The CD15 carbohydrate epitope is expressed in mature human neutrophils, monocytes, and promyelocytes. We aimed to determine the α1,3-fucosyltransferase responsible for the expression of CD15 in each subpopulation of leukocytes. Three α1,3-fucosyltransferases, FUT4, FUT7, and FUT9, are expressed in human leukocytes. We demonstrated that FUT9 exhibits 20-fold stronger activity for CD15 synthesis than FUT4, whereas FUT4 exhibits 4.5-fold stronger activity for CD65 synthesis than FUT9. By competitive reverse transcriptase-polymerase chain reaction, FUT9 was found to be strongly expressed in mature granulocytes and peripheral blood mononuclear cell, but not in monocytes. CD34<sup>+</sup> and CD15<sup>+</sup> cells in cord blood and myeloid cell lines (HL-60 and U937) did not express FUT9 at all. FUT4 transcripts were ubiquitously expressed in all blood cells and all cultured cell lines, with HL-60 and U937 cells in particular expressing a number of FUT4 transcripts. Transfection of the FUT9 gene into Jurkat and U937 cells demonstrated that FUT9 has the potential to express CD15 in myeloid and lymphoid cells. These findings suggest that the expression of CD15 in mature granulocytes is directed by FUT9, whereas it is determined in promyelocytes and monocytes by FUT4. Measurement of CD15 synthesizing activity in cell homogenates of each cell population using the polylactosamine acceptor further supported these conclusions.

There are three CD<sup>1</sup> markers of human leukocytes comprising fucosylated carbohydrate epitopes. As listed in Fig. 1 below, the distal lactosamine unit (LN; type 2 chain), Galβ1,4GlcNac, of the polylactosamine chain is fucosylated through α1,3-fucosyltransferase (α1,3FUT) activity to form the CD15 (Le<sup>x</sup>; Le<sup>x</sup>) epitope (1, 2). The CD15s (sialylated CD15; sialyl Le<sup>x</sup>; sLe<sup>x</sup>) and CDw65 (VIM-2) epitopes are also fucosylated structures related to CD15, i.e. CD15<sup>s</sup> is formed by α2,3-sialylation prior to the fucosylation of the distal LN unit of polylactosamine by α1,3FUT, and CDw65 is formed by fucosylation of the inner LN unit of α2,3-sialylated polylactosamine by α1,3FUT (2, 3).

The CD15 epitope is expressed in some tissues, such as epithelial cells of intestinal tissues (4–6), certain neurons and glial cells in the central nervous system (7, 8). In human leukocytes, CD15 is expressed preferentially in monocytes, mature neutrophils, and all myeloid cells from the promyelocyte stage onwards, making it a useful cell surface marker (9–11). CD15 is considered to be involved in neutrophil functions, that is, cell-cell interactions, phagocytosis, stimulation of degranulation, and respiratory burst, although the function of CD15 is not clear (12–16).

Six human α1,3FUT genes have been cloned to date, which are FUT3 (Fuc-TIII), FUT4 (Fuc-TIV), FUT5 (Fuc-TV), FUT6 (Fuc-TV), FUT7 (Fuc-TVI), and FUT9 (Fuc-TIX) (1, 17–23). FUT9, a new member of the human α1,3FUT family, which we have recently cloned, is expressed in human leukocytes, glanular compartments of the stomach, and forebrain (23). The FUT9 gene was mapped on chromosome 6q16 (24). Interestingly, only FUT9 has a highly conserved amino acid sequence between human and mouse, the level of conservation being equal to that of α-actin (25). Five human α1,3FUTs (FUT3, 4, 5, 6, and 7) share highly homologous sequences, whereas FUT9 has a different sequence altogether (23). This indicated that the substrate specificity of FUT9 is unique among the α1,3FUTs. In fact, we demonstrated in a previous study that FUT9 preferentially transfers a fucose to the GlcNAc residue at the distal LN unit of the polylactosamine chain, resulting in the Le<sup>x</sup> (CD15) structure, whereas the other α1,3FUTs preferentially transfer a fucose to the GlcNAc residue at the inner LN unit of the polylactosamine chain (25). This implied that FUT9 exhibits stronger activity than the other α1,3FUTs for forming the CD15 epitope that is recognized by anti-Le<sup>x</sup> (anti-CD15) antibodies.

It has been reported that FUT4 and FUT7 are expressed in human leukocytes, but FUT3, 5 and 6 are not (23, 26, 27). The carbohydrates modified by both FUT4 and FUT7 can function
**TABLE I**

| Target gene | Forward and reverse primers | Sizes of PCR products (bp) | Restriction enzymes of competitor DNA | Annealing temperature (°C) |
|-------------|-----------------------------|---------------------------|--------------------------------------|--------------------------|
| FUT4        | 5'-GAGAGGTCAAGCGCTTTTT3' | 516                       | SmaI-StuI                            | 65                       |
|             | 5'-GCCAGGCAATTTCGGAGGC-3' | 345                       |                                       |                          |
| FUT7        | 5'-CACTCAGCCCATCTCACTG-3' | 497                       | ApaI-BstXI                           | 65                       |
|             | 5'-GGTGTTACTGCTTCACTTATG-3' | 336                       |                                       |                          |
| FUT9        | 5'-GGCAAGCTTTTGAATGTCGAA-3' | 398                       | NheI-NsiI                            | 65                       |
|             | 5'-ACCCAAGCTTTTATTTCTTGAGG-3' | 285                       |                                       |                          |
| β-Actin     | 5'-GATATCCGCGCGCTGTCGAC-3' | 789                       | Eco0109I-BstEII                      | 60                       |
|             | 5'-CAGGAAGGAAACCCTGAAAGAC-3' | 639                       |                                       |                          |

