Effect of neuraminidase on reactivity of anti-myelin-associated glycoprotein (MAG) antiserum with human natural killer cells

Masami TANAKA, Shuzo SATO, Hiroko BABA and Tadashi MIYATAKE

Department of Neurology, Brain Research Institute, Niigata University, Niigata 951, Japan

ABSTRACT

We examined the effect of neuraminidase on the reactivity of anti-myelin-associated glycoprotein (MAG) antiserum with human natural killer cells. The percentage of MAG-positive cells was increased threefold by neuraminidase treatment, whereas the percentage of Leu-7-positive cells (natural killer cells) was unchanged. It is possible that the antigenic determinant masked by sialic acid is exposed after the neuraminidase treatment. Since most MAG-positive cells in neuraminidase-untreated mononuclear cells are also stained by anti-Leu-7, the antigenic determinants may differ between the treated and untreated cell membrane.

Myelin-associated glycoprotein (MAG) is a quantitatively minor component in both peripheral and central myelin sheaths (3). MAG is the first component of myelin to be degraded in demyelination in multiple sclerosis (6), and has been reported to be the antigen for a monoclonal IgM in demyelinating neuropathy (2, 12). Anti-MAG antibody has been detected in cerebrospinal fluid from patients with multiple sclerosis (17).

Recent studies of our own (10, 13-15) and other laboratories (7, 11) have demonstrated an antigenic determinant shared between MAG and natural killer (NK) cells. Previously we found that the same antigenic determinant exists on the surface of mononuclear cells in the human peripheral blood, which amounted to $9.1 \pm 2.1\%$ of mononuclear cells when stained by heterologous antiserum (14). In a two-color immunofluorescence study, more than 95% of MAG-positive human mononuclear cells were also stained with anti-Leu-7, a monoclonal antibody against human NK cells (1), but only 50 to 90% of Leu-7-positive cells were stained with anti-MAG antiserum (15). About 80% of MAG-positive cells were also stained with anti-Leu-11, a monoclonal antibody against IgG Fc receptor of human NK cells (8) (unpublished data). Human mononuclear cells conjugated with K-562 cells, target cells of human NK cells, were stained by anti-

Leu-7 and also by anti-MAG antiserum (15).

In the present report, we demonstrate that the percentage of MAG-positive cells in human peripheral blood is increased by neuraminidase treatment, but the percentage of Leu-7-positive cells is unchanged.

MAG and anti-MAG antiserum were prepared as previously described (10, 14). Immunofluorescence study of human mononuclear cells in the peripheral blood was performed as reported previously (14, 15). Mononuclear cells from healthy individuals were prepared by gradient centrifugation and washed with Hanks' balanced salt solution (HBSS). The cells (1×10^6 cells per 200 μ l) were incubated with 5 μ l of anti-MAG antiserum (diluted 1:50 with HBSS) for 30 min at 4°C and washed with HBSS. After incubation with 100 μ l of FITC-conjugated anti-rabbit IgG goat F(ab')₂ fragment (1:50 dilution; Cappel Lab., Cochranville) in HBSS, cells were washed with HBSS containing 10 mM NaN₃ and resuspended in 1-2 drops of Dulbecco's PBS containing 30% (v/v) glycerol. More than two hundred cells were examined with a fluorescence microscope (BHS-RF-A, Olympus, Tokyo).

Mononuclear cells (1×10^6 cells per 100 μ l in HBSS) were incubated with 5 μ l of anti-Leu-7 (Becton Dickinson, CA) as the first antibody, and with 100 μ l of FITC-conjugated anti-mouse IgM goat F(ab')₂ fragment (1:100 dilution; Cap-

pel Lab., Cochranville) as the second antibody.

Anti-MAG antiserum and all fluorescein-labeled antibodies were ultracentrifuged for 30 min at 105,000 *g* immediately before use to eliminate aggregates (5).

Treatment of mononuclear cells with neuraminidase was performed as reported by Honda *et al.* (5). After 1×10^6 cells suspended in 100 μ l of HBBS were incubated with 0.1 U of *Vibrio cholerae* neuraminidase (sialidase, *N*-acyl-neuraminylglycohydrolase, EC 3.2.1.18 from *Vibrio cholerae*; Behring Institute, Marburg, FRG) for 1 h at 37°C, cells were washed three times with HBSS and then used for immunofluorescence study as described above.

Percentage of MAG-positive cells was increased about threefold by neuraminidase treatment but that of the Leu-7-positive cells was unchanged (Table 1). The extent of percentage increase in MAG-positive cells differed in each individual. In healthy individuals, percentage of Leu-7-positive cells was always higher than that of MAG-positive cells (unpublished data). After neuraminidase treatment, however, percentage of MAG-positive cells became higher than that of Leu-7-positive cells. A similar finding was reported by Hakomori (4). He observed that the reactivity of human adult erythrocytes with anti-globoside antibody was greatly enhanced by both trypsin and neuraminidase treatment, and assumed that the reaction with anti-globoside antibody was usually hampered by the presence of a sialyl or sialomucopolypeptidyl group (4).

It is unknown why the percentage of Leu-7-positive cells was not increased after neuraminidase treatment. It is possible that the antigenic determinant exposed by neuraminidase differs from that of the untreated cell membrane, since

most MAG-positive cells in untreated mononuclear cells are also stained by anti-Leu-7 (15). There are limitations in studying the characteristics of the antigenic determinant in neuraminidase-treated or -untreated cell membrane when heterologous antiserum is used. However the antigenic determinants of anti-MAG antiserum may be relevant to the study of human NK cells for reasons given below.

