I iterative PKS and a putative enoyl reductase. This system appears to be all that is necessary for the 35 separate reactions [including a possible Diels-Alder cyclization reaction, heretofore precedented only once in nature (*21*)] postulated to be necessary for the biosynthesis of dihydromonacolin L from acetyl-CoA, malonyl-CoA, NADPH (the reduced form of nicotinamide adenine dinucleotide phosphate), and SAM. The LNKS/LovC system exhibits a remarkable discriminatory ability whose catalytic mechanism must be very different from the linear programming found in bacterial modular type I PKS systems (*4, 5*). Lovastatin biosynthesis is completed by the addition of the 2-methylbutyryl side chain to monacolin J by a specific transesterase encoded by *lovD*.

Bacterial type I PKSs have been manipulated genetically to produce novel compounds that are difficult to make by traditional chemical methods (*4–6*). Although much less is known about the molecular recognition powers of the PKSs that make reduced fungal metabolites like lovastatin, the methods of combinatorial biosynthesis may also be applicable to these enzymes. Elimination or inactivation and addition of domains to such PKSs will determine whether this approach can yield useful information on the substrate discriminatory properties of the LNKS/LovC complex in particular and will allow us to determine how it can be manipulated to produce novel compounds. Manipulation of *lovF* to produce compounds with different side chains is much easier to envisage. The deletion of activities in this gene or perhaps addition of further modules could allow the production of various lovastatin analogs in a predictive manner.

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UDP-GlcNAc 2-Epimerase: A Regulator of Cell Surface Sialylation

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Modification of cell surface molecules with sialic acid is crucial for their function in many biological processes, including cell adhesion and signal transduction. Uridine diphosphate-*N*-acetylglucosamine 2-epimerase (UDP-GlcNAc 2-epimerase) is an enzyme that catalyzes an early, rate-limiting step in the sialic acid biosynthetic pathway. UDP-GlcNAc 2-epimerase was found to be a major determinant of cell surface sialylation in human hematopoietic cell lines and a critical regulator of the function of specific cell surface adhesion molecules.

In eukaryotic cells, glycoproteins and glycolipids expressed at the cell surface can be modified to varying degrees by the addition of sialic acids [*N*-acetylneuraminic acid (NeuAc) or other N- and O-substituted neuraminic acids]. Because of their widespread distribution, structural versatility, and peripheral position on oligosaccharide chains of glycoconjugates, sialic acid residues are well suited as molecular determinants of specific biological processes. For example, they are involved in cell-cell interactions, T and B cell activation, and hematopoietic cell differentiation (*1–4*). Differential sialylation, that is, quantitative or linkage-specific differences in sialylation, of cell surface molecules is also implicated in the tumorigenicity and metastatic behavior of malignant cells (*5*).

Sialyltransferases, which reside in the Golgi apparatus, add cytidine monophosphate (CMP)–activated sialic acid residues to specific terminal nonreducing positions on oligosaccharide chains of proteins and lipids (*6*). The differential expression of sialyltransferases explains some (*4*, *7*) but not all examples of differential sialylation (*8*, *9*). In subclones of the human B lymphoma cell line BJA-B, differential sialylation accounts for a reduction of up to 73% in the incorporation of both α-2,6– and α-2,3–linked sialic acid residues into membrane glycoconjugates, even though the activity of the β-galactoside α-2,6-sialyltransferase, ST6Gal I, is not limited (*9*). Also, the different degrees of cell

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surface sialylation that have been described in variants of the HL-60 human myeloid leukemia cell line (*10*, *11*) cannot be attributed to differences in the expression of specific glycosyltransferases (*11*). We investigated whether differential sialylation could be a result of limitations in available intracellular CMP-NeuAc, a substrate of all sialyltransferases (Fig. 1A).

