Bisecting N-Acetylglucosamine on K562 Cells Suppresses Natural Killer Cytotoxicity and Promotes Spleen Colonization

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ABSTRACT

β1-4 N-acetylglucosaminyltransferase (GnT-III) catalyzes the formation of bisecting N-acetylglucosamine (GlcNAc) in the biosynthesis of N-linked oligosaccharides. To examine the effect of bisecting GlcNAc on the natural killer (NK) cytotoxicity, the GnT-III gene was introduced into NK-sensitive K562 cells that have no detectable GnT-III activity. We obtained three clones stably expressing high GnT-III (positive transfectants). Introduction of the GnT-III gene resulted in an increase of bisecting GlcNAc and a decrease of external asialic acid as well as tri- and tetra-antennary sugars, as judged by flow cytometry. Compared to controls, the NK cytotoxicity was completely blocked against positive transfectants. The binding of effector cells to positive transfectants was also decreased. After s.c. injection into nude mice, positive transfectants produced spleen colonization, although no spleen lesions were formed by control cells. In nude mice depleted of NK cells by anti-asialo GM1 antibody, both positive transfectants and controls produced spleen colonization equally. These results indicate that K562 cells expressing GnT-III are resistant to NK cytotoxicity, resulting in spleen colonization in nude mice.

INTRODUCTION

NK cells, which comprise 10–15% of the lymphocytes in human peripheral blood, are morphologically large granular lymphocytes with CD3−, CD16+, CD56+ and are able to lyse target cells without prior sensitization or MHC restriction (reviewed in Ref. 1). Many molecules have been reported to be involved in this spontaneous non-MHC-restricted cytotoxicity, such as the interaction of leukocyte function-associated antigen-1 (CD11a/CD18) or CD2 on NK cell surface, with their target cell ligands intercellular adhesion molecule-1 (CD54) or leukocyte function-associated antigen-3 (CD58), respectively (2, 3). These adhesion molecules are necessary for conjugate formation between NK cells and target cells for NK cells to recognize their targets. However, specific NK receptors, or receptor ligands on the target cells, have remained obscure, since NK cells do not recognize and kill all tumor targets equally. Conjugation alone is not sufficient for NK cytotoxicity, suggesting that further recognition events are involved in cytolysis of the target.

Previously, several investigators have reported that the potential target structures for NK cells are not only proteins but also carbohydrate determinants (especially N-oligosaccharide) in the interaction between NK and target cells. Biochemical analyses of Chinese hamster ovary cells restricted to high mannose-type and hybrid-type N-oligosaccharides have shown them to be more sensitive to cytolysis by NK cells than target cells with complex-type N-linked glycans (4). Other approaches, involving carbohydrate residues, have also been undertaken, such as using exo- or endoglycosidases to digest N-oligosaccharide on the cell surface, or using inhibitors of N-glycosylation or N-glycan processing. For example, the treatment of target cells with sialidase was found to suppress NK cytotoxicity, suggesting that the composition of sialic acid in the target cell membrane was inversely correlated with the NK susceptibility of the target cells (5). Sialic acid can act as a ligand for sialoadhesin on NK cells and NK cytotoxicity is increased by the addition of sialic acid to the target cell membrane (6). Sensitivity to cytolysis by NK cells was increased after the treatment of target cells with deoxymannojirimycin and swainsonine (N-glycan processing inhibitors that promote the accumulation of high mannose-type glycosides), whereas treatment with tunicamycin did not affect NK activity (6).

When target cells are reacted with the effector cells, NK activity is usually evaluated, based on measurement of 51Cr release (1). Because of their sensitivity to NK cytototoxicity and undetectable expression of MHC molecules, the K562 cell line has been commonly used as target cells for standard NK activity assays (7). In this paper, we used another approach of gene introduction to examine the effect of bisecting GlcNAc on NK cytotoxicity using transfection of GnT-III. This investigation was undertaken to examine the effect of bisecting GlcNAc on NK cytotoxicity by using K562 cells ectopically expressing high GnT-III, since GnT-III catalyzes the formation of bisecting GlcNAc (Fig. 1). We found that GnT-III-positive transfectants were more resistant to NK cytotoxicity, less bound by NK cells, resulting in the ability to colonize the spleen in nude mice after s.c. inoculation.

