P2 are derived from Vero cells used in a culture of measles virus. Anti-MAG monoclonal murine antibody (MC-P2) could NOT react with measles virus proteins. Although previously we have found in studying by inhibition assay, that MC-P2 bind directly to or close to the Leu-7 epitope, a chemical feature of the antigen of MC-P2 and Leu-7 seems to be different.

Key words: myelin-associated glycoprotein; measles virus; monoclonal antibody; glycoprotein; natural killer

INTRODUCTION

We previously demonstrated a shared antigen between myelin-associated glycoprotein (MAG) and human natural killer cells (Sato et al. 1983; M. Tanaka et al. 1984; K. Tanaka et al. 1984). MAG is lost early in the development of multiple sclerosis (MS) plaques (Itoyama et al. 1980; Johnson et al. 1986). In patients with MS, the reduced natural killer cell activity (Benczur et al. 1980) and elevated levels of antibody to the measles virus (Adams and Imagawa 1962) or anti-MAG antibody (Sato et al. 1986) in the peripheral blood have been reported. It has been demonstrated that MS patients have significantly lower response of measles virus-specific cytotoxic T cells than normal individuals (Jacobson et al. 1985). Thus, immunological relationship between MAG and measles virus is speculated in pathogenesis of MS. The reactivity of monoclonal antiviral antibodies with normal tissues is not an uncommon phenomenon. The examples are as follows. Of 635 monoclonal antibodies against eleven different viruses including measles, rabies, cytomegalovirus and others, 21 (3.5%) reacted with normal tissues (Srinivasappa et al. 1986). Shared antigens between measles virus phosphoprotein or 146 kDa protein of herpes simplex and vimentin have been found (Fujinami et al. 1983). Monoclonal antibodies against measles virus F protein which is one of two envelope glycoproteins of the measles virus can react with cellular stress protein (Sheshberadaran and Norrby 1984). Recently, homologous peptide sequences in encephalitogenic regions of myelin basic protein and in antigens of measles have been found (Jahnke et al. 1985). It has been also found that there is a homology of amino acid sequences between hepatitis B virus polymerase and rabbit myelin basic protein and acute experimental allergic encephalomyelitis develops in rabbits after the injection of hepatitis B virus polymerase (Fujinami and Oldstone 1985). These reports on the cross antigenicity between viruses and normal tissues led us to examined the reactivity of five anti-MAG murine monoclonal antibodies to measles virus glycoproteins.

METHODS

Enzyme-linked immunosorbent assay (ELISA)

Cross-reactivity between the measles virus and MAG was examined by ELISA in a fundamental study by Sato et al. (1983). Measles virus ELISA antigen (Lot. No. 509) prepared from Edmonston strain containing bovine serum albumin was purchased from Radioimmunoassay Inc. (Canada). Briefly, 100 μl of PBS, pH 7.1, containing 75 μg/ml of measles antigen was put into each well of a microplate (Immunoplate 1, Nunc, Denmark). After incubation at 4°C overnight, several diluted ascites containing anti-MAG murine monoclonal IgG or IgM, or murine monoclonal antibodies against human lymphocytes such as anti-Leu-4, Leu-7, Leu-11, Leu-12 (Becton Dickinson, Sunnyvale, CA,

USA) OKT3, T4, T8, T11, Ia1 and M1 (Ortho, Raritan, NJ, USA) or the serum of a patient with subacute sclerosing panencephalitis (SSPE) were put into each well and incubated for 1 hr at 25°C. After the wells were washed, either 100 μl of peroxidase-conjugated anti-mouse IgG or IgM goat serum or anti-human IgG goat $F(ab')_2$ (Cappel, Malvern, PA, USA) diluted to 1 : 3000 with PBS containing 2% bovine serum albumin was added to each well. The plate was again incubated for 1 hr at 25°C. The substrate solution was prepared with 0.1% (w/v) O-phenylenediamine and 0.03% (v/v) hydrogen peroxide in PBS just before use. The plate was washed and 200 μl of the substrate solution was added into each well. The reaction was stopped after 1hr incubation at 25°C by adding 50 μl of 4N HCl. The absorbance at 492 nm/630 nm (as reference) was measured in a dual-wavelength microplate (MTP-12, Corona Electric, Ibaragi, Japan). The value of reference wells not coated by the measles antigen was subtracted from the sample value.