To determine if sialic acid biosynthesis alters sialoglycan expression, we used two hyposialylated subclones of the BJA-B cell line, K20 and K6, in a metabolic complementation assay. On the basis of the known sialic acid biosynthesis pathway (Fig. 1A), cells were grown in the presence of different sialic acid precursors, and the overall sialylation of cell surface glycoconjugates, as well as the α-2,6-sialylation of specific B cell differentiation antigens, was analyzed (*12*, *13*). Representative flow cytometry results for subclone K20 are shown (Fig. 1, B to D). Cultivation of cells in either *N*-acetyl-D-mannosamine (ManNAc) or D-mannosamine (ManN) resulted in an increase in binding of sialic acid–specific *Limax flavus* agglutinin (LFA) (*14*) (Fig. 1B) and also induced an up-regulation in the expression of three α-2,6-sialyllactosamines tested, CDw75 (Fig. 1, C and D), CDw76, and EBU-65 (*15*). Binding of LFA or of monoclonal antibodies (mAbs) to CDw75 and CDw76 was reduced when cells grown in ManNAc were treated with *Vibrio cholerae* sialidase (*12*, *15*). Supplementation of the medium with *N*-acetyl-D-glucosamine (GlcNAc), D-glucosamine (GlcN), D-mannose (Man), or D-glucose (Glc) (*12*), which all enter the sialic acid biosynthetic pathway upstream of ManNAc (Fig. 1A), had no effect on sialoglycan expression (Fig. 1, B and C). Sialoglycan expression in K20 cells was dependent on the concentration of ManN present (*16*) and reached that of untreated K88 cells, which are highly sialylated (Fig. 1D and Table 1). The sialic acid content of ManNAc-treated K20 cells also reached that of K88 cells (*13*, *15*).

Sialyl-Lewis^x (sLe^x, CD15s) is an important sialylated component of carbohydrate ligands that bind to E- and P-selectin molecules. These ligands are expressed by leukocytes and are involved in leukocyte recruitment of selectin-expressing endothelial cells in response to injury or inflammation (*17*). Enhanced expression of sLe^x also correlates with metastatic progression of epithelial carcinomas (*18*). HL60-I, an HL-60 cell line variant, expressed mostly nonsialylated Lewis^x (Le^x, CD15) and less sLe^x on the cell surface, in addition to an overall hyposialylation of glycoconjugates (Fig. 2A and below) (*13*, *19*). In contrast, HL60-II, another HL-60 cell line variant, expressed greater than 100 times more sLe^x and was highly sialylated (Fig. 1E and below) (*10*). Both variant cell lines expressed equal amounts of the myelo-monocyte marker CD14 and major histocompatibility complex (MHC) class I (Fig. 1E). Growth of hyposialylated HL60-I cells in medium supplemented with ManNAc

Table 1. UDP-GlcNAc 2-epimerase activity and amount of membrane glycoconjugate-bound sialic acid (*13*) in BJA-B subclones and stable transfectants (*34*). Values shown are arithmetic means ± SD.

Cells (*N*)	UDP-GlcNAc 2-epimerase (μU/mg of protein)	NeuAc (nmol/mg of protein)
BJA-B (4)	16 ± 5	26 ± 6
K43 (4)	19 ± 3	31 ± 6
K88 (6)	18 ± 2	24 ± 4
K6 (4)	<0.2	9 ± 3
K20 (6)	<0.2	8 ± 3
K20Neo1 (3)	<0.2	7 ± 2
K20α2,6ST (3)	<0.2	9 ± 3
K20rEpi1 (5)	23 ± 6	24 ± 5
K20rEpi2 (5)	5 ± 1	19 ± 3
K88rEpi (4)	137 ± 32	25 ± 6