* The reaction mixture contained 5% dimethyl sulfoxide.

as ligands for E-selectin and P-selectin (28, 29). FUT4, named a myeloid-type α1,3FUT, is ubiquitously expressed in a variety of tissues and not only in leukocytes (23). In a previous study (23), we could not find any human tissues in which FUT4 is absent. However, a number of the tissues do not necessarily express the CD15 antigen. Overexpression of the FUT4 gene by its transfection resulted in the expression of CD15 on the cell surface (1, 22, 23, 30, 31), whereas FUT7 transfection resulted in the expression of CD15s but not CD15 (20, 21, 31). Therefore, FUT4 had been believed to be the enzyme solely responsible for the expression of CD15 in leukocytes before the finding of FUT9 in those cells. It has been investigated whether the expression of FUT4 and FUT7 correlated with the expression of CD15 and CD15s epitopes during hematopoiesis. Clarke et al. (26) reported that FUT7 definitely determined the expression of the CD15s epitope, consistent with other studies (22–24), however, the expression of CD15 does not correlate well with the expression of FUT4, which indicated that an unknown α1,3FUT is involved in the expression of CD15 in leukocytes. Marer et al. (27) also reported a discrepancy between the expression of FUT4 and that of CD15, in which the level of FUT4 expression does not necessarily correlate with the level of CD15 expression during myeloid cell maturation.

Previously (23), we cloned a human cDNA encoding FUT9, and examined the tissue distribution of the six α1,3FUTs. We found that FUT9 is expressed in peripheral blood leukocytes. This indicated that FUT9 might be the enzyme responsible for determining the expression of CD15 in leukocytes. In this study, we examined which α1,3FUT is responsible for the expression of CD15 in conjunction with the expression of CDw65, in human leukocytes and demonstrated that the expression of CD15 is determined differentially either by FUT9 or FUT4, depending on the subpopulation of leukocytes.

**EXPERIMENTAL PROCEDURES**

**Antibodies**—The monoclonal antibodies (mAbs) anti-human CD4 (PRA-T4), CD8 (PRA-T8), CD14 (clone M5E2), CD19 (HB19), and CD56 (B-159) were obtained from Becton Dickinson Immunocytometry Systems (San Jose, CA). The mAbs anti-human CD34 (BRMA-K3), CD15 (50B5), and CDw65 (VIM-2) were obtained from DAKO, Beckman Coulter, and Serotec, respectively.

**Cell Preparation**—Peripheral blood mononuclear cells (PBMCs) and polymorphonuclear cells obtained from normal donors were isolated from heparinized venous blood by density-gradient sedimentation over Lymphoprep and Polythymoprep (Nycomed Pharma, AS, Oslo, Norway), respectively. Cord blood samples obtained with permission from the umbilical vein at normal delivery were aspirated in heparinized plastic syringes. Mononuclear cells were also separated by density centrifugation over Lymphoprep (Nycomed Pharma, AS). The cells magnetically labeled with CD4, CD8, CD19, or CD34 Dynabeads (Dynal, Oslo, Norway) were isolated from mononuclear cell preparations and detached by DETACHaBEAD CD4/CD8, CD19, or CD34 in accordance with the manufacturer’s protocol. The cells magnetically labeled with CD14, CD15, or CD56 MicroBeads (Miltenyi Biotec, Auburn, CA) were selectively bound to an MS+ column using the MiniMACS and eluted from the column in accordance with the manufacturer’s instructions.

**Cell Culture and Transfection**—Human leukemic cell lines, HL-60, U937, Jurkat and Namalwa, were cultured in RPMI 1640 (Life Technologies) supplemented with 10% heat-inactivated fetal calf serum, 2 mCi glucose, 100 units/ml penicillin, and 100 μg/ml streptomycin. Jurkat, U937, and Namalwa cells were transfected with pAMo vectors containing FUT9 coding sequences by electroporation using a GenePulser (Bio-Rad) and selected in the presence of G418 (1 mg/ml) (Life Technologies) for 2–4 weeks to obtain stable transfectants. Limited dilution cloning was performed to stabilize and enhance the prevalence of transfectants with the desired phenotype that was examined by flow cytometric analysis using anti-CD15 antibodies, as described previously (22). Namalwa cells were also transfected with pAMo vectors containing each of the FUT4 and the FUT7 coding sequence and cloned by limited dilution. We selected one clone from each group, which expressed transcripts at almost an equal level, and used it for the following experiments (see Fig. 2D).