MATERIALS AND METHODS

Reagents. Mammalian expression vector pcAGGS was kindly provided by Dr. Ken-ichi Yamamura and Misao Suzuki (Kumamoto University School of Medicine, Kumamoto, Japan). A pSV2Neo vector, which has a neomycin-resistant gene, was supplied by Japanese Cancer Research Resources Bank (Tokyo, Japan). FITC-conjugated E-PHA, L-PHA, DSA, Con A, and LFA were purchased from EY Laboratories, Inc. (San Mateo, CA). Antibody to asialo GM1 was obtained from Wako Pure Chemical Industries, LTD. (Osaka, Japan). 2-[(N-morpholino)ethane sulfonate, HEPES, and MT was obtained from Sigma Chemical Co. (St. Louis, MO). Animals. Female athymic BALB/c nude mice (6 weeks old) were purchased from SLC Japan, Inc. (Shizuoka, Japan). The mice were caged in laminar flow under specific pathogen-free conditions and used at 8 weeks of age.

Establishment of GnT-III Transfectants. Rat GnT-III cDNA (8) was subcloned in the mammalian expression vector pcAGGS, as detailed (9). GnT-III gene expression in this construct, Act-3, was controlled by the actin promoter. Act-3 (20 µg) and pSV2Neo vector (2 µg) were cotransfected into K562 cells (a human erythroleukemia cell line), which were obtained from American Type Culture Collection (Rockville, MD) using an electroporation method. Infected K562 cells were incubated in DMEM containing G418 (1 mg/ml; GIBCO/BRL), and neo-resistant colonies were diluted to select clones expressing high levels of GnT-III. K562 cells and GnT-III transfectants were grown in RPMI 1640 supplemented with 10% FCS (GIBCO/BRL) and antibiotics in 5% CO2, humidified air at 37°C and were used within five passages throughout this investigation.
Enzyme Activity Assays for GnT-III, GnT-V, and Gal-T. GnT-III (10), GnT-V (11), and Gal-T (12) activities were assayed using a pyridylaminated biantennary sugar chain as a substrate (13). Briefly, cells were washed 3 times with cold PBS and sonicated for 10 min at 4°C. The crude enzyme preparations were incubated with a pyridylaminated biantennary sugar chain at 37°C for 2 h. The reaction buffer for the GnT-III assay consisted of 125 mM 2-[N-morpholino]ethane sulfonate buffer (pH 6.25) containing 0.77 mM substrate, 20 mM UDP-GlcNAc, 10 mM MnCl₂, 200 mM GlcNAc, and 0.5% Triton X-100. For the measurement of intrinsic GnT-III activity, 10 mM MnCl₂ was replaced by 10 mM EDTA to completely inhibit GnT-III activity, which requires Mn²⁺ (11). In the assay for Gal-T activity, the crude preparations were incubated in 10 mM HEPES buffer (pH 7.2) containing 0.77 mM substrate, 20 mM UDP-galactose, 10 mM MnCl₂, 33 mM NaCl, and 3 mM KCl. Samples were boiled for 1 min to stop the reactions, centrifuged at 15,000 rpm for 5 min to remove debris, and then subjected to HPLC; the amount of enzyme product was determined from the fluorescence intensity, as described previously (10–13). The enzyme activity was obtained by averaging the results of triplicate experiments and was expressed as pmol of GlcNAc transferred/h/mg protein. The protein concentration was determined with a BCA kit (Pierce, Rockford, IL).