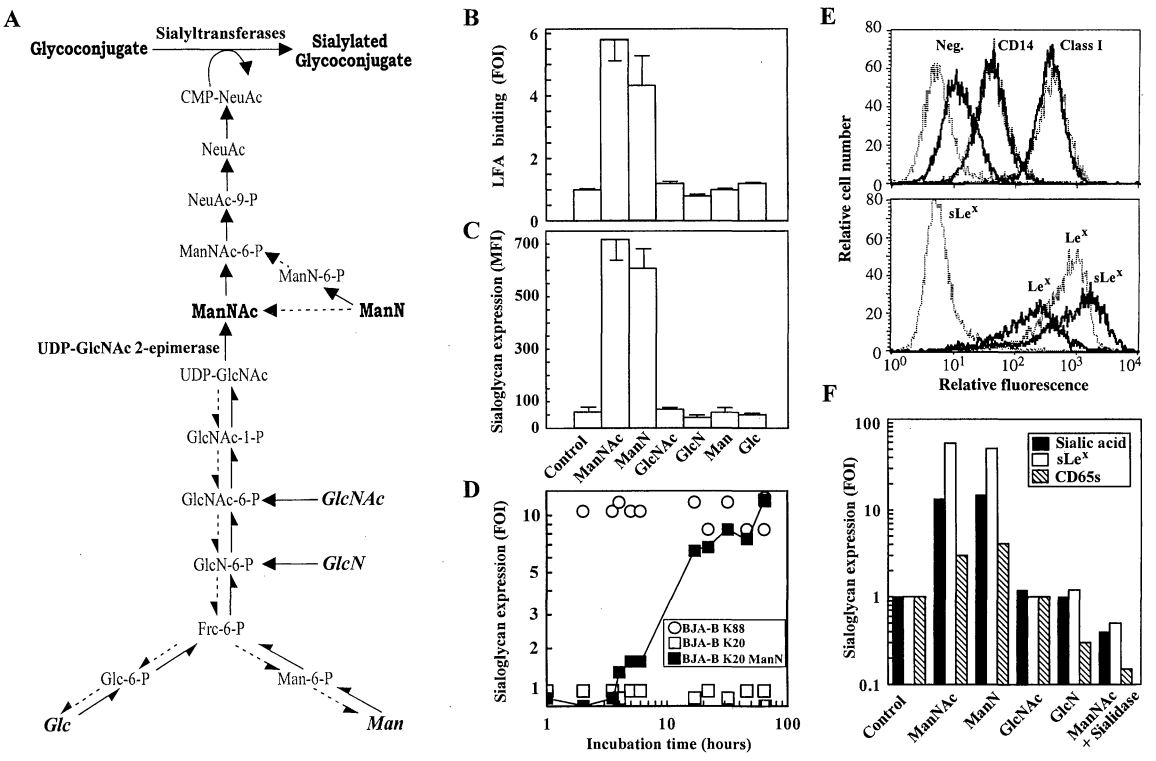

Fig. 1. Metabolic complementation studies indicate a control point in sialic acid biosynthesis. (**A**) Schematic representation of sialic acid metabolism. (**B** to **D**) Hyposialylated BJA-B cells were cultivated in the absence (control) or presence of various sugars (5 mM). MFI, mean flourescence intensity; FOI, factor of increase of MFI values relative to controls. (B) Binding of LFA to detect sialic acid residues irrespective of their linkage (*14*) (N = 3) after a 48-hour incubation with the indicated sugars. (C) Binding of mAb HH2 to detect CDw75 sialoglycan expression after a 48-hour incubation with the indicated sugars (N = 3) (*12*). (D) Binding of mAb HH2 to detect CDw75 expression on BJA-B K20 cells, cultivated in the presence (■) or absence (□) of ManN for indicated times and on untreated, highly sialylated BJA-B K88 cells (*9*) (○) (N = 2). (**E**) Overlay of FACScan histograms of HL60-I (dotted lines) and HL60-II (solid lines) (*19*). (Top) Negative control (Neg.), CD14, and MHC class I (Class I) molecules were determined with mAbs HD20, M5E2 and W6/32, respectively (*12*). (Bottom) mAbs 2H5 and HI98 to sLe^x and Le^x, respectively. Similar results to those obtained with mAb 2H5 were also obtained with mAb CSLEX1 (sLe^x) (*15*). (**F**) HL60-I cells, cultivated in the absence (Control) or presence of the indicated sugars (5 mM) for 70 to 74 hours and treated with *Vibrio cholerae* sialidase where indicated, were analyzed for sialic acid content by LFA binding (N = 4) and the expression of sLe^x (mAb 2H5) (N = 5) and CD65s (mAb VIM-2) (N = 3).