Immunoblotting study

Gel electrophoresis and immunostaining for blots were performed essentially as previously reported (Sato et al. 1983). One hundred and twenty micrograms of crude measles virus (Edmonston) proteins without serum and prepared from a cell-free culture media, which were kindly presented by Dr. M. Nakajima (Denka Seiken Co., Tokyo, Japan), or the same content of Vero cell homogenates were loaded on each well and electrophoresis was done on 10% SDS-polyacrylamide slab gels. After electrophoresis, proteins in the gels were transferred to nitrocellulose papers (Bio-Rad, Trans-Blot TM cell). The homogenates of Vero cells used in cultures of the measles virus were prepared as follows: Vero cells washed with Eagle's MEM twice were homogenized by a glass homogenizer, the homogenates were put into an ultrasound bath for 10 min and frozen and thawed three times. The protein concentration of Vero homogenates from a tissue culture flask (75 cm^2, Falcon) was 1.7 mg by Lowry's method.

Immunostaining of the blots was performed autoradiographically as reported previously (Hall and Choppin 1981; K. Tanaka et al. 1986). Briefly, the blotted nitrocellulose papers were washed with TBS (10 mM Tris-HCl, pH 7.4, 150 mM NaCl) and incubated with TBSA (TBS containing 3% bovine serum albumin) overnight at 25°C. Then the blots were incubated with an anti-MAG murine monoclonal antibody (MC-P2) or Ig reference mouse serum (Miles Sci., Naperville, IL, USA) diluted to 25 μg/ml or serum from a patient with SSPE diluted 1 : 100 by TBSAG (TBSA containing 10% decomplemented goat serum) at 37°C for 1 hr followed by incubation at 25°C overnight. The blots were rinsed with TBS for 90 min by changing the buffer every 15 min. The second antibody was ^{125}I-anti-mouse Ig or anti-human Ig $F(ab')_2$ goat (Amersham International plc, Buckinghamshire, England) which was incubated with the blots at the concentration of 1.5 μCi/ml for 2 hr at 37°C and then for 6 hr at 25°C. Finally, the blots were rinsed, air dried, placed flush against LKB Ultrofilm ^{3}H, and allowed to stand at −80°C for 48 hr.

RESULTS

Enzyme-linked immunosorbent assay

The basic characterization of anti-MAG murine monoclonal antibodies such as their immunoglobulin class and their reactivity with deglycosylated antigen has been previously reported (Nishizawa, et al. 1986.) All three antibodies recognizing the carbohydrate moiety of MAG (GC-J4, MC-P2 and MC-P4) could react with human lymphocytes. Clone MC-P2 and MC-P4 were of the IgM class and GC-J4, IgG. Clone MC-P2 was used in our previous study showing that human natural killer cell activity was reduced by treatment of the anti-MAG murine antibody and complement (M. Tanaka et al. 1985). One of these antibodies (MC-P2) could react with measles ELISA antigen but two monoclonal antibodies (IgG) recognizing the peptide moiety of MAG (GP-D3, GP-H2) could not. Eleven murine monoclonal antibodies including anti-Leu-7 against human lymphocytes and the anti-MAG monoclonal IgM from a patient with demyelinating polyneuropathy could not react with measles ELISA antigen. The absorbance of MC-P2, MC-P4, Leu-7 and mouse Ig reference serum at the same content of mouse IgM (10 μg/ml) was 0.236, 0.035, 0.012,

