The Macrophage C-type Lectin Specific for Galactose/N-Acetylgalactosamine Is an Endocytic Receptor Expressed on Monocyte-derived Immature Dendritic Cells*

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Lectins on antigen presenting cells are potentially involved in the antigen uptake and the cellular recognition and trafficking. Serial analysis of gene expression in monocyte-derived dendritic cells (DCs), monocytes, and macrophages revealed that 7 of the 19 C-type lectin mRNA were present in immature DCs. Two of these, the macrophage mannose receptor and the macrophage lectin specific for galactose/N-acetylgalactosamine (MGL), were found only in immature DCs, as confirmed by reverse transcriptase-PCR and flow cytometric analysis. By subcloning and sequencing the amplified mRNA, we obtained nucleotide sequences encoding seven different human MGL (hMGL) subtypes, which were apparently derived from alternatively spliced mRNA. In addition, the hMGL gene locus on human chromosome 17p13 contains one gene. A single nucleotide polymorphism was identified at a position in exon 3 that corresponds to the cytoplasmic region proximal to the transmembrane domain. Of all the splicing variants, the hMGL variant 6C was expressed at the highest levels on immature DCs from all donors tested. Immature DCs could incorporate α-GalNAc-modified soluble acrylamide polymers, and this was significantly inhibited by pretreatment of the cells with an anti-hMGL monoclonal antibody that blocks the lectin-carbohydrate interaction. We propose that hMGL is a marker of imDCs and that it functions as an endocytic receptor for glycosylated antigens.

Dendritic cells (DCs)† play a pivotal role in the immune system by processing and presenting a variety of antigens to T cells (1). The uptake of exogenous antigens is the first step in this process and is therefore a critical event that influences DC function. The uptake of glycogen conjugates by DCs is potentially mediated by lectins, which are carbohydrate-binding proteins. There are at least four distinct lectin families in animal cells known as the C-, S-, P-, and I-type lectins (2). Some lectins are known to participate in molecular and cellular trafficking in a manner that is dependent on the lectin type, its molecular architecture, and its subcellular localization. DCs are known to express a variety of lectins, particularly C-type lectins, but as yet their biological roles in DC function are unclear.

How glycosylated antigen presentation is regulated and how this affects the subsequent immune responses has not yet been clarified. This is an important issue to investigate as it may improve our understanding of, for example, anti-tumor immunity to MUC1. MUC1 is a glycosylated membrane protein that frequently expresses truncated O-glycans such as the T (Galβ1-3GalNAc-Thr/Ser) and Tn (GalNAc-Thr/Ser) antigens. MUC1 is an important candidate vaccine antigen as it is an antigen that is often overexpressed in solid tumors, including carcinoma of the breast, lung, pancreas, colon, and ovaries. MUC1-specific cytotoxic T lymphocytes have been isolated from draining lymph nodes of pancreatic and breast cancer patients, ascitic fluids of ovarian cancer patients, and peripheral blood mononuclear cells (PBMCs) of multiple myeloma patients (3–6). It is important to understand how this naturally acquired MUC1-specific immune response is raised as this might allow us to optimize MUC1 immunization strategies for cancer immunotherapy. Thus, how glycosylated antigens are recognized by antigen-presenting cells (APCs), how they are taken up by lectin-dependent pathways, and how these antigens can be processed and presented with MHC molecules are all important issues that deserve investigation.

A unique C-type lectin specific for clusters of galactose and/or N-acetylgalactosamine has been identified in mice (7), rats (8), and humans (9). This molecule has been denoted the macrophage (MØ) galactose/N-acetylgalactosamine (Gal/GalNAc)-specific C-type lectin (MGL). Molecular cloning and characterization of both the human and murine MGLs revealed that MGL is a type II transmembrane glycoprotein with a single extracellular C-type carbohydrate recognition domain (CRD) (7, 9). MGL is unique among the mammalian lectins because its capacity for carbohydrate recognition has been extensively ex-
MGL and endocytic receptor for Gal/GalNAc-modified proteins, migration in the body. With regard to the second role of MGL as a recognition molecule are our previous studies that showed that recombinant human MGL (hMGL) was able to bind to a carbohydrate chains derived from a mucin (11). Such carbohydrate binding capacity would provide MGLs with a dual function as a recognition molecule and as an endocytic receptor for glycosylated antigens. Supporting its role as a recognition molecule is the rapid from the application site (17). Such MGL cells and MGL-transfected cells have been shown to internalize glycosylated proteins via MGL-dependent endocytosis (12, 21). Supporting this proposed function of MGL is the fact that MGL and its rat counterpart contain in their cytoplasmic regions the tyrosine motif that is required for interaction with clathrin-coated vesicles and thus endocytosis (7–9).