**Purification of FUT Proteins Fused with FLAG Peptide**—The putative catalytic domain of each of FUT4, FUT7, and FUT9 was expressed as a secreted protein fused with FLAG peptide in Namalwa cells. pAMoF2 is an expression vector derived from pAMo and contains a fragment encoding signal peptide of human immunoglobulin κ (MH-FQVQIFLLASVIMSREG) and FLAG peptide (DYKDDDDK). Namalwa cells were transfected with pAMoF2 vector containing each FUT coding sequence by electroporation and selected with G418 as described above. The recombinant enzyme secreted in culture medium was purified on anti-FLAG (M1) and anti-FLAG (F7) affinity columns (Sigma Chemical Co.) in accordance with the manufacturer’s protocol.

**Competitive RT-PCR Assay**—Competitive RT-PCR assay was performed to determine the amount of transcripts of FUT4, FUT7, and FUT9 and β-actin in lymphocytes as previously described (22, 23). In brief, total RNAs were isolated from the cells using ISOGEN (Nippon Gene, Tokyo, Japan) as recommended by the manufacturer. cDNAs were synthesized with oligo(dT) primers from RNAs in a total volume of 20 μl of reaction mixture using SuperScript II reverse transcriptase in accordance with the superscript premammalianization system protocol (Life Technologies). Competitive RT-PCR was performed using Ampli Taq Gold with GeneAmp (PerkinElmer Life Sciences) in a total volume of 50 μl of reaction buffer containing 10 μl of standard plasmid DNA or sample cDNA, 10 μl of competitor DNA at optimal concentration, and 0.2 μl of each primer of the gene-specific primer sets (Table I). After competitive RT-PCR, a 10-μl aliquot was electrophoresed in 1% agarose gel, and the bands were visualized by ethidium bromide staining. The intensities of the amplified fragments were quantified by scanning positive pictures. Measurement of the β-actin transcripts in each sample was performed using the same competitive RT-PCR method. The values for the transcripts were plotted on the respective standard curves to obtain the actual amount of each transcript. The actual amount of transcript of each glycosyltransferase (fg/μl) was divided by that of β-actin (pg/μl) for normalization.

**Western Blot Assay**—We also determined the amount of CD15 antigen in each subpopulation of peripheral blood cells from healthy individuals by Western blot analysis, as described previously (35). Briefly, cell pellets were solubilized in a 20 mM HEPES buffer (pH 7.2) containing 2% Triton X-100 by brief sonication. Proteins (20 μg) separated by...
NeuAc lactosamines (LN) for the enzyme activity assays (see Fig. 3). We measured the amount of each FUT-FLAG by Western blot with a fluorescence spectrophotometer (JASCO FP-920; Nihon Bunkoh, Tokyo, Japan). We measured the amount of each FUT-FLAG by Western blot detection agents (Amersham Pharmacia Biotech) as recommended by the manufacturer.

**RESULTS**

**FUT9 in Granulocyte**

7.5% SDS-polyacrylamide gel electrophoresis were transferred to an Immobilon polyvinylidene difluoride membrane (Millipore, Bedford, MA) using a Transblot SD cell (Bio-Rad, Richmond, CA). The membrane was blocked with phosphate-buffered saline containing 5% skim milk at 4°C overnight and then incubated with anti-CD15 antibody. The membrane was stained with the ECL Western blot detection agents (Amersham Pharmacia Biotech) as recommended by the manufacturer.

**Fig. 1.** Terminal carbohydrate structures of the LN unit and three CD markers (CD15, CD15s, and CDw65).

**Fig. 2. A,** flow cytometric analysis of Namalwa-FUT9, Namalwa-FUT4, and Namalwa-FUT7 cells with anti-CD15 and anti-CDw65 mAbs. **B,** profiles of α1,3FUT activity toward 3LN-2AB of each of the Namalwa transfectants. **C,** profiles of α1,3FUT activity toward S3LN-2AB of each of the Namalwa transfectants. **D,** relative amounts of transcripts of FUT4, FUT7, and FUT9. The transcripts in each Namalwa transfectant were measured by competitive RT-PCR. The actual amount of each glycosyltransferase (fg/μl) was divided by that of β-actin (pg/μl) for normalization.

substrates (Fig. 2B). The area of P1, P2, and P3 indicates the synthesizing activity of each product. The carbohydrate structure of the P1 peak corresponds to CD15, as described under “Experimental Procedures.” As seen in Fig. 2B, the cell homogenate of Namalwa-FUT9 exhibited about 20-fold larger P1 peak than that of Namalwa-FUT4. FUT9 was found to have about 20 times stronger activity for CD15 synthesis than FUT4. Both enzymes exhibit almost the same level of activity for the fucosylation of the inner LN unit of 3LN-2AB (P2). Namalwa-FUT4 showed a very small P3 peak, indicating the bifucosylation of distal and inner LN units, which is also recognized by anti-CD15 antibody. Namalwa-FUT7 showed no activity for the fucosylation of neutral oligosaccharide, S3LN-2AB.