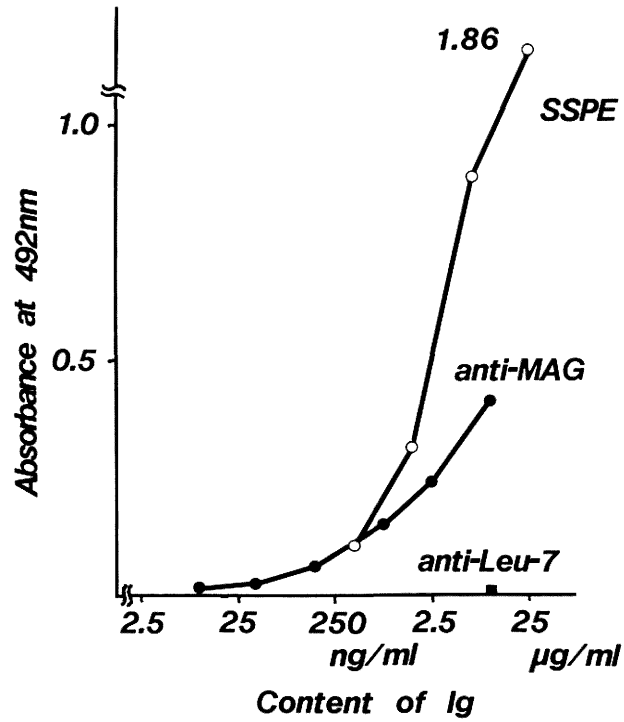

Fig. 1. Comparison of antibody titer against measles ELISA antigen between serum from a patient with subacute sclerosing panencephalitis and anti-MAG mouse monoclonal antibody (MC-P2).

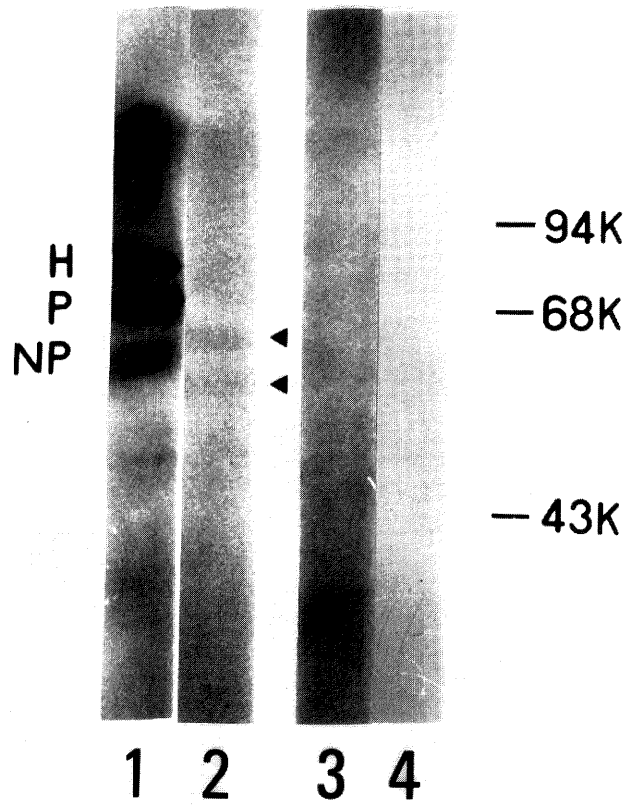

Fig. 2. Immunostaining of measles virus antigen and Vero cell homogenates.
Measles virus proteins (lane 1-3) and Vero cell homogenates (lane 4) were electrophoresed by SDS-polyacrylamide gel electrophoresis, transferred onto nitrocellulose sheets, and immunostained with serum from a patient with subacute sclerosing panencephalitis (SSPE) (lane 1), anti-MAG mouse monoclonal antibody (MC-P2) (lane 2, 4) and the same mouse immunoglobulin content of normal mouse serum (lane 3). The bands, 56 kDa and 63 kDa, stained by the anti-MAG mouse monoclonal antibody were not the same as those — H (78 kDa), P (69 kDa) and NP (60 kDa) -stained by the serum of a patient with SSPE.

0.014, respectively. Fig. 1 shows a comparison of antibody titers against the measles ELISA antigen between serum from a patient with SSPE and the anti-MAG murine monoclonal antibody (MC-P2). The titer of the anti-MAG antibody was lower than that of the SSPE patient.

Immunoblotting study

Since the measles ELISA antigen was crude, we examined the antigen recognized by the anti-MAG monoclonal antibody using an immunoblotting study. Two bands, 56 kDa and 63 kDa, were weakly but constantly stained by the anti-MAG antibody (MC-P2) in

three different experiments, but these were not the same as the one stained by the serum from a patient with SSPE (Fig. 2).