RNA Preparation and Northern Analysis. Total cellular RNAs were extracted from K562 cells and GnT-III transfectants by acid guanidinium isothiocyanate and phenol/chloroform as detailed (14). Extracted RNAs (20 μg) were electrophoresed on formaldehyde-agarose gel, transferred onto the filter (Zeta-probe; Bio-Rad, Hercules, CA) and hybridized with 32P-labeled rat GnT-III cDNA. After washing, the filter was autoradiographed at -80°C for 2 days, and the intensity of the signals was measured with a densitometer (CS-9000; Shimazu, Tokyo, Japan).

Flow Cytometry. The surface N-oligosaccharides of K562 and GnT-III transfectants were analyzed by the binding of lectins using a series of FITC-conjugated lectins. Cells (1 × 10⁶) were washed and resuspended in 50 μl PBS containing 0.5% BSA and 0.05% sodium azide. FITC-conjugated lectin (20 μg/ml) was added and incubated for 30 min on ice. In controls, the cells were not stained with FITC-conjugated lectins. After two washes, a total of 10,000 events were analyzed by flow cytometry on FACScan (Becton Dickinson, CA), and the mean fluorescence intensity was obtained using LYSIS computer software. Each fluorescence intensity of the cells stained with FITC-lectins was obtained by subtracting that given by the appropriate controls.

NK Cytotoxicity. The susceptibility of the target cells to NK-mediated cytolysis was assayed by a standard 4-h ⁵¹Cr-release assay. From heparinized peripheral blood of five healthy individuals, mononuclear cells were isolated by density gradient centrifugation using Lymphoprep (Nycomed, Oslo, Norway). After a 1-h incubation in culture dishes, the nonadherent cells were harvested in RPMI 1640 containing 2% FCS, suspended in 96-well round-bottomed plates (Corning, NY) at the concentration of 1–5 10⁶ cells/well, and used as the effector cells. These effector cells are referred to as NK cells in this study. K562 cells and GnT-III transfectants, as the targets, were labeled with 100 μCi of Na₂[¹⁵⁴]CrO₄ (ICN Radiomedicals, Irvine, CA) at 37°C for 1 h. After extensive washing to remove the excess ¹⁵⁴Cr, cells (1 × 10⁶) were resuspended and mixed with the effector cells at the various E:T ratios in a volume of 200 μl using triplicate assays. After a 4-h incubation at 37°C in 5% CO₂, plates were centrifuged, and the supernatants were counted in a gamma counter (ARC300; Aloka). NK cytotoxicity was calculated using the following equation:

\[
\%\text{ lysis} = \frac{\text{cpm spontaneous} - \text{cpm each experiment}}{\text{cpm maximal} - \text{cpm spontaneous}} \times 100
\]

where cpm spontaneous and cpm maximal were determined by measuring cpm of the supernatants of the target cells alone in the presence of assay medium or in the presence of 1% SDS, respectively. Spontaneous release was below 8% of the maximal release throughout this experiment.

E:T Binding Assay. The binding of effector cells to the targets was analyzed, as described (15). In brief, effector cells (1 × 10⁶/100 μl) and target cells (1 × 10⁶/100 μl) were combined in 96-well, round-bottomed plates, centrifuged at 400 rpm for 2 min, and incubated in triplicate at 37°C for 15 min. In each well, more than 200 effector cells were counted, and the number of effector cells that were bound to the target or free was quantitated by visual inspection with a phase contrast microscope. The binding ratio (%) was calculated as the ratio of effector cells bound to the targets and represented the mean ± SD of effector cells from five independent individuals.

Tumorigenicity and Spontaneous Metastasis in Nude Mice. To evaluate tumorigenicity and spontaneous metastasis, original K562 cells and GnT-III transfectants were inoculated s.c., as described previously (16). Briefly, cells were harvested by a brief treatment with PBS containing 0.02% EDTA, washed, and resuspended in HBSS. A group of five mice were given the s.c. injections in the heel pads with 5 × 10⁶ cells in 100 μl HBSS. Mice were sacrificed 6 weeks after cell inoculation. Tumor colonization was examined macroscopically in the spleen, lymph nodes (cervical, axilial, and inguinal), bone marrow, liver, and heel pads, where the cells were injected; finally, the spleen and the liver were weighed. For microscopic analysis, the spleen, bone marrow, liver, and lymph nodes were fixed in 10% formalin-PBS, embedded in paraffin, and then sectioned, and stained with hematoxylin-eosin. For the preparation of NK-depleted nude mice, the mice received i.p. administration of anti-asialo GM1 (10 μg/head) four times: three days (day -3) and the day (day -1) prior to s.c. inoculation; the day (day 0) at the time of injection; and two days (day +2) after inoculation. At three weeks after s.c. inoculation of the cells, NK-depleted mice were sacrificed and analyzed in the same manner as described above.