FUT4 and FUT9 also exhibited positive activity for synthesizing the CDw65 (VIM-2) structure by a fucose transfer to the internal LN unit of a 2,3-sialylated polylactosamine, S3LN-2AB (P2s in Fig. 2C). The cell homogenate of Namalwa-FUT4 exhibited about a 4.5-fold larger area of peak than that of Namalwa-FUT9, demonstrating that FUT4 had about 4.5 times stronger activity for the CDw65 synthesis than FUT9. The cell homogenate of Namalwa-FUT7 exhibited only a small, but apparently positive peak (P1s) that is different from the P2s peak (Fig. 2C). The P1s peak area indicated synthesizing activity for CD15s, as described under “Experimental Procedures.”

Then we analyzed the relative activities of each FUT for
3LN-2AB and S3LN-2AB using the same amount of each FUT-FLAG protein (Fig. 3C). The 20 times stronger activity for the CD15 synthesis of FUT9-FLAG than that of FUT4-FLAG was again confirmed (P1 peaks in Fig. 3A). Both FUT4-FLAG and FUT9-FLAG again showed positive activity for the CDw65 synthesis. FUT4-FLAG exhibited about a 2.5-stronger activity for the CDw65 synthesis than FUT9-FLAG (P2s in Fig. 3B). FUT7-FLAG exhibited positive activity for synthesizing the CD15s structure (P1s in Fig. 3B) and also the CDw65 structure (P2s in Fig. 3B). FUT7-FLAG exhibited about a 20-fold larger area of P1s than P2s.

**Differential Expression of FUT9 in CD15^+ Cells in Peripheral and Cord Blood Cells**—We evaluated the transcript levels of three α1,3FUTs, FUT4, FUT7, and FUT9, in CD15^+ cells in peripheral and cord blood cells. CD14^- monocytes and mature granulocytes in peripheral blood cells showed dull staining and bright staining with anti-CD15 mAb, respectively (Fig. 4A). Interestingly, monocytes did not express FUT9 at all, whereas granulocytes expressed substantial amounts of FUT9 (Fig. 4B). The difference of the CD15 staining intensity between monocytes and granulocytes could be explained by the strong activity of FUT9 for the CD15 synthesis. Both cells were brightly stained with CDw65, probably directed by FUT4 activity (Fig. 4A). Two populations, CD15^- and CD15^+, were sorted from cord blood mononuclear cells. Neither expressed detectable amounts of FUT9 (Fig. 5A). Thus, the expression of CD15 in immature promyelocytes in cord blood is not directed by FUT9, and probably directed by FUT4.

As seen in Fig. 4A, HL-60 was brightly stained with anti-CD15 mAb. U937 and Jurkat cells were stained to a lesser degree with anti-CD15 mAb. FUT9 could not be detected in HL-60, U937, or Jurkat cells, at all, whereas FUT4 and FUT7 were clearly detected (Fig. 4B). HL-60 cells expressed enormous amounts of FUT4 transcripts (18.6). The CD15 intensity in these cell lines correlated with the amount of FUT4 transcripts. All these cell lines expressed substantial amounts of
the CD65 epitope. This indicated that the expression of CD15 and CD65 in these cell lines is directed by FUT4.

The expression of CD15 epitope on cell surfaces induced by FUT9 in myeloid and lymphoid cell lines—Stable FUT9 transfectants were established in Jurkat and U937 cells, and named Jurkat-FUT9 and U937-FUT9, respectively. Both transfectants showed increases in the expression of CD15 on flow cytometric analysis, but different results in the expression of CD65 (Fig. 4A). U937-FUT9 exhibited an increase in CD65 expression in correlation with the increase of CD15, but Jurkat-FUT9 showed a prominent decrease in the expression of CD65. The transfection of the FUT9 gene to U937 and Jurkat cells demonstrated that FUT9 could potentially express CD15 epitope on their surfaces in myeloid and lymphoid cells.

HL-60 exhibited a pattern of 1,3FUT activity directed by FUT4, whereas Jurkat-FUT9 cells exhibited a FUT9-specific pattern—Upon measurement of 1,3FUT activity in the cell homogenates using 3LN-AB as an acceptor, HL-60 exhibited a typical pattern of 1,3FUT activity directed by FUT4. As seen in Fig. 4, the enormous amounts of FUT4 transcript (18.6) in HL-60 cells gave a very large P2 peak, leading to the bright expression of CD65, and a relatively small, but substantial P1 peak that is enough for the bright expression of CD15. Jurkat cells also exhibited a FUT4-specific pattern of 1,3FUT activity, in which the P1 and P2 peak areas were well correlated with the amount of FUT4 transcripts (Fig. 4). The relatively low amount of FUT4 in Jurkat cells, in comparison to that in HL-60 cells, would explain the very weak expression of CD15 and CD65 in Jurkat cells. The profile of 1,3FUT activity in Jurkat-FUT9 cells was almost converted to the FUT9-specific pattern. The large P1 peak in Jurkat-FUT9 cells gave rise to the bright expression of CD15, and interestingly, decreased the expression of CD65 (Fig. 4).

