Localization of a Glycosphingolipid, Asialo GM1, in Rat Immunocytes

Takashi MOMOI, Kumiko NAKAJIMA, Kooko SAKAKIBARA, and Yoshitaka NAGAI
Department of Pathobiochemical Cell Research, Institute of Medical Science, The University of Tokyo, Shirokanedai, Minato-ku, Tokyo 108

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One of the TLMA (rat T-lymphocyte-macrophage-associated antigens) was characterized as asialo GM1, Gal(β 1-3)GalNAc(β 1-4)Gal(β 1-4)Glc(β 1-1)-Cer by two assay methods using monospecific anti-asialo GM1 antibody, immunocytotoxicity testing and immunostaining.

Nylon column-enriched T lymphocytes (non-adherent cells) were highly cytolized by anti-asialo GM1 antiserum and complement as compared with adherent cells (non T cells). Forty % of peritoneal exudate macrophages and 20 % of granulocytes were also cytolyzed by anti-asialo GM1 antiserum. On the other hand, 5 % of thymic cells and 60-80 % of lymph node T cells were immunostained by anti-asialo GM1 antibody. These asialo GM1-positive cells in thymus were found to be localized in the cortico-medulla junction. About 20 % of macrophages and 2) % of granulocytes were also immunostained by anti-asialo GM1 F(ab')2 fragment. These results strongly support the previous conclusion that TLMA can be identified as asialo GM1. Asialo GM1 was universally expressed on a certain population of macrophages of various tissues, but not on all of the population. Alveolar macrophages were also asialo GM1-positive.

It has been shown that the glyco portion of glycolipids as well as glycoproteins is deeply involved in the expression of the antigenicity of cell surfaces, and that the glycoantigens can therefore be useful as one of the surface-related differentiation markers to characterize the subpopulations of cells of various types (I). Recently, asialo GM1, gangliotetraosylceramide, has been characterized

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Abbreviations: Glycosphingolipids are named according to the recommendations of the IUPAC-IUB Lipid Nomenclature Document (1976) by combining the notation of their carbohydrate with Cer=ceramide. Oligosaccharides residues of glycosphingolipids are abbreviated as follows:

Lac = lactose = Galβ1-4Glc; GbOse3 = globotriaose = Galα1-4Galβ1-4Glc; GbOse4 = globotetraose = GalNAcβ1-3Galα1-4Galβ1-4Glc; GgOse3 = gangliotriaose = asialo GM2 = GalNAcβ1-4 Galβ1-4Glc; GgOse4 = gangliotetraose = asialo GM3 = Galβ1-3 GalNAcβ1-4 Galβ1-4Glc.

Other gangliosides are designated according to Svennerholm (1963) J. Neurochem. 10, 613–623.

Other abbreviations: HP-silica gel TL plate, high performance-silica gel thin layer plate; TLMA, T-lymphocyte-macrophage associated antigen; FITC, fluorescein isothiocyanate; ELISA, enzyme-linked immunosorbent assay.
as a specific antigen of immature thymocytes and natural killer cells in mice (2-4).

In rats, however, it has been shown that the cytotoxic activity of anti-rat lymph node Ig- cells antiserum (anti-TLMA) which was specifically directed to lymph node Ig- cells rather than thymocytes (5), was selectively absorbed with asialo GM1 (6). Furthermore, this antigen has been shown to be expressed not only on lymph node Ig- cells but also on peritoneal exudate macrophages (5). Therefore, asialo GM1 has been considered to be a common antigen of the rat T lymphocytes, in particular mature T cells and macrophages (6). The visualization by the chemical microdetection method using fluorography of a band corresponding to asialo GM1 on a thin-layer chromatogram confirmed that asialo GM1 is actually present in rat thymocytes, lymph node Ig- cells and macrophages, but not in Ig+ cells or erythrocytes (7).

Therefore, it is of interest to know more precisely the localization or subpopulation of asialo GM1-positive cells in rats. For this purpose, we prepared highly specific anti-asialo GM1 antibody.

The present paper deals with the antigenic activity and localization of asialo GM1 in various tissues and cell types, which were studied by using this monospecific anti-asialo GM1 antibody.