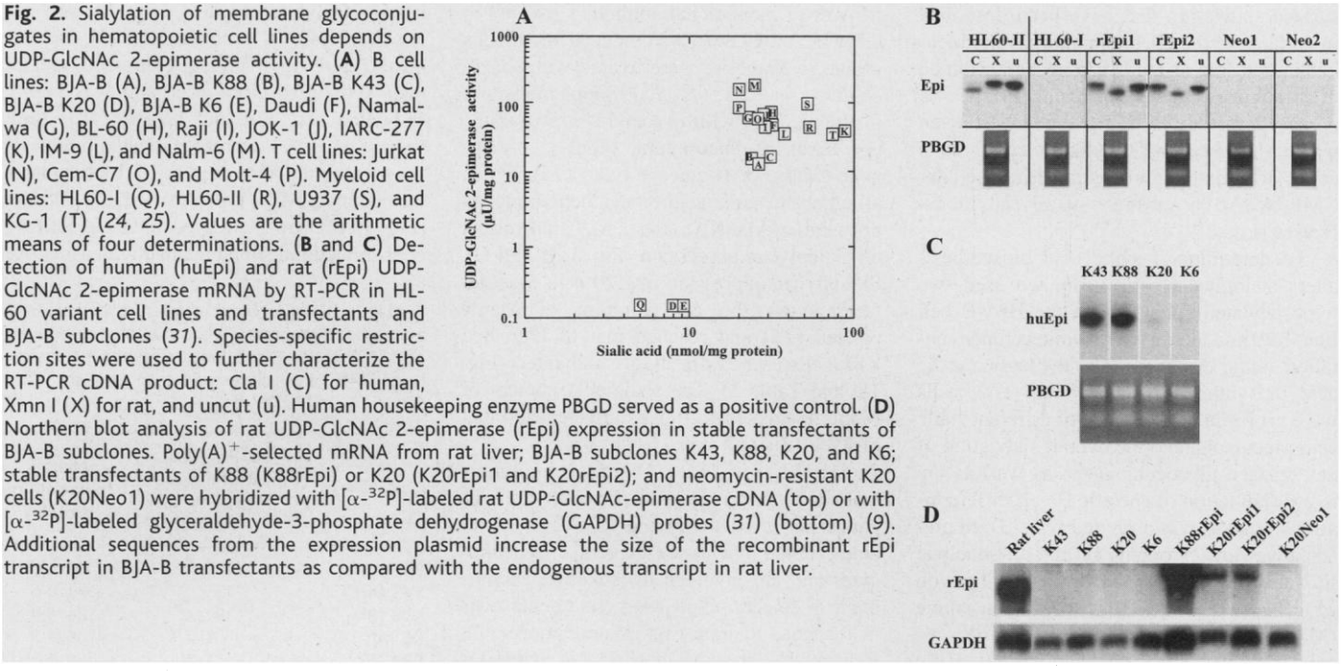

Fig. 2. Sialylation of membrane glycoconjugates in hematopoietic cell lines depends on UDP-GlcNAc 2-epimerase activity. (**A**) B cell lines: BJA-B (A), BJA-B K88 (B), BJA-B K43 (C), BJA-B K20 (D), BJA-B K6 (E), Daudi (F), Namalwa (G), BL-60 (H), Raji (I), JOK-1 (J), IARC-277 (K), IM-9 (L), and Nalm-6 (M). T cell lines: Jurkat (N), Cem-C7 (O), and Molt-4 (P). Myeloid cell lines: HL60-I (Q), HL60-II (R), U937 (S), and KG-1 (T) (*24*, *25*). Values are the arithmetic means of four determinations. (**B** and **C**) Detection of human (huEpi) and rat (rEpi) UDP-GlcNAc 2-epimerase mRNA by RT-PCR in HL-60 variant cell lines and transfectants and BJA-B subclones (*31*). Species-specific restriction sites were used to further characterize the RT-PCR cDNA product: Cla I (C) for human, Xmn I (X) for rat, and uncut (u). Human housekeeping enzyme PBGD served as a positive control. (**D**) Northern blot analysis of rat UDP-GlcNAc 2-epimerase (rEpi) expression in stable transfectants of BJA-B subclones. Poly(A)$^+$-selected mRNA from rat liver; BJA-B subclones K43, K88, K20, and K6; stable transfectants of K88 (K88rEpi) or K20 (K20rEpi1 and K20rEpi2); and neomycin-resistant K20 cells (K20Neo1) were hybridized with [α-^{32}P]-labeled rat UDP-GlcNAc-epimerase cDNA (top) or with [α-^{32}P]-labeled glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probes (*31*) (bottom) (*9*). Additional sequences from the expression plasmid increase the size of the recombinant rEpi transcript in BJA-B transfectants as compared with the endogenous transcript in rat liver.