Differential expression of FUT9 in each subpopulation of PBMC—Each subpopulation of peripheral blood mononuclear cells (PBMC) was isolated from peripheral blood by immunomagnetic labeling. Flow cytometric analysis revealed that monocytes (CD141) showed dull staining with anti-CD15 mAb, and CD561 cells slightly expressed CD15, but CD41 T cells, CD81 T cells, and B cells (CD191) did not express it (data not shown). We again found the ubiquitous expression of FUT4 and FUT7 in all subpopulations of PBMC, whereas FUT9 was expressed in subpopulations of PBMC except monocytes (Fig. 5A). The average levels of relative transcripts of FUT4 and FUT7 were not so different from each other. FUT4 transcript was in the range of 0.5 to 1.7, and FUT7 transcript was in the range of 0.8 to 2.5. CD41 and CD81 T cells showed the maximal and minimal amounts of FUT7 transcripts, respectively, among the subpopulations examined. B cells (CD191), monocytes (CD141), and natural killer cells (CD561) expressed FUT7 at moderate levels. In contrast to the FUT4 and FUT7 expression, the average level of transcript of FUT9 in each subpopulation was different and ranged 0.02 to 0.3 (Fig. 5A). Monocytes did not express FUT9, although they expressed CD15 on their surface. On the other hand, CD81 T cells, B cells, and CD561 cells, which showed negative or faint staining with CD15 by flow cytometry, expressed FUT9 in an amount similar to that of FUT4 and FUT7 in each subpopulation (Fig. 5A).

CD15 Antigen Is Present Intracellularly in All Subpopulations of Peripheral Blood Cells—We also determined the expression of the CD15 antigen in subpopulations of PBMC by Western blot analysis (Fig. 5B). An apparent molecular mass of about 200 kDa was detected by anti-CD15 mAb in all subpopulations, including CD41 T cells, CD81 T cells, B cells (CD191), monocytes (CD141), NK cells (CD561), and mature granulocytes. Although CD41 T cells and B cells (CD191) showed two additional bands, they were not CD151 glycoprotein, but mouse IgM derived from magnetic beads. Thus, the subpopulations, which do not express CD15 antigen on the cell surface, apparently possess CD15 antigen intracellularly.

DISCUSSION

The CD15 antigen is expressed on mature granulocytes and on myeloid cells from the promyelocyte stage onwards. Anti-CD15 antibody reacts with promyelocytes, and less strongly with myelocytes and metamyelocytes, but not bone marrow myeloblasts, as detected by flow cytometric analysis and an immunohistochemical method (10, 11, 36). CD15 epitopes are carried as terminal sequences on the oligosaccharide chains in both glycoproteins and glycolipids on mature granulocytes (37). Some anti-CD15 antibodies also have a high immunoreactivity for normal peripheral blood monocytes (10), but the majority of T and B lymphocytes do not express CD15 antigens (36).

The preferential activity of FUT9 to transfer fucose to the distal GlcNAc residue suggested that FUT9 synthesizes the CD15 epitope more efficiently in vivo than FUT4. The relative initial rate of transfer to the distal LN unit of neutral polylactosamine, 3LN-2AB, was ~20 times higher for FUT9 than FUT4. On the other hand, FUT4 preferentially fucosylates the inner LN unit of polylactosamine on both the neutral and α2,3-sialylated polylactosamine chains, so that FUT4 can efficiently synthesize CD65 epitope (2, 25). In the present study, we demonstrated that FUT9 can also transfer a fucose to the inner LN unit of the sialylated polylactosamine chain with a relative activity one fifth that of FUT4, resulting in the synthesis of CD65 epitope (Fig. 2). In fact, the FUT9-transfected cells, Namalwa-FUT9 and U937-FUT9 cells, showed increased CD65 expression. This confirmed the FUT9 activity to transfer fucose to the inner LN unit of the sialylated polylactosamine chain.

Human mature granulocytes strongly express CD15 and CD65 on their surface (10, 11, 38). Lactosaminoglycan on glycoprotein is a major carrier for the CD15 epitope in human granulocytes, because the CD15 epitope is three times more abundant in glycoproteins than in glycolipids (37, 39). A typical feature of granulocyte lactosaminoglycan was a multiple fucosylated structure on LN units. Also, some of the side chains contain two or more fucose residues. However, the majority of fucose-containing neutral oligosaccharides possess a Galβ1–4Fucα1–3GlcNAc terminal structure (37). This is consistent with the finding in the present study that mature granulocytes express substantial amounts of FUT9, which preferentially fucosylates the GlcNAc residue at the distal LN unit (25). The repertoire of a1,3FUTs expressed in human mature granulocytes is distinct from that in promyelocytes, that is, a substantial amount of FUT9 transcript was detected in mature granulocytes, but not in promyelocytes. FUT4 can also be detected in mature granulocytes and promyelocytes, although its activity for the synthesis of CD15 is known to be minor in contrast to that for the synthesis of CD65.