MATERIALS AND METHODS

Materials—Rats (Sprague-Dawley, 6 weeks) were obtained from Shizuoka Experimental Animal Co. (Hamamatsu, Japan). A nude rat (outbred) was generously donated by Prof. Tamaoki (Tokai University, Sagamihara, Japan). Ficoll-hypaque was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden). AH-Sepharose, CNBr-Sepharose, Sephacryl S-300 and DEAE-Sephadex were also from Pharmacia Fine Chemicals. NaCNBH3 was from Aldrich Chem. (Milwaukee, U.S.A.). FITC- or peroxidase conjugate goat anti-rabbit IgG was from Maruzen Seikiyu Co. (Tokyo), and Miles Laboratories Ltd. (Slough, England), respectively. Pepsin [EC 3.4.23.1] and collagenase Type IV [EC 3.4.24.3] were obtained from Sigma (Saint Louis, U.S.A.). Zap-Oglobin and Isoton II were from Coulter Diagnostics (Hialeah, Florida, U.S.A.). Guinea pig complement was from Toshiba Co. (Tokyo). Nylon wool was obtained from Wako Chemicals (Tokyo). Florisil and HP-silica gel TL plates were obtained from E. Merck (Darmstadt, F.R.G.).

Preparation of Rat Immunocytes—Rat peritoneal exudate macrophages and granulocytes were induced by injection of 20 ml of 3% thioglycollate into the peritoneal cavity. After 15-20 h and 4 days, granulocytes and macrophages were obtained from the peritoneal cavity, respectively. Thymocytes and lymph node lymphocytes were mechanically released from the tissues and filtered through a 150 mesh platinum filter to remove connective tissues. Spleen lymphocytes were separated from contaminating erythrocytes by the Ficoll-hypaque method after spleen cells had been released from the tissues as described above. Spleen and lymph node lymphocytes (T and B cells) were further subfractionated on a nylon column according to the method of Julis et al. (8). Lymphocyte T cells were obtained in the nylon wool non-adherent cell fraction, while B cells (nylon adherent cells) were released by mild mechanical shaking in PBS from nylon wool. Fetal liver hematopoietic cells were obtained by 0.01% (w/v) collagenase digestion at 37°C for 15 min following the Ficoll-hypaque method. Bone marrow cells were obtained from femurs and contaminating erythrocytes were removed by hypotonic shock with 0.83% (w/v) NH4Cl.

Preparation of Glycolipids—More than 10⁸ cells each of macrophages and granulocytes were lyophilized, and the lipids were extracted with 10 ml of chloroform-methanol-water (20 : 10 : 1, v/v/v), (10 : 10 : 1, v/v/v), and (30 : 60 : 8, v/v/v), respectively. The extracts were combined, and the total extract was submitted to Folch-Pi (9) partition by adjusting the ratio of chloroform, methanol, and water to 8 : 4 : 3 (v/v/v) by addition of methanol and water. The lower phase was evaporated down and the residue was dried over P2O5 and then peracetylated with pyridine-acetic anhydride (2 : 1, v/v). Peracetylated glycolipids were isolated according to the method of Saito and Hakomori (10), combined with the upper phase fraction of Folch-Pi, and then treated with 0.1 N NaOH for 1 h room temperature. The total glycolipids thus obtained were dialyzed against water, evaporated to dryness and applied to a DEAE-Sephadex column to separate neutral and
acidic glycolipids according to the method of Ledeen and Yu (11).

**Thin Layer Chromatography**—Neutral and acidic glycolipids isolated from about $5 \times 10^7$ cells were applied to a high performance silica gel TL plate, and developed with chloroform–methanol–water, 60:30:5 and 60:40:9 (v/v/v), respectively. Neutral glycolipids were detected with orcinol reagents and gangliosides with resorcinol reagents.

Authentic standards of neutral glycolipids of the globo series (GlcCer, LacCer, GbOse$_4$Cer, and GbOse$_4$Cer, globoside) and ganglio series (GlcCer, LacCer, GgOse$_2$Cer, asialo GM2, and GgOse$_2$Cer, asialo GM1) were obtained by partial hydrolysis (0.3 n HCl, 60°C for 45 min) of globoside and asialo GM1, respectively. Globoside was prepared from human erythrocyte membrane. Asialo GM1 was obtained from GM1 by formic acid hydrolysis (1 M formic acid, 100°C for 1 h). After dialysis and lyophilization, the desired compound was purified by iatrobeads and silica gel column chromatography. GM1 and other gangliosides were obtained from human brain.