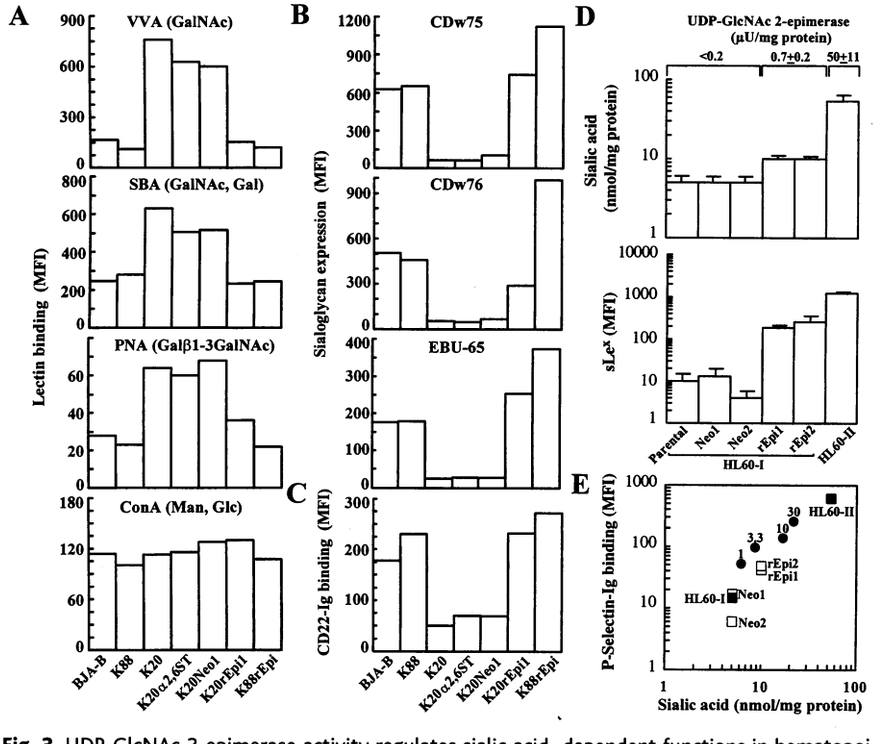

Fig. 3. UDP-GlcNAc 2-epimerase activity regulates sialic acid–dependent functions in hematopoietic cell lines. BJA-B subclones and transfectants were analyzed by flow cytometry, and arithmetic means of MFI are given ($N > 2$). (**A**) Binding of lectins VVA, SBA, PNA, and concanavalin A (ConA) and (**B**) the expression of α-2,6–sialylated B cell differentiation antigens CDw75, CDw76, and EBU-65 (*12*). (**C**) Binding of CD22-Ig to cells (*40*). (**D**) Sialic acid content (top) (*13*) and sLe^x expression (bottom) (*12*) in hyposialylated HL60-I cells, their stable rEpi transfectants (rEpi1 and rEpi2), control neomycin-resistant transfectants (Neo1 and Neo2), and highly sialylated HL60-II cells. The UDP-GlcNAc 2-epimerase activity (*13*) is indicated above the top panel. (**E**) Correlation of sialic acid content and P-selectin-Ig binding (*41*) in HL60-I and -II cells (■), HL60-I transfectants (□), and HL60-I cells (●) grown in 1 mM, 3.3 mM, 10 mM, or 30 mM ManNAc as indicated for 3 days.

or ManN also enhanced overall cell surface sialylation and the sialylation of two α-2,3-sialoglycans, sLe^x and CD65s (Fig. 1F and below) (*20*).

On the basis of the known sialic acid biosynthetic pathway (Fig. 1A), these metabolic complementation data indicate that the epimerization of uridine diphosphate-*N*-acetylglucosamine (UDP-GlcNAc) to ManNAc by UDP-GlcNAc 2-epimerase (*21*) might be a rate-limiting step in the generation of CMP-NeuAc in hyposialylated BJA-B and HL-60 cells. This enzyme has been proposed as a regulator of sialylation in various primary tissues (*22*). The activity of UDP-GlcNAc 2-epimerase correlated with CMP-NeuAc levels (*23*) as well as with sialic acid content in BJA-B and HL-60 cells (*13*) (Fig. 2A, Table 1, and below). In all hematopoietic cell lines tested, UDP-GlcNAc 2-epimerase activity and sialic acid content were concordant (Fig. 2A) (*24*, *25*).