Cell Growth Assay. The cell growth of parental K562 cells and GnT-III transfectants was evaluated by MTT assay, as described (17).

RESULTS

Establishment of K562 Sublines Stably Expressing High GnT-III Activity. From eleven G-418-resistant clones, three clones with elevated GnT-III activity were chosen at random and designated as K562-III-1, -2, and -3 (positive transfectants). Similarly, two G-418-resistant clones with undetectable GnT-III were selected and designated as K562-neo-1, and -2 (control transfectants). As shown in Fig. 2, original K562 and control transfectants showed undetectable levels of GnT-III activity (below 0.05 mmol/h/mg), whereas positive transfectants had GnT-III activity, ranging from 4.56–8.29 mmol/h/mg. The activity of GnT-V and Gal-T were not significantly changed in
E-PHA to the cell surface was increased in the positive transfectants; 63% increase in comparison to original K562 cells. L-PHA (19) and DSA (20) recognize tri- or tetraantennary structures of complex-type asparagine-linked sugar chains. The binding of L-PHA and DSA to the cells was decreased in positive transfectants: 60.3 and 55.1% of original K562 cells, respectively. LFA binds preferentially to the sialic acid at the terminal end of N-oligosaccharides (21), and Con A has an affinity for the core mannose of biantennary structures of complex-type and high-mannose type N-glycans. This affinity for the core mannose is suppressed by bisecting GlcNAc (22). The binding of LFA and Con A was also decreased in positive transfectants: 38.3 and 70.5% of the original K562 cells, respectively. These lectin-binding profiles suggested that, in K562 cells expressing high GnT-III, the composition of bisecting GlcNAc was increased on the N-oligosaccharides due to the ectopically expressed GnT-III (as confirmed by increased E-PHA binding and decreased Con A binding), while tri- or tetraantennary structures and terminal sialic acid of N-oligosaccha-

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**Table 1** Lectin binding to parental K562 cells and GnT-III transfectants, as measured by flow cytometry using FITC-lectins

<table>
<thead>
<tr>
<th>Cells</th>
<th>E-PHA</th>
<th>L-PHA</th>
<th>DSA</th>
<th>LFA</th>
<th>Con A</th>
</tr>
</thead>
<tbody>
<tr>
<td>K562</td>
<td>51.5(5.4)</td>
<td>31.4(3.1)</td>
<td>62.0(3.1)</td>
<td>51.9(4.5)</td>
<td>55.4(3.5)</td>
</tr>
<tr>
<td>K562-neo-1</td>
<td>52.7(6.3)</td>
<td>34.3(2.9)</td>
<td>63.5(3.9)</td>
<td>48.6(4.1)</td>
<td>53.7(4.0)</td>
</tr>
<tr>
<td>K562-neo-2</td>
<td>54.8(5.8)</td>
<td>33.9(2.5)</td>
<td>65.3(4.1)</td>
<td>50.7(4.8)</td>
<td>59.6(3.1)</td>
</tr>
<tr>
<td>K562-III-1</td>
<td>82.6(6.8)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.9(2.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.1(3.4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.2(4.1)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.0(3.8)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>K562-III-2</td>
<td>89.2(6.6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.4(2.4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>36.5(3.9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.4(4.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>38.7(3.8)&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>K562-III-3</td>
<td>76.6(7.6)&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.8(2.4)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>32.5(4.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.0(3.8)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.1(3.7)&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> P < 0.05 versus K562.
<sup>b</sup> P < 0.02 versus K562.
Bisecting GlcNAc and NK Sensitivity