Given these dual roles, MGL may facilitate both the close contact with tumor cells and the subsequent uptake of glycosylated tumor antigens by DCs if it is expressed on these cells. Thus, the identification of MGL is an important goal, as it should help to understand the regulatory mechanism of the immune system and aid the development of vaccination protocols. We report here the identification of MGL on various potential APCs in humans by serial analysis of gene expression (SAGE) (22–25). MGL and the macrophage mannose receptor (MMR) were found to be expressed exclusively on immature DC as far as the cell populations we tested. The MGL on immature DCs was observed to act as an endocytic receptor. Spliced MGL variants in immature DCs were also identified and their relative frequencies in the cells determined. The possibility that targeting MGL by attaching Gal/GalNAc residues to a candidate antigen might improve specific antigen presentation by DCs and thus improve vaccine immunogenicity is discussed.

**TABLE I**

| Lectins                          | Monocytes | GM-CSF M/CS | M-CSF M/CS | Immature DCs | Mature DCs | SAGE tag          | UniGene cluster |
|---------------------------------|-----------|-------------|------------|--------------|------------|-------------------|-----------------|
| DEC-205                         | 0         | 0           | 0          | 1            | 7          | AAGGAAATGTG        | 153,563         |
| M5 mannose receptor, C-type 1   | 0         | 0           | 0          | 17           | 0          | TCCGAGACAA         | 75,182          |
| Langerin                        | 0         | 0           | 0          | 0            | 0          | ATCCCACCTTT        | 167,741         |
| DC-SIGN                         | 0         | 0           | 0          | 17           | 0          | CCTCACCACC         | 153,563         |
| hMGL                            | 0         | 0           | 3          | 17           | 0          | CTCACCACCT         | 75,182          |
| CLECSF1                         | 0         | 0           | 0          | 0            | 0          | GGCACGACG          | 167,741         |
| CLECSF2                         | 0         | 0           | 0          | 0            | 0          | TGGATGTACC         | 153,563         |
| CLECSF5, MDL-1                  | 0         | 0           | 0          | 0            | 0          | TGGATGTACC         | 153,563         |
| CLECSF6, DCIR                   | 7         | 0           | 2          | 24           | 0          | GTCTCTCTCTT         | 115,515         |
| CLECSF12, decidin-1             | 3         | 3           | 0          | 15           | 1          | TGCTGATTTG         | 161,786         |
| CD23                            | 0         | 0           | 2          | 20           | 1          | GATACAGCCA         | 1,418           |
| CD62L, L-selectin               | 0         | 0           | 3          | 20           | 1          | CATTTCGCA          | 82,848          |
| CD69                            | 2         | 0           | 0          | 0            | 0          | CATTTCGCA          | 82,848          |
| CD94                            | 4         | 4           | 2          | 8            | 5          | ACTGTAATCC         | 41,682          |
| asialoglycoprotein receptor 1    | 0         | 0           | 0          | 0            | 0          | ACCAAGGACT         | 12,056          |
| asialoglycoprotein receptor 2    | 0         | 0           | 0          | 0            | 0          | GTCTGACTCA         | 1,259           |
| endocytic receptor (M5 mannose receptor family) | 0 | 0 | 1 | 1 | 0 | GCCCTCTCTT | 7,935 |
| Scavenger receptor with C-type lectin | 0 | 0 | 2 | 0 | 0 | GAGGAAAAGA | 29,423 |
| Stem cell growth factor; lymphocyte secreted C-type lectin | 0 | 0 | 1 | 1 | 0 | GGGCTCTGGGG | 105,927 |
| Man-6-P receptor (cation-dependent) | 2 | 18 | 15 | 7 | 12 | GCTCACCGTG | 75,709 |
| Siadhesin                       | 0 | 0 | 2 | 1 | 7 | CTCGATTGCC | 31,689 |
| CD33                            | 1 | 1 | 1 | 7 | 0 | GAAAAACCA | 83,731 |
| Ficolin 1                       | 244 | 3 | 13 | 0 | 0 | CCCACACACT | 252,136 |
| Ficolin 3                       | 1 | 0 | 0 | 0 | 0 | GACACCGAGG | 333,383 |
| Galectin 1                      | 96 | 170 | 225 | 76 | 127 | GCCCCCAATA | 198,261 |
| Galectin 2                      | 12 | 0 | 0 | 0 | 0 | TCCCTCTCTA | 113,987 |
| Galectin 3                      | 29 | 180 | 211 | 69 | 16 | TTCCTCTCTA | 621 |
| Galectin 8                      | 0 | 0 | 2 | 0 | 4 | GCACCCACAC | 4,082 |
| Galectin 9                      | 9 | 13 | 8 | 7 | 12 | CTCTGCACCCC | 81,337 |