Roles of sialic acid in the cell membrane in lymphocyte function have been reported (5, 16). Masking of the cell surface by sialic acid occurs during maturation of human T cells (9). In the plasma of patients with systemic lupus erythematosus, anti-desialized T cell antibodies were found, which recognize β -galactosyl residue of desialized T cells (5). When human NK cells or K-562 cells were pretreated with neuraminidase, binding and percentage of lytic cells among binders were increased (16). In this study, we found that the percentage of MAG-positive cells was increased after neuraminidase treatment but that of Leu-7 positive cells was unchanged. Anti-MAG antiserum may be useful for studying the human NK cell lineage.

This study was supported by a grant from the Neuro-immunological Disorders Research Committee, the Ministry of Health and Welfare, Japan.

Received for publication 2 April 1984

REFERENCES

1. ABO T. and BALCH C. M. (1981) A differentiation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). *J. Immunol.* **127**, 1024-1029
2. BRAUN P. E., FRAIL D. E. and LATOV N. (1982) Myelin-associated glycoprotein is the antigen for a

Table 1 *Effects of Neuraminidase Treatment on the Reactivity of Anti-MAG Antiserum and Anti-Leu-7 with Human Mononuclear Cells in the Peripheral Venous Blood*

Donor	Untreated		Neuraminidase-treated	
	Leu-7	MAG	Leu-7	MAG
1	20.0 ^a	8.2	16.1	25.2
2	13.5	6.0	14.2	31.1
3	9.4	5.2	8.5	18.2
4	NE ^b	8.7	NE	18.3
Mean	14.3	7.0	12.9	23.2

^aEach value represents the percentage of Leu-7-positive or MAG-positive cells in mononuclear cells. For immunofluorescence examination, see the text.

^bNot examined. MAG: myelin-associated glycoprotein. Anti-Leu-7: monoclonal antibody against human natural killer cells

- monoclonal IgM in polyneuropathy. *J. Neurochem.* **39**, 1261-1265
3. FIGLEWICZ D. A., QUARLES R. H., JOHNSON D., BARBARASH G. R. and STERNBERGER N. H. (1981) Biochemical demonstration of the myelin-associated glycoprotein in the peripheral nervous system. *J. Neurochem.* **37**, 749-758
 4. HAKOMORI S.-I. (1969) Differential reactivities of fetal and adult human erythrocytes to antisera directed against glycolipids of human erythrocytes. *Vox Sang.* **16**, 478-485
 5. HONDA M., SAKANE T., STEINBERG A. D., KOTANI H., TSUNEMATSU T., MORIYAMA K. and FUKASE M. (1982) Studies of immune functions of patients with systemic lupus erythematosus. Antibodies to desialized, rather than intact, T cells preferentially bind to and eliminate suppressor effector T cells. *J. Clin. Invest.* **69**, 940-949
 6. ITOYAMA Y., STERNBERGER N. H., WEBSTER H. DEF., QUARLES R. H., COHEN S. R. and RICHARDSON E. P. (1980) Immunocytochemical observations on the distribution of myelin-associated glycoprotein and myelin basic protein in multiple sclerosis lesions. *Ann. Neurol.* **7**, 167-177
 7. MCGARRY R. C., HELFAND S. L., QUARLES R. H. and ROPER J. C. (1983) Recognition of myelin-associated glycoprotein by the monoclonal antibody HNK-1. *Nature* **306**, 376-378
 8. PHILLIPS J. H. and BABCOCK G. F. (1983) NKP-15: a monoclonal antibody reactive against purified human natural killer cells and granulocytes. *Immunol. Lett.* **6**, 143-149
 9. SAKANE T., HONDA M., TANIGUCHI Y., KOTANI H. and NIWA Y. (1982) Characterization of human peripheral blood T lymphocytes bearing receptors for autologous erythrocytes and T lymphocytes lacking these receptors. *J. Clin. Immunol.* **2**, 20-29
 10. SATO S., BABA H., TANAKA M., YANAGISAWA K. and MIYATAKE T. (1983) Antigenic determinant shared between myelin-associated glycoprotein from human brain and natural killer cells. *Biomedical Res.* **4**, 489-494
 11. SCHULLER-PETROVIC S., GEBHART W., LASSMANN H., RUMPOLD H. and KRAFT D. (1983) A shared antigenic determinant between natural killer cells and nervous tissue. *Nature* **306**, 179-181
 12. STECK A. J., MURRAY N., MEIER C., PAGE N. and PERRUISSEAU G. (1983) Demyelinating neuropathy and monoclonal IgM antibody to myelin-associated glycoprotein. *Neurology* **33**, 19-23
 13. TANAKA K., TANAKA M., SATO S. and MIYATAKE T. (1984) The expression of Leu-7 (HNK-1) antigen on human myelin of peripheral nervous system. *Biomedical Res.* **5**, 225-228
 14. TANAKA M., SATO S. and MIYATAKE T. (1984) Identification of myelin-associated glycoprotein (MAG) on the cell surface of mononuclear cells in human peripheral blood by a heterologous antiserum. *Jap. J. Exp. Med.* **54**, 9-15
 15. TANAKA M., SATO S., YANAGISAWA K. and MIYATAKE T. (1984) Myelin-associated glycoprotein (MAG): Expression on the surface of human natural killer cells. *Biomedical Res.* **5**, 71-76
 16. TIMONEN T., ORTARDO J. R. and HERBERMAN R. B. (1982) Analysis of natural killer activity of human large granular lymphocytes at a single cell level. In *NK Cells and Other Natural Effector Cells* (ed. HERBERMAN R. B.) Academic Press, New York, pp. 9-16
 17. WAJGT A. and GÓRNY M. (1983) CSF antibodies to myelin basic protein and to myelin-associated glycoprotein in multiple sclerosis. Evidence of the intrathecal production of antibodies. *Acta Neurol. Scand.* **68**, 337-343