Fig. 4. Susceptibility to the NK cytotoxicity in K562 cells and GnT-III transfectants. 51Cr-labeled target cells were separately exposed to each effector cell prepared from five individuals, and released 51Cr was measured in triplicate. Cytolysis was calculated as described in "Materials and Methods." The cytotoxicity of the bidecting GlcNAc on the susceptibility to NK cytotoxicity, using standard 51Cr-release assays. Six target lines (K562, K562-neo-1, -2, K562-III-1, -2, -3) were exposed to the effector from the same individuals simultaneously. % cytolyysis at the various E:T ratios was the mean of five separate assay values with effector cells from five individuals, as shown in Fig. 4. NK effector cells were able to cytoxide control transfectants as well as original K562 cells, and cytotoxicity correlated with the E:T ratio. Lysis by the effector cells was, however, completely blocked when the positive transfectants were exposed to the NK effector cells. The cytotoxicity to positive transfectants tended to be elevated at a higher E:T ratio.

Next, binding of human effector cells to the original K562 cells and GnT-III transfectants was also assayed, since NK cytotoxicity is initiated by the interaction of the effector cells with the targets (1). As shown in Fig. 5, the binding to positive transfectants was significantly decreased, as compared to original K562 cells and control transfectants, suggesting that lower binding of effector cells to positive transfectants was responsible for the decreased NK cytotoxicity to the positive transfectants.

Tumor Formation by GnT-III-positive K562 Cells in the Spleen of the Nude Mice. In athymic nude mice, T lymphocyte-mediated cytotoxicity is severely impaired because of the deficiency of T lymphocytes, whereas NK cells function normally. To evaluate the susceptibility to NK cytotoxicity in vivo, original K562 cells and GnT-III transfectants were s.c. inoculated into nude mice. At 6 weeks after the inoculation of the cells, the mice were sacrificed, and the number of tumor formations were examined to evaluate tumorigenicity and metastatic potential. No tumorigenicity was observed in lymph nodes, bone marrow, liver, and s.c. heel pads (injection sites) after the injection of the original K562 cells and positive and control transfectants. As listed in Table 2, all nude mice injected with positive transfectants developed tumor colonies on the surface of their enlarged spleens (five of five). On the other hand, the original K562 cells and control transfectants did not display any splenic lesions. Microscopic analysis revealed that in all nude mice that were injected with positive transfectants, the spleen was occupied with many large neoplastic cells with the destruction of the spleen architecture, such as cortex and follicles in the medulla (Fig. 6, A and B).

No neoplastic cells were detected in the random section of the spleen taken from nude mice injected with original K562 cells or control transfectants (Fig. 6, C and D). Neoplastic cells were not found microscopically in the lymph nodes, liver, bone marrow, and s.c. injection site at the heel pad in nude mice injected with original K562 cells and positive and control transfectants in the random sections (data not shown).

Spleen colonization was also assayed by s.c. inoculation of the cells into nude mice that were depleted of NK cells by i.p. administration of antibody to asialo GM1. At 3 weeks after the inoculation of the cells, the mice were sacrificed, and the splenic lesions were evaluated.