Cultivation of HL60-I and K20 cells in serum-free medium resulted in a loss of glycoconjugate sialylation (<2 nmol of NeuAc per milligram of protein) (*26*). In contrast, the sialylation of glycoconjugates in HL60-II and K88 cells, which display high UDP-GlcNAc 2-epimerase activity, was unaffected under these conditions. Thus, the residual sialylation in UDP-GlcNAc 2-epimerase–deficient cells likely occurs through a salvage pathway that recruits sialic acid from serum sialoglycoconjugates in the media (*27*) and not through an alternative pathway of de novo sialic acid biosynthesis (*28*). These results confirm that the activity of the UDP-GlcNAc 2-epimerase is rate-limiting to the

biosynthesis of sialic acid and show that this enzyme is required for normal sialylation in hematopoietic cells.

Different tissues from rodents display considerable variations in steady-state levels of UDP-GlcNAc 2-epimerase transcripts (*29*, *30*). In BJA-B and HL-60 cells, epimerase activity correlated with transcript levels as determined by reverse transcriptase–polymerase chain reaction (RT-PCR) (Fig. 2, B and C) (*31*–*33*). We established HL60-I and BJA-B transfectants that constitutively express the rat homolog of this enzyme (rEpi) (*31*, *34*). Epimerase mRNA in transfectants was detected by RT-PCR or by Northern (RNA) blot analysis (Fig. 2, B to D). In the BJA-B transfectant K20rEpi, an increase in UDP-GlcNAc 2-epimerase activity was accompanied by an increase in the sialic acid content (Table 1). Enzyme activity and sialylation were comparable to those of highly sialylated BJA-B cells. Lectin-binding analyses with fluorochrome-coupled *Vicia villosa* (VVA), soybean (SBA), and peanut (PNA) agglutinin, which specifically bind to the indicated saccharide residues (*12*), confirmed an increased masking of these penultimate saccharides by sialic acid in K20rEpi1 (Fig. 3A) and K20rEpi2 cells (*15*). K20rEpi1 cells also expressed more of the α-2,6-sialyllactosamines CDw75, CDw76, and EBU-65 on the cell surface than did hyposialylated K20 and K20Neo1 cells (Fig. 3B). Overexpression of ST6Gal I sialyltransferase (*9*) did not affect expression of these antigens (Fig. 3B).