**Preparation of Monospecific Anti-Asialo GM1 Antibody**—Rabbit anti-asialo GM1 antiserum was obtained as described previously (3). $\gamma$-Globulin fraction was precipitated with 33% ammonium sulfate from total rabbit serum. After dialysis against PBS (pH 7.2), an aliquot of the retenate was applied to a Sephacryl S-300 column to separate IgG fraction from IgM and rabbit serum albumin. Anti-BSA antibody, which was present in the rabbit antiserum because of the use of BSA in immunization as a carrier for the haptenic glycolipid antigen, was removed on a BSA-coupled Sepharose affinity column. The pass-through fraction was applied to a gangliotetraitol-Sepharose affinity column (5 ml) prepared according to the method of Taki et al. (12). After washing with 50 column volumes of PBS, monospecific anti-asialo GM1 was eluted with 3 M NaSCN in PBS. F(ab')$_2$ fragment was prepared according to the procedure described by Nisonoff et al. (13).

**Enzyme-Linked Immunosorbent Assay (ELISA)**—The specificity of anti-asialo GM1 antibody was examined by the ELISA method as described elsewhere (14). Briefly, 50 $\mu$l of antigen solution (10 $\mu$g of glycolipid in 1 ml of 0.1% deoxycholate containing PBS) was added to a microtiter plate (96 wells) and incubated at 37°C for 1 h for antigen absorption. Then, the plate was washed three times with Buffer A (0.05% Tween 20 containing PBS). 50 $\mu$l of anti-asialo GM1 antibody in Buffer B (2% BSA containing Buffer A) was applied to the plate, which was then incubated at 37°C for 30 min. The plate was washed again with Buffer A, then a second antibody (peroxidase conjugate anti-rabbit IgG) in Buffer B (50 $\mu$l) was added and the plate was incubated for another 30 min at 37°C. 300 $\mu$l of 3% (v/v) 5-aminosalicylic acid in 0.05% H$_2$O$_2$ was added as a substrate and after 1 h, the difference of absorption at 450 nm from that at 660 nm was read.

**Cytotoxic Activity Test**—Cytotoxic activity of anti-asialo GM1 antiserum toward various rat immunocytes was examined by a Coulter Counter cytotoxicity assay technique which was newly developed in our laboratory (15). Briefly, 100 $\mu$l of target cell suspension ($5 \times 10^6$ cells/ml), 100 $\mu$l of anti-asialo GM1 antiserum and 100 $\mu$l of guinea pig complement were mixed and incubated at 37°C for 1 h, followed by incubation with 0.3 ml of pronase E at 37°C for 30 min. A 0.5 ml aliquot was mixed with 10 ml of Isoton and 0.1 ml of Zap-Oglobin solution and counted with a Coulter Counter. The counting conditions were as follows: A (1/amplification) = 0.5 I (1/aperture current) = 0.354, TL = 15, TU = 60. Under these conditions, only remaining nuclei were counted.

**Immunostaining of Cells**—Various rat immunocytes were stained by the indirect method using anti-asialo GM1 IgG or its F(ab')$_2$, and FITC conjugate goat anti-rabbit IgG. 2 $\times$ 10$^6$ cells in 50 $\mu$l PBS were incubated with anti-asialo GM1 IgG or its F(ab')$_2$ at 0°C for 1 h, then 200 $\mu$l of cold PBS was added, and the whole was centrifuged at 16,000 rpm for 5 min. This procedure was repeated another two times. 50 $\mu$l of FITC conjugate goat-anti-rabbit IgG (1/200 dilution) was added to the cell pellet and after mixing well, this aliquot was allowed to stand on ice for 30 min. Then 30 $\mu$l of cold PBS was added, and the mixture was centrifuged at 16,000 rpm for 5 min. This centrifugation procedure was repeated again. FITC-positive cells were counted under a fluorescence microscope (Nikon Co., Ltd., Tokyo).