Table 2. Metastatic potential of K562 cells and GnT-III transfectants

<table>
<thead>
<tr>
<th>Cells</th>
<th>Liver weight (g)</th>
<th>Spleen weight (g)</th>
<th>Incidence</th>
<th>No. of nodules</th>
</tr>
</thead>
<tbody>
<tr>
<td>K562</td>
<td>1.53 ± 0.31</td>
<td>0.09 ± 0.02</td>
<td>0/0</td>
<td>0, 0, 0, 0, 0,</td>
</tr>
<tr>
<td>K562-neo-1</td>
<td>1.49 ± 0.27</td>
<td>0.10 ± 0.02</td>
<td>0/0</td>
<td>0, 0, 0, 0, 0,</td>
</tr>
<tr>
<td>K562-neo-2</td>
<td>1.61 ± 0.41</td>
<td>0.08 ± 0.01</td>
<td>0/0</td>
<td>0, 0, 0, 0, 0,</td>
</tr>
<tr>
<td>K562-III-1</td>
<td>1.56 ± 0.33</td>
<td>0.39 ± 0.07</td>
<td>5/5</td>
<td>35, 29, 17, 15, 13,</td>
</tr>
<tr>
<td>K562-III-2</td>
<td>1.44 ± 0.29</td>
<td>0.41 ± 0.08</td>
<td>5/5</td>
<td>26, 20, 15, 13, 9,</td>
</tr>
<tr>
<td>K562-III-3</td>
<td>1.60 ± 0.40</td>
<td>0.37 ± 0.06</td>
<td>5/5</td>
<td>27, 20, 13, 11, 9,</td>
</tr>
</tbody>
</table>

a P < 0.02 versus K562.
Fig. 6. Spleen of nude mice in tumorigenicity experiments. A, spleen after s.c. injection of K562-III-1 cells showing the extensive infiltration of neoplastic cells in the spleen (×200). B, higher magnification of A showing large neoplastic cells with atypical nucleus of irregular margin and two or three distinctive nucleoli (×400). C, spleen sections from nude mice after inoculation of original K562 cells showing no destruction of the spleen (×200). D, higher magnification of C showing small splenic cells without invasion of neoplastic cells (×400). Hematoxylin and eosin staining of formalin-fixed sections.

The splenic colony numbers (represented as the mean ± SD of five mice) were as follows: K562, 14 ± 4; K562-neo-1, 20 ± 7; K562-neo-2, 23 ± 8; K562-III-1, 17 ± 6; K562-III-2, 25 ± 8; and K562-III-3, 19 ± 7, showing that original K562 cells, control transfectants, and positive transfectants produced splenic lesions equally in NK-depleted nude mice.

In vitro growth rate was determined by MTT assay. The half times (days) of cell growth are as follows: K562, 2.3 ± 0.2; K562-neo-1, 2.2 ± 0.2; K562-neo-2, 2.1 ± 0.2; K562-III-1, 2.0 ± 0.2; K562-III-2, 2.1 ± 0.2; and K562-III-3, 2.2 ± 0.2. No significant difference in growth was found among these cells examined, indicating that the specific colonization of the spleen by positive transfectants was not due to cell proliferation.

DISCUSSION

In this study, ectopic expression of GnT-III resulted in an altered composition of N-oligosaccharides on surface glycoproteins, as detected by flow cytometry. The alteration of lectin-binding patterns was coincident with the increase in bisecting GlcNAc residues on N-glycans, presumably because positive transfectants were more E-PHA bound and less Con A bound. Once a bisecting GlcNAc residue is transferred to a core mannose structure by GnT-III, other N-acetylglucosaminyltransferases, such as GnT-V, are poorly able to transfer GlcNAc at the β1–6 position of the core mannose (23). This initial step is requisite for the formation of tri- or tetraantennary structures (24). Therefore, the introduction of a GnT-III gene might suppress the formation of elongated sugar chains, such as tri- or tetraantennary, and resulted in an increase in shortened N-oligosaccharides. The shortened sugar chain is more likely to be terminated by the core mannose with a bisecting GlcNAc in K562 cells expressing GnT-III. In contrast, sialic acid, GlcNAc, and galactose, which are usually located at the terminal of tri- or tetraantennary of the elongated N-glycans (25), are seen less often in positive transfectants than controls. These assumptions were supported by the decreased L-PHA, DSA, and LFA affinities of in the positive transfectants.
Previously, it was reported that sensitivity to NK cells was increased by treatment of the cells with sialidase (4), indicating that the content of surface sialic acid is inversely correlated with sensitivity to NK cytotoxicity. In that study, the authors concluded that NK cells were able to conjugate the target more easily following sialidase treatment of the target, because of their increased sensitivity to NK cytotoxicity. In our study, however, expression of GnT-III decreased the content of sialic acid, as confirmed by LFA binding, while NK cytolyis and E:T binding were decreased in the positive transfectants. In K562 cells expressing GnT-III, it was speculated that the addition of bisecting GlcNAc resulted in the lower binding of NK cells to the target, although the sialic acid content was decreased.