The absence of FUT9 in the CD151 and CD341 cells indicated that FUT4 is mainly responsible for the CD15 expression in the immature promyelocytes in cord blood cells. The human promyelocytic leukemic cell line, HL-60, which is composed of cells arrested at the promyelocytic stage, showed strong staining with both anti-CD15 and CD65 antibodies, as described by others (26). The enormous amount of FUT4 expressed in HL-60 cells was able to give rise to bright CD15 and CD65 staining of HL-60 even in the absence of FUT9. HL-60 cells are able to differentiate into myeloid mature cells and monocytes when cultured in the presence of dimethyl sulfoxide (Me2SO) and all-trans-retinoic acid (RA), respectively (40, 41). However, we could not detect FUT9 transcripts in Me2SO-differenti-
ated and RA-differentiated HL-60 cells (data not shown). FUT9 was not involved in the CD15 expression even in the differentiated HL-60 cells. It is difficult to exclude the presence of unidentified α1,3FUT in HL-60 cells and monocytes. However, each profile of the peaks (P1, P2, and P3) of HPLC in Fig. 2 is very characteristic of each α1,3FUT, enabling one to demonstrate that the α1,3FUT activity detected in the HL-60 cell homogenates corresponded to a typical pattern of FUT4 specificity. The peak areas were large enough for the CD15 and CDw65 expression, respectively, on the cell surface. These findings are consistent with the interpretation that FUT4 is responsible for the CD15 expression in the myeloid cells at the promyelocyte stage (26).

U937 and Jurkat cells expressed less FUT4 (9.5 and 7.0, respectively) than HL-60 cells and no detectable FUT9 leading to the weaker expression of CD15 and CDw65 on the cell surface. The transfection of the FUT9 gene to U937 cells demonstrated that FUT9 can potentially synthesize the CD15 epitope in myeloid cells. In U937-FUT9 cells, the intensity of CDw65 staining increased in conjunction with the increase of CD15 reactivity. The transcript levels for FUT4 and FUT9 genes expressed in Jurkat-FUT9 cells were found to be almost the same as those in mature granulocytes isolated from peripheral blood cells. The profile of α1,3FUT activity in Jurkat cells was typical of that of FUT4, and the profile in Jurkat-FUT9 cells converted to that of FUT4 plus FUT9. The P1 peak responsible for the CD15 synthesis was demonstrated to be directed by FUT9 in the Jurkat-FUT9 cells, not by FUT4. This fact strongly suggested that the CD15 expression in mature granulocytes is directed by FUT9, not by FUT4.

The CDw65 expression in the Jurkat-FUT9 cells contrasted with that in the U937-FUT9 cells. The CDw65 intensity in the Jurkat-FUT9 cells decreased in comparison to that in the wild-type Jurkat cells, even though the FUT9 activity was increased. One explanation for this discrepancy may be that U937 cells possess enough of the precursor structure, i.e., the sialylated polylactosamine chains, to be fucosylated at the inner LN unit resulting in the CDw65 expression. Thus, FUT4 and FUT9 in the U937-FUT9 cells additively fucosylated the sialylated polylactosamine chains for the CDw65 expression. On the other hand, Jurkat cells may not possess enough of the precursor structure, because of weak activity of sialyltransferases, which is supplied for both FUT4 and FUT9 activity. In such a case, FUT9 may preferentially fucosylate the distal LN unit by overwhelming the sialyltransferase activity, resulting in the CD15 increase and CDw65 decrease.

FUT9 was detected in each subpopulation of human PBMCs, although the expression level differed among the populations. Unexpectedly, monocytes do not express FUT9 despite expressing CD15 on their surface. The flow cytometric analysis indicated that the CD15 intensity in monocytes was dull and lower than that of CDw65, consistent with the substrate specificity of FUT4. FUT4 was found to synthesize CDw65 more than CD15, because P2a is 3.5 times larger than P1 (Fig. 2, B and C). Monocytes expressed FUT4 to almost the same extent as granulocytes, however, granulocytes showed bright CD15 staining with a much higher level of CD15 expression than monocytes due to the additional expression of FUT9. CD4+ T cells, CD8+ T cells, B cells, and CD56− cells expressed FUT9, although flow cytometric analysis did not usually show CD15-positive staining in those cells. However, the transfection of the FUT9 gene to Jurkat cells demonstrated that FUT9 can potentially express the CD15 epitope on the cell surfaces of lymphoid cells (Fig. 5). Western blot analysis clearly demonstrated that all subpopulations of PBMCs can produce CD15-carrying glycoproteins intracellularly, which are not transported to be expressed on the cell surface. Considering the stronger activity of FUT9 for the synthesis of the CD15 epitope, the intracellular CD15 epitopes in PBMCs must be mainly synthesized by FUT9. The reasons why there were very few CD15 epitopes on the cell surface of PBMCs is unclear, but it is possible that either the intracellular CD15-positive glycoproteins are not translocated to the surface of resting PBMCs for some reason, or the expression level of FUT9 in each subpopulation of PBMCs is not sufficient to synthesize enough CD15-positive glycoproteins to be expressed on the cell surface. In fact, the level of FUT9 expression in PBMCs is lower than that in granulocytes and Jurkat-FUT9 cells. Among many Jurkat clones transfected with the FUT9 gene, we have selected some, which expressed FUT9 at a range of 0.5 to 1.0. These clones with a relatively low expression level of FUT9 did not express CD15 on their cell surface (data not shown). These findings indicated that peripheral lymphoid cells do not express a high enough level of FUT9 to induce the cell surface expression of CD15.