**EXPERIMENTAL PROCEDURES**

**Preparation of Cells—**PBMCs were isolated from venous blood drawn from normal healthy volunteers at the Tokyo Metropolitan Red Cross Blood Center (Tokyo, Japan) (24). Briefly, PBMCs were isolated by centrifugation on a Ficoll-Metrizoate density gradient (d = 1.077 g/ml; Lymphoprep, Nycomed, Oslo, Norway) and suspended in RPMI 1640 medium containing 7.5% heat-inactivated fetal calf serum (FCS) (In-vitrogen; The FCS contained 100 units/ml penicillin. To purify monocytes, the PBMCs were incubated with an anti-CD14 mAb-coated microbeads and then passed through a magnetic cell separation system (MACS, Miltenyi Biotec, Bergisch Gladbach, Germany) with column-type VR. The cell suspensions were then aliquotted into plastic tissue culture plates and incubated for 30 min at 37 °C, 5% CO2 to obtain highly purified cells. More than 99% of the cells were monocytes as determined by their morphology, positive...
staining with a CD14 mAb (LeuM3, Becton Dickinson, San Jose, CA), and nonspecific esterase staining (24).

MØs were prepared from the CD14+ monocytes by culturing them for 7 days in RPMI 1640 medium with 7.5% FCS and 100 units/ml of M-CSF (Morinaga Milk Industry Co. Ltd., Tokyo, Japan) or 500 units/ml of GM-CSF (Kirin Brewery Co. Ltd., Tokyo, Japan). The purity of the MØ cell population was confirmed by morphology and flow cytometric analysis using specific markers as described in a previous article (24). Immature DCs were prepared from the CD14+ monocytes by culturing them for 7 days in RPMI 1640 medium containing 7.5% FCS, 500 units/ml of GM-CSF (Kirin Brewery Co.), and 100 units/ml of interleukin-4 (Ono Pharmaceutical Co. Ltd., Osaka, Japan). Mature DCs were induced from immature DCs by culturing them for 48 h in RPMI 1640 medium containing 7.5% FCS and lipopolysaccharide (100 µg/ml). Expression of DC cell surface markers was confirmed by flow cytometric analysis with mAbs to the following antigens: CD86 (2331; PharMingen), CD80 (MAB104; Coulter, Fullerton, CA), CD83 (HB15a; Coulter), and CD33 (B8.12.2; Immunotech, Marseille, France). Down-regulation of CD14 was detected with an anti-CD14 mAb (M5E2; PharMingen). The flow cytometric analyses were performed with the Epics Elite (Beckman Coulter, Fullerton, CA) (23).

**SAGE Protocol**—Total RNA from monocytes, MØs, and monocyte-derived DCs obtained from at least six donors was isolated by direct lysis in RNAzol B (Tel-Test, Inc., Friendswood, TX). Poly(A) RNA were isolated using the FastTrac mRNA purification kit (Invitrogen) according to the manufacturer's instructions. The SAGE was performed as described (26–28). SAGE libraries were generated using 2.5 µg of poly(A) RNA, which was converted to cDNA with an Invitrogen synthesis kit following the manufacturer’s protocol, with the inclusion of primer biotin-58-T18–38. The cDNA was cleaved with NlaIII restriction enzyme, and the 35-terminal cDNA fragments were bound to streptavidin-coated magnetic beads (Dynal, Oslo, Norway). After ligasation to oligonucleotides containing recognition sites for BsmF1, the linked cDNAs were released from the beads by BsmF1 digestion. The released tags were ligated to one another, concatenated, and cloned into the SpIhi site of pZero, version 1.0 (Invitrogen). Colonies were screened with polymerase chain reaction (PCR) using M13 forward and M13 reverse primers. PCR products containing inserts that were greater than 400 bp were sequenced with the T7/T7 Dye terminator kit and analyzed using a 377-ABT automated sequencer (PerkinElmer Life Sciences). All electropherograms were reanalyzed by visual inspection to check for ambiguous bases and to correct misreadings.