**Immunostaining of Tissues**—The thymus and lungs were removed from a young adult male rat.
under anesthesia with ether, dissected, and quickly frozen in liquid nitrogen. The frozen tissues were cut in a cryostat at a thickness of 6-8 μm, deposited on slides, and immersed in acetone for 20 min at room temperature. The tissue sections were incubated with anti-asialo GM1 antibody for 30 min at 37°C, followed by washing in PBS. The antibodies bound to the sections were then visualized by the indirect immunoperoxidase method, including a 30-min incubation in a 1:20 dilution of peroxidase-labeled anti-rabbit immunoglobulin raised in goat, a 15-min wash in PBS, and a 5-min exposure to a mixture of 3,3-diaminobenzidine and hydrogen peroxidase in Tris-HCl at pH 7.6. The sections were finally washed in distilled water, dehydrated in ethanol and xylene and mounted in synthetic resin. As a control, the tissue sections were exposed to non-immune rabbit serum and subjected to indirect immuno-peroxidase staining in each experiment.

RESULTS

For the study of the distribution and localization of asialo GM1 on the cell surface, we prepared highly purified monospecific anti-asialo GM1 antibody and applied it to cytotoxicity testing and immunostaining.

Cytotoxic Activity Test—Cytotoxic activity of anti-asialo GM1 antisera was tested against rat various immunocytes. The titer of this anti-serum determined by the peroxidase-ELISA method was 1280. As shown in Fig. 1a, about 60% of peripheral lymphocyte and thymocyte populations was cytolyzed by anti-asialo GM1 in the presence of complement, and 40-50% of lymph node lymphocytes and spleen lymphocytes were lysed under the same conditions. However, after separation of the subpopulations of lymph node and spleen cells on a nylon column, about 80% of nylon non-adherent lymph node lymphocytes, which are mainly considered to have T cell function, were lysed by anti-asialo GM1 antiserum together with complement. Nylon non-adherent lymphocytes in spleen were also cytolyzed by anti-asialo GM1 antiserum more than the spleen cells before separation. Furthermore, 40% and 20% of macrophages and granulocytes, respectively, were lysed by anti-asialo GM1 antiserum (Fig. 1b).

Immunostaining—Affinity purified monospecific rabbit anti-asialo GM1 antibody was used together with FITC conjugate anti-rabbit IgG serum for the detection of asialo GM1-positive cell population. Anti-asialo GM1 IgG was eluted with 3 M NaSCN in PBS from a gangliotetraitol-Sepharose 4B affinity column. The titer

![Fig. 1. Cytotoxic activity of anti-asialo GM1 antiserum. a: Cytotoxic activity of anti-asialo GM1 antiserum against various rat cells. •, lymph node T cells; ○, lymph node lymphocytes; ■, spleen T cells; □, spleen lymphocytes; Δ, peripheral lymphocytes; ——, thymocytes. b: Cytotoxic activity of anti-asialo GM1 antiserum against peritoneal exudate macrophages and granulocytes. •, peritoneal exudate macrophages; ○, peritoneal exudate granulocytes.](https://academic.oup.com/jb/article-abstract/91/1/301/784537)
Fig. 2. Specificity of anti-asialo GM1 antibody purified on an affinity column. Cross reactions of anti-asialo GM1 antibody with other glycolipids were checked by the ELISA technique. a, asialo GM2; b, asialo GM1; c, GM1; d, Fuc-GM1; e, GD1a. Numbers indicated the degree of dilution. –Ab, without antibody; –Ag, without antigen (glycolipid).

of this fraction was found to be 562 by the peroxidase-ELISA method. As shown in Fig. 2, no cross reaction was observed with GM1, GD1a, Fuc-GM1, asialo GM2, or globoside.

Usually about 80% of lymph node nylon non-adherent cells (T cells) were asialo GM1-positive as shown in Fig. 3a. On the other hand, in the nylon adherent cell (non-T cell) fraction, the content of asialo GM1-positive cells was not more than 10% (Fig. 3b). 30-40% of spleen and peripheral lymphocytes, and fetal liver hematopoietic cells were also stained by anti-asialo GM1 antibody. Furthermore, in contrast with the results of cytotoxic activity testing, only 5% of thymocytes were asialo GM1-positive. In fetal thymocytes (20-day gestation) and bone marrow, the content of asialo GM1-positive cells was not more than 10%.

In mice, asialo GM1 has been characterized as a selective marker of natural killer cells (1, 4) which are mainly present in the spleen of nude mice. Therefore, it is of interest to examine the asialo GM1-positive cell population in nude rat spleen cells. 30-40% of spleen cells in nude rat were stained by anti-asialo GM1 antibody and FITC-conjugate goat anti-rabbit IgG.