Although the function of CD15 in leukocytes is still not clear, CD15 may be involved in cell-cell interaction. The carbohydrate structure associated with CD15 on myeloid cells may be another ligand for human CD2 (16). As reported by others (14, 42, 43), the CD15-positive glycoproteins in mature granulocytes showed characteristically broad bands spanning 140–180 kDa and 95–110 kDa on Western blot analysis. Surface-labeling studies revealed that only the 165-kDa and 105-kDa CD15-reactive glycoproteins are localized on the cell surface (43, 44). They were identified to be a member of the LFA1/CR3/p150,95 (CD11/CD18) family (12, 14) and NCA160 (44). We found that bands corresponding to the molecular sizes of CD11/CD18 members and NCA160 were present not only in mature granulocytes but also in all subpopulations of PBMCs. Monocytes showed the highest density of these bands, compatible with the strong cell surface expression of CD15.

Recently, it has been reported that not only FUT7 but also FUT4 generate selectin ligands that support in vivo rolling of some leukocytes, whereas FUT7-dependent carbohydrates determine the rolling fraction for most leukocytes (28, 29). FUT9 can synthesize the CD15 epitope with very strong activity by transferring a fucose to the distal GlcNAc residue, although FUT7 can only fucosylate this precursor after siaylation by α2,3-sialyltransferases. This distinct substrate preference may lead to competition between the three α1,3FUTs if the availability of acceptor substrates is limited. This would result not only in the synthesis of CD15 but also in the interference of the generation of some carbohydrate epitope(s), such as CD15s and CDw65. The availability of FUT9 provides a tool for investigating the biological functions of the polylactosamine chain with or without fucosylation in human leukocytes under certain inflammatory conditions.