Sequence files were analyzed with the SAGE software, CGAP SAGE data base (www.ncbi.nlm.nih.gov/SAGE/), the NCBI’s sequence search tool (Advanced BLAST search, www.ncbi.nlm.nih.gov/BLAST/), and DNAvis software (Takara, Shiga, Japan). After the elimination of linker sequences and the repeated ditags, a total of 261,256 tags, which included 57,560, 57,463, 55,856, 58,540, and 31,837 tags from monocytes, GM-CSF-induced MØs, M-CSF-induced MØs, immature DCs, and mature DCs, respectively, were analyzed (22–24).

**Reverse Transcriptase-PCR (RT-PCR)**—Total RNAs (200 ng) were prepared using RNAzol B. The RNA was reverse-transcribed in 50 µl of 10 mM Tris-HCl (pH 8.3), 6.5 mM MgCl2, 50 mM KCl, 10 mM dithiothreitol, each dNTP at 2 µM random hexamer, and 2.4 units/µl Moloney murine leukemia virus reverse transcriptase for 1 h at 42°C. The cDNA, corresponding to 40 ng of total RNA, was boiled for 3 min and quenched with 1 µl of AmpliTaq polymerase (Applied Biosystems, Tokyo). The PCR primers used were: hMGL, sense 5'-CACATCTCGTGTCTGTTGATTTTC-3' and antisense 5'-GCATAGTCGTTGCTCAGTCTC3'-antisense, and dTTP each at 200 µM, dGTP, dATP, dCTP, and dTTP each at 200 µM (Amersham Biosciences), 20 µM hMGL primers buffer, 2 µM MgCl2, and 0.1 µM of AmpliTaq polymerase (Applied Biosystems, Tokyo). The PCR primers used were: hMGL, sense 5'-GCACCTGGTCGCCAGAGGATGA-3' and antisense 5'-ACAATTGCCCAACAGCTCAT-3'. Reverse transcribed PCR products were subjected to agarose gel electrophoresis. In immature DCs, one intense band (~530 bp) and one weak band just above the intense one (~610 bp) were detected in the amplified materials. Mature DCs, MØs, and monocytes were negative for hMGL mRNA expression. Equivalent results were observed in the RT-PCR analysis of DCs from three normal donors. CAP, adenylyl cyclase-associated protein. B, flow cytometric analysis of immature DCs. Immature DCs harvested at day 8 were stained with anti-hMGL mAb MLD-1. Equivalent MGL staining was observed in DCs from six independent donors.

**Screening with Polymerase Chain Reaction (PCR)** Using M13 forward and M13 reverse primers, the amplified cDNAs were released from the beads by 1 µl of AmpliTaq polymerase (Applied Biosystems, Tokyo). The PCR primers used were: hMGL, sense 5'-CACATCTCGTGTCTGTTGATTTTC-3' and antisense 5'-GCATAGTCGTTGCTCAGTCTC3'-antisense, and dTTP each at 200 µM, dGTP, dATP, dCTP, and dTTP each at 200 µM (Amersham Biosciences), 20 µM hMGL primers buffer, 2 µM MgCl2, and 0.1 µM of AmpliTaq polymerase (Applied Biosystems, Tokyo). The PCR primers used were: hMGL, sense 5'-GCACCTGGTCGCCAGAGGATGA-3' and antisense 5'-ACAATTGCCCAACAGCTCAT-3'. Reaction mixtures were incubated in a PerkinElmer DNA Thermal Cycler (33 cycles, denaturation at 94°C for 45 s, annealing at 58°C for 45 s, extension at 72°C for 60 s).

**Determination of Nucleotide Sequences of PCR-Produced Products**—PCR was performed in a 20-µl solution containing 2 µl of template cDNA from monocytes, MØs, or DCs, 2 µl of 10× buffer, 2 µl of 2.5 mM each dNTP, 1 unit of AmpliTaq polymerase, and 0.5 µl hMGL primers (sense, 5'-AATTGACACCTCCTCCAGTCC3'-antisense, and antisense, 5'-TCACCAAGAGGGCAGCTCAGT-3'). The PCR was performed with 40 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 30 s, and extension at 72°C for 30 s. The amplified products were electrophoresed on 2% agarose gels. Bands corresponding to the products were recovered and ligated into the pGEM-T-easy vector. Competent Esche-
The amino acid residue at position 35 was originally identified as Arg (9). The numbers in parentheses indicate the percentage of all the clones obtained from each individual.