For the further study of asialo GM1-positive cells in T cell differentiation, the thymus was stained with anti-asialo GM1 and FITC or peroxidase conjugate goat anti-rabbit IgG (Figs. 4, 5a, b). These pictures show that asialo GM1 was not localized in the region of the cortex or medulla, but rather in the junction region between the cortex and medulla with colony formation.

Macrophages and granulocytes, which have Fc receptor, were immunostained by using F(ab')2 fragment of anti-asialo GM1 IgG. 20% of macrophages and granulocytes were asialo GM1-positive (Fig. 3c, d, e, f). This result is consistent with the cytotoxic test data, indicating that asialo GM1 also appears on the myeloid cells. But in contrast with mature T cell population, only a certain population of myeloid cells was asialo GM1-positive. In thymus, spleen, and lymph node, some but not all of the plastic adherent cells were asialo GM1-positive (unpublished observation). Histochemical staining using anti-asialo GM1 antibody and peroxidase conjugate goat anti-rabbit IgG showed that the alveolar macrophages were also strongly asialo GM1-positive (Fig. 5c, d).

Glycolipids of Rat Macrophages and Granulocytes—Cytotoxic activity testing and immunostaining of macrophages and granulocytes by anti-asialo GM1 antiserum and F(ab')2 of anti-asialo GM1 IgG strongly suggested the presence of asialo GM1 on these myeloid originated cells as well as rat mature T cells. Therefore, the glycolipid profiles of macrophages and granulocytes were compared. A band corresponding to asialo GM1 was clearly detected in macrophages.
Fig. 3. Immunostaining of rat lymph node lymphocytes, macrophages, and granulocytes with anti-asialo GM1 antibody and goat anti-rabbit IgG. a, lymph node T cells (nylon non-adherent cells) with anti-asialo GM1 antibody and FITC conjugate goat anti-rabbit IgG; b, lymph node B cells (nylon adherent cells) with anti-asialo GM1 antibody and FITC conjugate goat anti-rabbit IgG; c, peritoneal exudate macrophages with anti-asialo GM1 F(ab')2 fragment and FITC conjugate goat anti-rabbit IgG; d, peritoneal exudate macrophages with normal serum and FITC conjugate goat anti-rabbit IgG; e, peritoneal exudate granulocytes with anti-asialo GM1 F(ab')2 fragment and FITC conjugate goat anti-rabbit IgG; f, peritoneal exudate granulocytes with normal serum and FITC conjugate goat anti-rabbit IgG.
and also in granulocytes, but the amount of asialo GM1 in granulocytes were much smaller than that of macrophages.

As shown in Fig. 6, other glycolipids of these cells were also distinctly different from each other. Comparable spots of glob-iso-tetraosyl ceramide (indicated by a single arrow) and fucosyl-ganglio-iso-hexaosyl ceramide (indicated by double arrows) which were previously characterized by Hanada et al. (16) were also detected in the glycolipids of macrophage preparation in this experiment. Both glycolipids were more prominent on macrophages than on granulocytes. The major ganglioside corresponding to GM3 was commonly present in the macrophages and granulocytes. On the other hand, an unknown ganglioside (indicated by a single star) was detected in granulocytes but not in macrophages, and another unknown glycolipid (indicated by double stars) which was detected in glycolipids of macrophages, was not visualized in glycolipids of granulocytes on thin layer chromatogram under the conditions used in this experiment.

DISCUSSION

Both rat T lymphocytes (particularly mature T cells) and macrophage-associated antigen (TLMA)
were recently characterized as asialo GM1 immunologically by absorption of cytotoxic of anti-TLMA antiserum and chemically by a microchemical detection method (6, 7). Both results clearly showed that asialo GM1 has the character of TLMA. However, it is still not certain whether asialo GM1 is actually present on the cell surface of mature T cells and macrophages as TLMA. In this respect, the present paper has shown that the distribution of asialo GM1 in rat immunocytes is consistent with that of TLMA by using a specific anti-asialo GM1 antibody.

The methods used in this experiment were cytotoxic activity testing and immunostaining by anti-asialo GM1 antiserum or antibody. Both methods could demonstrate that asialo GM1 is expressed on the cell surface of T-lineage cells, such as thymocytes, spleen, and lymph node nylon non-adherent cells, fetal hematopoietic cells, fetal thymocytes, and bone marrow cells. In particular, the observation of high expression of asialo GM1 on lymph node nylon non-adherent cells supported the view that asialo GM1 mainly appears in the post thymus differentiation stage as TLMA antigen.