Discussion

It has been reported that shared antigenic determinants with MAG were found in retinal Muller cells (Stefansson et al. 1984), rat astrocytes and neurons (Lisak et al. 1984), neural cell adhesion molecules (Kruse et al. 1984), the dorsal root ganglion in chicken (Omlin et al. 1985), mouse B cells (Dal Canto and Barbano 1986), cell lines derived from lung small cell carcinoma (Willison et al. 1986), and lung adenocarcinoma (Hozumi et al. 1987). Leu-7, the surface marker of human natural killer cells, was found in neuroectodermal cells (Lipinski et al. 1983), thyroid medullary carcinoma (Caillaud et al. 1984), and lung small cell carcinoma (Bunn et al. 1985). Furthermore, it is reported that between these two antigens, MAG and Leu-7 share an antigenic determinant as follows (McGarry et al. 1983; Sato et al. 1983). Anti-Leu-7 can react with the carbohydrate determinant of MAG (Inuzuka et al. 1984; Kruse et al. 1984). Monoclonal antibodies against the carbohydrate determinant of MAG can stain human natural killer cells (Dobersen et al. 1985; M. Tanaka et al. 1985). Numerous similarities between anti-Leu-7 and the human anti-MAG IgM paraproteins associated with neuropathy have been reported (Inuzuka et al. 1984; Kruse et al. 1984; Murray and Steck 1984; Nobile-Orazio et al. 1984; Ilyas et al. 1984; Chou et al. 1985). An acidic glycolipid reacting with human IgM paraproteins was shown to be a glucuronic acid-containing sulfated glycosphingolipid with five sugars but without sialic acid, although the acid labile sulfate group was required for reactivity with anti-Leu-7 (Chou et al. 1985).

Most of the human mononuclear cells stained by anti-MAG antibody (MAG^+ cells) can be stained by anti-Leu-7 (M. Tanaka et al. 1984). In pathological conditions such as patients with multiple sclerosis, some patients show a reduced number of MAG^+ cells (stained by polyclonal antisera) but a normal number of $Leu\text{-}7^+$ cells (M. Tanaka et al. 1985). We found a patient with chronic T cell leukemia with a NK phenotype (M. Tanaka et al. 1986). Her large granular lymphocytes could be stained by anti-Leu-7 and MC-P2. Since we have not found $MAG^+Leu\text{-}7^-$ or MAG^- $Leu\text{-}7^+$ cells in immature lymphocytes of developmental organs or from patients with leukemia, we don't know whether MAG^+ cell population is exactly the same as $Leu\text{-}7^+$ cell population. MC-P2 bind directly to or close to the leu-7 epitope of mononuclear cells from a healthy volunteer since MC-P2 inhibit the binding of anti-Leu-7 to mononuclear cells and anti-Leu-7 inhibit the binding of MC-P2 (Arai et al. 1987). But we have shown here that the chemical feature of the antigen stained by MC-P2 is not the same as Leu-7 or this certain patient's anti-MAG monoclonal IgM. The antibody titer of serum from a patient with SSPE was higher than that of clone MC-P2 (Fig. 1), since the content of antigens reacting with the patient's serum, polyclonal anti-measles virus antibody, was more than that stained by clone MC-P2 reacting with the carbohydrate determinant of measles ELISA antigen. Since two

bands, 56 kDa and 63 kDa, were stained by MC-P2, which differed from those-H (78kDa), P (69 kDa) and NP (60 kDa)—stained by the serum of a patient with subacute sclerosing panencephalitis, clone MC-P2 could not react with measles virus proteins. The two bands stained by MC-P2 may be derived from Vero cells used in a culture of measles virus. It may also be possible that the anti-MAG antibody (MC-P2) is not able to stain Vero cell homogenates by immunoblot due to the small amount of carbohydrate antigens reacting with MC-P2.

Three types of immunologic cross reactions were brought to attention by Waksman (1984): 1) autoantigen and an exogenous agent (bacterial or viral); 2) autoantigen and another tissue element such as tumors; and 3) autoantigen of nervous tissue and a membrane component of immunologically active lymphoid cells. The same carbohydrate antigen is present between onco-developmental and normal tissues (Feizi, 1985), however, there is no cross-reactivity between MAG and CA-19-9 (Magnani et al. 1982) or CA-125 (Bast et al. 1981), which are both carbohydrate antigens used as tumor marker since anti-CA-19-9 or CA-125 monoclonal antibody do not react with MAG by radioimmunoassay (unpublished data).

It has not been known how many murine monoclonal antibodies reacting with the carbohydrate determinant of MAG and natural killer cells can be generated by immunization with MAG. We have demonstrated here that at least two kinds of monoclonal antibodies in reactivity with measles ELISA antigen are produced. The antigenic determinant reacting with anti-MAG antibody, not with Leu-7, may be expressed on the surface of human NK cells.

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