Table II
Expression frequency of the seven hMGL subtypes

| Subtype       | Size of amplified product | Donor 1 | Donor 2 | Donor 3 | Donor 4 | Donor 5 | Percentage |
|---------------|---------------------------|---------|---------|---------|---------|---------|------------|
|               | bp                        |         |         |         |         |         |            |
| hMGL          | 1075                      | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (2.0) | 0.6 ± 0.7 |
| hMGL(6A)      | 974                       | 2 (20.0)| 3 (21.6)| 1 (5.7) | 4 (20.4)| 6 ± 0.2  |
| hMGL(6B)      | 1066                      | 2 (22.6)| 1 (10.4)| 2 (13.3)| 8 (25.6)| 22 ± 2.4 |
| hMGL(6C)      | 1065                      | 9 (12.6)| 10 (14.7)| 1 (1.9) | 1 (1.9) | 2 ± 0.2  |
| hMGL(6A/8A)   | 982                       | 1 (6.7) | 0 (0.0) | 2 (3.0) | 3 (5.4) | 0.6 ± 0.6 |
| hMGL(6C/8A)   | 974                       | 1 (6.7) | 0 (0.0) | 2 (3.0) | 3 (5.4) | 0.6 ± 0.6 |
| hMGL(8A)      | 1066                      | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0.6 ± 0.6 |

Table III
Sequences at the intron/exon boundaries

| Intron | Splice donor | Splice acceptor | Length |
|--------|--------------|-----------------|--------|
| Intron 1 | gtagcttgcc----atatccccag | CTCCACCCCA.. | 1166 bp |
| Intron 2 | gtagcttgcc----cctcttgcag | GGCCTCTTCC.. | 632 bp |
| Intron 3 | gtagcttgcag----acctgtttag | ATCCCAATT.. | 1009 bp |
| Intron 4 | gtagcttgcag----tgcttccccag | GGCACGCTT.. | 87 bp |
| Intron 5 | gtagcttgcag----tgcttccccag | GGCACGCTT.. | 580 bp |
| Intron 6 | gtagcttgcag----tgcttccccag | GGCACGCTT.. | 661 bp |
| Intron 7 | gtagcttgcag----tgcttccccag | GGCACGCTT.. | 90 bp |
| Intron 8 | gtagcttgcag----tgcttccccag | GGCACGCTT.. | 264 bp |

RESULTS

mRNA for Macrophage Mannose Receptor and MGL Are Specifically Expressed in Immature DCs—The mRNA expression frequencies of candidate endogenous lectins in monocytes, MØ, and DCs prepared from human PBMCs were determined by SAGE. Results were obtained from 57,560 monocyte tags, 57,463 GM-CSF-induced MØ tags, 55,856 M-CSF-induced MØ tags, 58,540 immature DC tags, and 31,837 mature DC tags derived from six donors. The expression frequency of mRNAs for the lectins was normalized and quantified as shown in Table I. Among the lectins analyzed, two were found to be specifically expressed in immature DCs, namely, the MMR and the macrophage lectin specific for galactose/N-acetylgalactosamine (hMGL). Five other lectins, namely, CLEC6F (DCIR), CLEC6F12 (dectin-1), DC-SIGN, CD23, and CD94, were also preferentially expressed on immature DCs, but they were also expressed in other cells of the same lineage, albeit to a less extent.

MGL Protein Is Expressed on Immature DC Surfaces—Although MMR and MGL are both C-type lectins, their molecular architectures are distinct. MMR has been well characterized as
being an endocytic receptor on DCs (29, 30) whereas the expression profiles and functions of MGL were unknown. The SAGE analysis clearly showed that MGL expression occurs exclusively in immature DCs. However, as the analyzed mRNA specimens were derived from at least six volunteers, it was not clear whether all humans express MGL in their immature DCs. It is possible that the positive MGL tags could have been derived from only one donor because of genetic polymorphism or a subclinical disease. To confirm that MGLs are expressed in immature DCs in at least the majority of human individuals, FIG. 3.