In s.c. inoculation into NK-depleted nude mice, no significant differences in the spleen colonization were observed between controls and positive transfectants. In the presence of NK cells, the spleen colonization of control cells was completely suppressed, whereas positive transfectants were also able to colonize the spleen. These findings indicate that NK cells in nude mice are unable to cytolyze K562 cells expressing GnT-III and that bisecting GlcNAc can protect K562 cells from NK-mediated cytosis.

Recently, several investigators reported a group of surface transmembrane proteins that are expressed predominantly on NK cells and considered as the likely candidates for NK cell receptors (26). In NK receptor protein-1 (27), one of these molecules has an extracellular domain that contains carbohydrate recognition domains, which are found in C-type (calcium-dependent) animal lectins. Binding and inhibition assays, using monosaccharides and neoglycoconjugates, indicated that NK receptor protein-1 is a lectin with a preference order of N-acetylgalactosamine > GlcNAc > fucose > galactose > mannose (28). Introduction of the GnT-III gene caused an increase in the composition of bisecting GlcNAc and a decrease in the elongated tri- and tetraantennary structure, as well as terminal sialic acid at the terminal end. We speculate that bisecting GlcNAc might also be inhibitory for the binding of NK receptor protein-1.

The K562 cell line was established from a patient with chronic myelogenous leukemia. Following the s.c. inoculation of nude mice, we hypothesized that NK-resistant GnT-III transfectants would colonize and grow in the hematopoietic organs such as bone marrow, spleen, and liver, because cells of hematopoietic origin would normally show orthotopic affinity to the hematopoietic tissues, and because hematopoiesis progresses in the liver and the spleen during embryonic development, as well as in the bone marrow of the fetus at late development and after birth. Actually, no tumor formation was observed in bone marrow, lymph nodes, and liver for all cells examined. It is postulated that cells of human leukemic origin are unlikely to colonize and proliferate in the hematopoietic tissues (bone marrow, lymph nodes, and liver) of nude mice because of species differences, although several human tumor cells are transplantable and passagable in nude mice. Unexpectedly, positive transfectants displayed specific colonization in the spleen of nude mice. In addition, tumorigenicity of GnT-III-positive transfectants displayed specific localization in the spleen after s.c. inoculation into nude mice. Further investigations are being undertaken to identify and characterize the molecules that are involved in NK resistance and specific colonization of the spleen in nude mice.

ACKNOWLEDGMENTS

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10. Nishikawa, A., Fujii, S., Sugiyama, T., and Taniguchi, N. A method for the determination of lung metastasis of GnT-III-positive B16-hm cells. On the contrary, this study showed that K562 cells expressing GnT-III were less bound to and less cytolyzed by NK cells, as compared to controls, which resulted in the increase of spleen colonization. The evidence indicates that the introduction of the GnT-III gene does not affect the NK sensitivity of all of the target cells.

In summary, we have demonstrated that ectopic expression of GnT-III in K562 cells results in an increase of bisecting GlcNAc and a decrease of external sialic acid and tri- and tetraantennary structures. These alterations in oligosaccharides are associated with the decreased susceptibility to NK cytotoxicity. Our findings may be highly relevant to the control of organ rejection during transplantation or graft-versus-host disease, since NK cells may be more activated under such pathological conditions and attack the transplant graft (1). Moreover, GnT-III-positive transfectants displayed specific localization in the spleen after s.c. inoculation into nude mice. Further investigations are being undertaken to identify and characterize the molecules that are involved in NK resistance and specific colonization of the spleen in nude mice.

4 Unpublished observation.
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