It is interesting to know whether asialo GM1 is also expressed on natural killer cells in rats, as described in mice. Therefore, nude rat spleen (which was expected to be rich in natural killer cells) was subjected to immunostaining. As shown in normal rat spleen, 30-40% of asialo GM1 is present in nude rat spleen. As yet we have no direct evidence as to whether these positive cells are natural killer cells or not, but considering that T cells are almost wholly deficient in the nude rat (17), these asialo GM1-positive cells may be natural killer cells. However, since asialo GM1 is expressed on most mature T cells, and also some of the myeloid cells such as macrophages and granulocytes, the appearance of asialo GM1 seems not to be restricted to natural killer cells. However, natural killer cells could form a part of the population of asialo GM1-positive cells.

Asialo GM1 was detected on mouse peripheral T lymphocytes by using anti-asialo GM1 antiserum by Stein et al. (18). Recently Nakano et al. found that anti-asialo GM1 antiserum was capable of eliminating suppressor T cell function of mice as well as natural killer cell activity in the presence of complement (19). In addition, asialo GM1 was detected in a mouse myeloid leukemia cell line, M-1 (20). These data support the view that asialo GM1 is not a selective marker of natural killer cells even in mice, contrary to the previous expectation.

However, it should also be noted that sensitivity toward antibody or serum may be different with immunostaining and with cytotoxicity assays. In this experiment, for instance, about 60% of
thymocytes were cytolyzed by anti-asialo GM1 antiseraum, whereas only 5% of thymocytes were stained by anti-asialo GM1 together with goat FITC conjugate rabbit IgG. A possible explanation is that the presence of a small number of molecules, giving a low density of asialo GM1 on the cell surface, may be enough for detection by cytotoxicity testing whereas a large number (giving a high density) may be necessary for immunostaining. Therefore, we should be cautious in comparing the data obtained by cytotoxic assay testing and immunostaining.

The distribution of asialo GM1-positive cells in the thymus is interesting in relation to T cell differentiation. The picture obtained by peroxidase staining showed that asialo GM1 is not localized in the cortex or the medulla, but rather at the cortico-medullary junction within the thymus (Fig. 4, Fig. 5a, b). It is of interest to note that thymic macrophages capable of stimulating maturation in vitro, leading to the development of immunocompetence, are localized in this region as described by others (21, 22). The observation that plastic adherent cells obtained from the thymus were also asialo GM1-positive, may allow us to speculate that some of the asialo GM1-positive cells shown in Figs. 4 and 5a, b are thymic macrophages.

Cytotoxic activity testing and immunostaining of macrophages and granulocytes by anti-asialo GM1 antiseraum and F(ab')2 of anti-asialo GM1 IgG antibody strongly suggested that asialo GM1 is expressed on these myeloid cells as well as on rat mature T cells. It is interesting that asialo GM1 is universally distributed on rat myeloid cells in the peritoneal cavity and reticulum cells in the lung, spleen, and thymus. Although the distribution and localization of asialo GM1 on rat immunocytes, were quite similar to that of TLMA, the population of macrophages in the peritoneal cavity showed different sensitivity toward TLMA and asialo GM1, being 60% positive for TLMA and 20% positive for asialo GM1 (7). This difference may be due to the glycoprotein antigen, the carbohydrate moiety of which is similar to the asialo GM1 sugar moiety, since Gal (β 1-3)GalNAc of the terminal disaccharide of asialo GM1, which is the PNA binding site, is common in glycoprotein as well as asialo GM1 (23). TLMA may recognize these disaccharides as PNA, whereas the specificity of anti-asialo GM1 may be stricter. Thus, as it is possible to consider the presence of glycoprotein which cross-reacts with anti-asialo GM1 antibody, it is necessary to visualize asialo GM1 in these myeloid cells. A band corresponding to asialo GM1 was detected in macrophages, but the amount was less than that in granulocytes, as shown in Fig. 6a.

In addition to asialo GM1, the different glycolipid profiles of these myeloid cells make it possible that such a glycolipid antigen is useful not only as a surface marker of T cells in the rat, but also as a marker for distinguishing the subpopulation of myeloid cells. Further studies on glycolipids antigens of myeloid cells and their characterization are in progress in our laboratory.

REFERENCES