Fig. 3. Genetic structure of the hMGL gene locus. Positions of the PCR primers used for RT-PCR (Fig. 1) and those used for subcloning and sequencing the product (Fig. 2) are shown by arrows with dotted and solid lines, respectively. Nucleotides that are deleted in some of the alternatively spliced variants are boxed. Single nucleotide polymorphisms and the resulting amino acid heterogeneity at residue 35 are also indicated.
we performed RT-PCR and flow cytometric analyses on the various APC populations from three individuals. The RT-PCR analysis showed that all three individuals had MGL mRNA expression in their immature DCs and that this expression was lacking in mature DCs and monocytes. Interestingly, however, the PCR-amplified products were heterogeneous in size. All five individuals showed a major band at the position of 530 bp and a minor band at the position of 610 bp. In addition, immature DCs derived from six donors examined by flow cytometric analysis using an anti-hMGL mAb all expressed MGL protein on their cell surface. Thus, hMGL is expressed at the mRNA and protein levels in immature DCs from all subjects examined (Fig. 1).

Heterogeneity of hMGL Subtypes due to Alternative Splicing—The diversity of the PCR-amplified products suggested that at least two different subtypes of hMGL are expressed in the immature DC preparation. To identify these hMGL subtypes, we recovered the PCR-amplified products, subcloned them into the pGEM-T-easy vector, and determined their nucleotide sequences. We obtained nucleotide sequences encoding seven different hMGL subtypes that result from various deletions at three potential deletion sites, namely, either a 81-bp deletion in the neck domain, a 9-bp deletion at the beginning of the CRD, or a 12-bp deletion inside the CRD. As discussed further below, the human genome has only one hMGL, and thus the heterogeneity of the PCR-amplified products reflects alternatively spliced mRNAs that encode hMGL. The nucleotide structures of the seven variants are indicated in Fig. 2B. This shows that three of the variants, namely, hMGL(8A), -(6A/8A), and -(6C/8A), apparently differ from the others in their CRDs in that four amino acids are missing. The frequencies of each of the seven hMGL mRNA species in the immature DCs from five individuals was determined by sequencing a number of PCR-amplified products ligated into the pGEM-T-easy vector. This revealed that each variant occurred at an equivalent frequency in the five donors and that the hMGL variant 6C is particularly frequently expressed (Table II).

Genomic Structure of hMGL Gene and Single Nucleotide Polymorphism—The hMGL gene locus has been identified to be 17p13 on the human genome. There is only one hMGL gene. Alignment of the nucleotide sequences of the seven hMGL mRNA subtypes including the originally determined subtype (9) demonstrates the whole hMGL gene structure and the various splicing patterns of hMGL mRNA (Fig. 2A). The exon numbers were assigned based on the exon numbering of the rat hepatic lectin-1 gene (31). The gene for hMGL is composed of 9 exons and 8 introns. The nucleotide sequences at the 5′ donor and 3′ acceptor sites of all introns conform to the GT-AG rule (Table III). In addition, a single nucleotide polymorphism was found in exon 3 (CGC or TGC, which give rise to Cys and Arg, respectively) at a site that corresponds to amino acid residue 35 in the cytoplasmic region proximal to the transmembrane domain.

hMGL Is a Functional Endocytic Receptor—Because recombinant hMGL binds to carbohydrate ligands (9), it has been assumed that the physiological functions of hMGL are to act as an endocytic receptor, to recognize other cells in the immune system (similar to DC-SIGN (32)), or to participate in cell trafficking. To assess the first possibility, we tested the ability of immature DCs to bind soluble FITC-labeled polyacrylamide polymers that contain multiple α-GalNac residues. When they were incubated at 37 °C, immature DCs bound and incorporated the α-GalNac-soluble polyacrylamide polymers in a time-dependent manner (Fig. 4). The uptake continued for at least 120 min and could be inhibited by the addition of mAb MLD-1, an anti-hMGL mAb that blocks hMGL-carbohydrate interactions. Confocal microscopy of similarly treated DCs revealed that the increase in fluorescence intensity of the DCs over time is indeed due to the internalization of the α-GalNac polymers (Fig. 4). The fluoresceinated ligand was also shown to be concentrated largely in intracellular granular structures rather than dispersed throughout the cell.