REFERENCES

1. Lowe, J. B., Kuokskaja Latallo, J. F., Nair, R. P., Larsen, R. D., Markes, R. M., Macer, B. A., Kelly, R. J., and Ernst, L. K. (1991) J. Biol. Chem. 266, 17467–17477
2. Nimmerjahn, R., Natunen, J., Majuri, M. L., Maheimo, H., Helin, J., Lowe, J. B., Renkonen, O., and Renkonen, R. (1998) J. Biol. Chem. 273, 4021–4026
3. Kono, M., Ohyama, Y., Lee, Y. C., Hamamoto, T., Kojima, N., and Tsuji, S. (1997) Glycoconjugate J. 7, 469–479
4. Hakomori, S., Nudelman, E., Levery, S. B., and Kannagi, R. (1984) J. Biol. Chem. 259, 4672–4680
5. Itkowitz, S. H., Yuan, M., Fukushima, Y., Palekar, A., Phelps, P. C., Shamsuddin, A. M., Trump, H. F., Hakomori, S., and Kim, Y. S. (1986) Cancer Res. 46, 2627–2632
6. Hakomori, S. (1992) Histochem. J. 24, 771–776
7. Streit, A., Yuen, C. T., Loreless, R. W., Lawson, A. M., Finne, J., Schmitz, B., Feizi, T., and Stern, C. D. (1996) J. Neurochem. 66, 834–844
8. Ashwell, K. W. S., and Mai, J. K. (1997) Cell Tissue Res. 289, 17–23
9. Ordel, R., Le Pendu, J., and Mollicone, R. (1980) Vox Sang. 51, 161–171
10. Hanjan, S. N., Kearney, J. F., and Cooper, M. D. (1982) Clin. Immunol. Immunopathol. 23, 172–188
11. Civin, C. I., Mirro, J., and Banquerigo, M. L. (1981) Blood 57, 842–845
12. Melnick, D. A., Nauseef, W. M., Markowitz, S. D., Gardner, J. P., and Malech,
13. Melnick, D. A., Meshulam, T., Manto, A., and Malech, H. L. (1986) *Blood* **67**, 1388–1394
14. Skubitz, K. M., and Snook, R. W. (1987) *J. Immunol.* **139**, 1631–1639
15. Forsyth, K. D., Simpson, A. C., and Levinsky, R. J. (1989) *Eur. J. Immunol.* **19**, 1331–1334
16. Warren, H. S., Altin, J. G., Waldron, J. C., Kinnear, B. F., and Parish, C. R. (1996) *J. Immunol.* **156**, 2866–2873
17. Kukowska Latallo, J. F., Larsen, R. D., Nair, R. P., and Lowe, J. B. (1990) *Genes Dev.* **4**, 1288–1303
18. Weston, B. W., Nair, R. P., Larsen, R. D., and Lowe, J. B. (1992) *J. Biol. Chem.* **267**, 4152–4160
19. Weston, B. W., Smith, P. L., Kelly, R. J., and Lowe, J. B. (1992) *J. Biol. Chem.* **267**, 24575–24584
20. Sasaki, K., Kurata, K., Funayama, K., Nagata, M., Watanabe, E., Ohta, S., Hanai, N., and Nishii, T. (1994) *J. Biol. Chem.* **269**, 14730–14737
21. Natsuka, S., Gersten, K. M., Zenita, K., Kannagi, R., and Lowe, J. B. (1994) *J. Biol. Chem.* **269**, 16789–16794
22. Kudo, T., Ikebara, Y., Toyoguchi, A., Kaneko, M., Hiraga, T., Sasaki, K., and Narimatsu, H. (1998) *J. Biol. Chem.* **273**, 26729–26738
23. Kaneko, M., Kudo, T., Iwasaki, H., Ikebara, Y., Nishihara, S., Nakagawa, S., Sasaki, K., Shina, T., Inoko, H., Saitou, N., and Narimatsu, H. (1999) *FEBS Lett.* **452**, 237–242
24. Kaneko, M., Kudo, T., Iwasaki, H., Shina, T., Inoko, H., Kozaki, T., Saitou, N., and Narimatsu, H. (1999) *Cytogenet. Cell Genet.* **86**, 329–330
25. Nishihara, S., Iwasaki, H., Kaneko, M., Tawada, A., Ito, M., and Narimatsu, H. (1999) *FEBS Lett.* **462**, 289–294
26. Clarke, J. L., and Watkins, W. M. (1996) *J. Biol. Chem.* **271**, 10317–10328
27. Marer, N. L., Clarke, J. L., Pulie, M. M., Davies, D., and Skaoel, P. O. (1997) *Glycobiology* **7**, 357–365
28. Wening, W., Ulfman, L. H., Cheng, G., Souchkova, N., Quackenbush, E. J., Lowe, J. B., and von Andrian, U. H. (2000) *Immunity* **12**, 665–676
29. Huang, M. C., Zollner, O., Mell, T., Maly, P., Thall, A. D., Lowe, J. B., and Vestweber, D. (2000) *J. Biol. Chem.* **275**, 31353–31360
30. Goetz, S. E., Hess, C., Goff, D., Griffiths, B., Tizard, R., Newman, B., Chi Rosso, G., and Lobb, R. (1990) *Cell* **63**, 1349–1356
31. Kimura, H., Shinya, N., Nishihara, S., Kaneko, M., Irimura, T., and Narimatsu, H. (1997) *Biochim. Biophys. Res. Commun.* **237**, 131–137
32. Hiraiwa, N., Dohi, T., Kawakami-Kimura, N., Yumen, M., Ohnori, K., Maeda, M., and Kannagi, R. (1996) *J. Biol. Chem.* **271**, 31556–31561
33. Knibbs, R. N., Craig, R. A., Natsuka, S., Chang, A., Cameron, M., Lowe, J. B., and Steelman, L. M. (1996) *J. Cell Biol.* **133**, 911–920
34. Maly, P., Thall, A. D., Petryniak, B., Rogers, C. E., Smith, P. L., Marks, R. M., Kelly, R. J., Gersten, K. M., Cheng, G., Saunders, T. L., Camper, S. A., Camphausen, R. T., Sullivan, P. X., Isegal, Y., Hinds, O., von Andrian, U. H., and Lowe, J. B. (1996) *Cell* **86**, 643–653
35. Nishihara, S., Hiraga, T., Ikebara, Y., Kudo, T., Iwasaki, H., Morozumi, K., Akamatsu, S., Tachikawa, T., and Narimatsu, H. (1999) *Glycobiology* **9**, 607–616
36. Schienle, H. W., Stein, N., and Muller-Ruchholtz, W. (1982) *J. Clin. Pathol.* **35**, 959–966
37. Spooncer, E., Fukuda, M., Klock, J. C., Oates, J. E., and Dell, A. (1984) *J. Biol. Chem.* **259**, 4792–4801
38. Macher, B. A., Buehler, J., Scudder, P., Knapp, W., and Feizi, T. (1988) *J. Biol. Chem.* **263**, 10186–10191
39. Fukuda, M. N., Dell, A., Oates, J. E., Wu, P., Klock, J. C., and Fukuda, M. (1989) *J. Biol. Chem.* **260**, 1067–1082
40. Collins, S. J., Ruscetti, F. W., Gallagher, R. E., and Gallo, R. C. (1978) *Proc. Natl. Acad. Sci. U. S. A.* **75**, 2458–2462
41. Nojiri, H., Takaku, F., Tetsuka, T., Motoyoshi, K., Miura, Y., and Saito, M. (1984) *Blood* **64**, 534–541
42. Teterosio, P. A., Mulder, A., Landorp, P. M., Zola, H., Baker, D. A., Visser, F. J., and von dem Borne, A. E. (1984) *Eur. J. Immunol.* **14**, 1089–1095
43. Albrechtsen, M., and Kerr, M. A. (1989) *Br. J. Haematol.* **72**, 312–320
44. Stocks, S. C., Albrechtsen, M., and Kerr, M. A. (1990) *Biochem. J.* **268**, 275–280
CD15 Expression in Mature Granulocytes Is Determined by α1,3-Fucosyltransferase IX, but in Promyelocytes and Monocytes by α1,3-Fucosyltransferase IV
Fumiaki Nakayama, Shoko Nishihara, Hiroko Iwasaki, Takashi Kudo, Reiko Okubo, Mika Kaneko, Mitsuru Nakamura, Masataka Karube, Katsutoshi Sasaki and Hisashi Narimatsu

J. Biol. Chem. 2001, 276:16100-16106.
doi: 10.1074/jbc.M007272200 originally published online February 23, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M007272200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 44 references, 26 of which can be accessed free at
http://www.jbc.org/content/276/19/16100.full.html#ref-list-1