DISCUSSION

Immature DCs are thought to express a variety of recognition molecules that are involved in the uptake of exogenous antigens. Among these are endogenous lectins that bind specific oligosaccharides and thus can bind to glycans. To determine the lectins that are specifically expressed on immature DCs but not on other APCs, we performed SAGE analysis. We found that only immature DCs express MMR and hMGL out of the five leukocyte cell fractions tested. That immature DCs are the only cells to express MMR is consistent with two previous reports (29, 30). That the immature DCs from a number of different donors specifically express hMGL mRNA and proteins was confirmed by RT-PCR and flow cytometric analysis. The immature DCs were also found to take up soluble polyacrylamide polymers that contain multiple α-GalNac residues, and application of an anti-hMGL antibody showed that this process was dependent on hMGL. Thus, we conclude that hMGL is an immature DC-specific lectin that is involved in receptor-mediated endocytosis of glycosylated proteins. Supporting this notion is a histochemical study of healthy human skin, which showed that approximately half of the dermal CD1c+ DCs express hMGL (33). This indicates that hMGL+ DCs are present in vivo and that our in vitro manipulations in preparing the DCs have not resulted in abnormal hMGL expression. Murine MGL has recently been characterized as a marker of bone...
Lectin Expressions on Dendritic Cells

It has been proposed that a number of other C-type lectins, including DEC-205, DCIR, Langerin, DC-SIGN, dectin-1, and dectin-2, are expressed predominantly on particular DC subpopulations (32, 34–40). Our SAGE analysis showed that some of these lectins are indeed expressed on either immature (DCIR, DC-SIGN, dectin-1, CD23) or mature (DEC-205) DCs. These lectins are not, however, specific for immature DC as they are also expressed at low levels in monocytes, M-CSF-, or GM-CSF-induced MØs, or mature DCs, but their expression is nonetheless highest in immature DCs. Expression of Langerin was not detected by the SAGE analysis, probably because of its low expression level. That MMR, hMGL, and the other DC-preferential lectins are specifically or predominantly expressed in one or the other DC subpopulations suggests that these molecules may be useful markers that distinguish DC subpopulations, especially those of myeloid origin.

The distinct patterns of lectin expression by the various cell types tested in our SAGE analysis may relate to the changes in functional ability that have been reported for MØs and DCs that are at distinct stages of differentiation. When immature DCs differentiate from monocytes, their ability to take up antigen and to migrate becomes enhanced. When immature DCs mature, however, they lose their capacity for antigen uptake and instead become superbly capable of interacting with naive T cells. Like MMR, hMGL has a tyrosine motif in its cytoplasmic region and is involved in receptor-mediated endocytosis of glycosylated antigens (Fig. 4). Thus, the fact that these two lectins are up-regulated at the immature DC stage but down-regulated at the mature stage suggests they may be partly responsible for the known enhanced antigen uptake of immature DCs. Variations in the expression of other lectins in other monocyte differentiation pathways may also contribute to altered functional capacities. We found that L-selectin is expressed on monocytes but that this expression was down-modulated after differentiation into MØs and DCs, as has been previously reported (41, 42). L-selectin is involved in the extravasation of immune cells into local inflammatory sites and contributes to the distribution of immature DCs in peripheral organs. Thus, the loss of L-selectin after differentiation results in the inability of the cell to migrate through vessel walls.

We found that hMGL in immature DCs is present as seven different subtypes. These subtypes result from alternative splicing because the mRNA encoding each subtype is edited in accordance with the GT-AG rule (Table III). That there is only one hMGL gene in the human genome further supports the notion of such alternative splicing. We wished to determine the expression frequency of each hMGL subtype by sequencing a number of PCR-amplified products from immature DCs cultured from five separate donors. To avoid the possibility that the size of the amplified products affects the amplification efficiency and subsequent ligation, we designed new PCR primers that amplify longer (973–1075 bp) products (Fig. 3, Table II). It appears that hMGL(8C) is the major hMGL subtype. Our next objective is to determine whether the hMGL subtypes are variously expressed on different DC subpopulations.

All of the identified hMGL subtypes are potential endocytic receptors because they contain the intracellular tyrosine motif (YENP) that is required for interaction with clathrin-coated vesicles (43). It is interesting that the hMGL splicing variants showed structural microdiversity in both the neck domain and the CRD. Insertion in the neck domain would affect oligomer formation and sensitivity to enzyme digestion. The neck domain consists of heptad repeats that contain hydrophobic amino acid residues at discrete positions. Such a structure is involved in the folding of an α-helical coiled-coil and subsequent oligomer formation, as has been shown for several C-type lectins, including the mannoside-binding protein, the asialoglycoprotein receptors, CD23, and DC-SIGN (44–46). In our previous paper we showed that the neck domain was required for the trimer formation of hMGL because recombinant hMGL with the whole neck domain forms homotrimers, whereas the CRD alone does not (11). With regard to the variation in the CRD between the hMGL subtypes, three subtypes, namely, hMGL(8A), (6A/8A), and (6C/8A), had a deletion of 12-bp nucleotides in the CRD. This may serve to alter the specificity of carbohydrate recognition. Alignment of the hMGL amino acid sequence with that of the mannoside-binding protein indicates that the deletion occurs at the end of helix α2, which is the most variable element in the secondary structure of the known C-type lectin-like folds (47, 48). Since such altered conformation could possibly modify the carbohydrate recognition site, it would be interesting to know whether the alternative splicing in hMGL indeed facilitates diverse carbohydrate recognition.

We also found another microheterogeneity in the sequence of hMGL in that there is a single amino acid substitution (Arg23 or Cys235) at the proximal portion of the cytoplasmic region due to the polymorphism of a single nucleotide. A number of C-type lectins possess a cysteine residue at this position. In the asialoglycoprotein receptor, the cysteine residue is modified with palmitate (49), which would enable the cytoplasmic domain to attach closely to the plasma membrane. The deacylation of this residue was reported to inactivate the receptor and decrease its ligand binding capacity, possibly because the spatial arrangement of the subunits had been altered (50). The microheterogeneity we observed in hMGL might also alter the carbohydrate binding capacity of this lectin. It is known that the different subtypes of human hepatic asialoglycoproteins that result from alternative splicing also differ in their intracellular trafficking, stability, and phosphorylation (51). It is thus quite possible that the various hMGL subtypes that we have identified may differ similarly.

We showed that immature DCs could take up a FITC-labeled GalNAc-conjugated carbohydrate ligand. The uptake was slow, continuing steadily for 120 min, and could be partially but significantly inhibited by the addition of a blocking antibody against hMGL. Confocal microscopy showed that the fluoresceinated ligand was not dispersed within the cell but instead was largely concentrated in intracellular granular structures (Fig. 4). Thus, hMGL probably functions as an endocytic receptor on immature DCs. That the anti-hMGL antibody only partially inhibits uptake suggests that other mechanisms such as pinocytosis might also be involved in the ligand uptake.

Among the lectins that are expressed specifically or preferentially on DCs, hMGL is unique in its recognition of carbohydrates that bear terminal Gal/GalNAc residues. In particular, it can recognize clusters of truncated O-linked carbohydrate chains known as the T and Tn antigens that are well known carcinoma-associated epitopes. With regard to the other lectins expressed on DCs, the carbohydrate-binding capacities of only a few have been characterized. MMR, DC-SIGN, and Langerin have been shown to be specific for mannose (29, 30, 32, 37). Thus, one strategy for specifically targeting immature DCs with vaccine antigens may be to employ hMGL-mediated antigen uptake by modifying the candidate antigens with Gal/GalNAc residues. If such a strategy would target immunogenic DCs but not tolerogenic DCs, this would be a major advance in vaccinology.

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2 Denda-Nagai, K., Kubota, N., Kamata, M., Tsuiji, and Irimura, T. (2002) Glycobiology, in press.
The recognition and capture of malignant cells may be another physiological function of hMGL. In previous studies, we showed that murine MGL^+ cells recognize and capture malignant cells through the MGL-carbohydrate interaction (12, 13). These cells also accumulate selectively in tumor-bearing sites, as do MGL transfectants (14, 15). This accumulation can be inhibited in part by the administration of an anti-murine MGL mAb that blocks the MGL-carbohydrate interaction (16). Considering the dual functions of hMGL in antigen recognition and endocytosis, we hypothesize that MGL^− immature DCs could be involved in the generation of anti-tumor immunity and the clearing of apoptotic cells because of their capacity to accumulate in tumor-bearing sites and their ability to incorporate malignant cells or apoptotic cells with exposed Gal or GalNAc residues resulting from altered glycosylation. It is also important to know whether and how the hMGL-dependent carbohydrate recognition system contributes to the trafficking of cells in the body.

In conclusion, we have found that hMGL is a lectin that is exclusively expressed on immature DCs. We have also shown that hMGL acts in carbohydrate binding and as an endocytic receptor. We postulate that targeting hMGL by linking antigens with Gal/GalNAc residues might selectively focus antigen delivery to immature DCs and thus modulate the immunogenicity of the antigens.

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