CD22 is a B lymphocyte–specific adhesion and signaling molecule that is involved in the regulation of B cell activation. It binds to α-2,6-sialyllactosamines (*35*–*37*), which are the products of the ST6Gal I. CD22 interacts with CDw75 (*38*) and possibly with other α-2,6–linked B cell antigens to mediate B cell–B cell interactions (*37*, *39*). We investigated the binding of CD22 to BJA-B subclones and transfectants with a soluble fusion protein made up of the extracellular domains of CD22 and the Fc portion of human immunoglobulin G1 (IgG1) (CD22-Ig) (*40*). Binding of CD22-Ig to BJA-B cells correlated with UDP-GlcNAc 2-epimerase activity (Fig. 3C and Table 1). Compared with K20 or K20Neo1 cells, the parental BJA-B and the highly sialylated K88, K20rEpi1, and K88rEpi cells all bound greater amounts of CD22-Ig (Fig. 3C). Overexpression of ST6Gal I in epimerase-negative cells (K20α-2,6ST) did not affect CD22-Ig binding. An increase in the sialylation of several specific surface molecules in K88rEpi cells (Fig. 3B) raised the possibility of a hierarchy in glycoconjugate sialylation, with some acceptor sites being sialylated only under conditions of sialic acid excess. Although the activity of ST6Gal I is a prerequisite for generating α-2,6–sialylated B cell differentiation antigens in this B cell line (*35*–*37*), the activity of UDP-GlcNAc 2-epimerase is the only limiting factor.

In spite of a marginal increase in UDP-GlcNAc 2-epimerase activity (0.7 ± 0.2 μU/mg) (Fig. 3D), HL60-I rEpi transfectants showed an increase in sialic acid and sLe^x expression by 2 and 20 times, respectively, compared with parental cells and HL60-I Neo transfectants (Fig. 3D). When hyposialylation was either metabolically (ManNAc) or genetically (rEpi transfection) complemented in the HL60-I variant, binding of a P-selectin-Ig chimeric molecule (*41*) to cells also increased. This correlated with an increase in sialic acid content and sLe^x surface expression (Fig. 3, D and E). The increased P-selectin-Ig binding was sialidase sensitive (*12*, *15*). Thus, subtle changes in UDP-GlcNAc 2-epimerase activity affected sLe^x expression and P-selectin-Ig binding in this myeloid cell line.

Our data show that UDP-GlcNAc 2-epimerase, which catalyzes a rate-limiting step in the biosynthesis of sialic acids, is an important regulator of cell surface glycoconjugate sialylation in hematopoietic cell lines. Activity of the enzyme can be controlled at transcription and can affect the sialylation and function of specific cell surface molecules expressed on B cells and myeloid cells. Further insight into the regulation of sialylation by UDP-GlcNAc 2-epimerase in normal human cells and primary tumors may contribute to the understanding of physiological as well as pathological sialic acid–dependent processes in adhesion, signaling, differentiation, and metastasis.

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12. Sources of antibodies and lectins, specificity references, staining procedures, and FACScan analyses have been reported (*7*, *9*). Antibodies were fluorescein isothiocyanate (FITC)–conjugated mAb HI98 to Le^x, mAb 2H5 to sLe^x, and phycoerythrin-conjugated mAb M5E2 to CD14 (Pharmingen, San Diego, CA); mAbs HH2 and HD66 to α-2,6-sialyllactosamines CDw75 and CDw76, respectively; and mAb EBU-65 to an unclustered α-2,6-sialyllactosamine, FITC-conjugated goat $F(ab')_2$ fragments to mouse IgG and IgM heavy and light chains (Dianova, Hamburg, Germany). mAbs HD20 and W6/32 (MHC class I molecules) were negative and positive controls, respectively, in all antibody studies. ManNAc, ManN, GlcNAc, GlcN, Man, and Glc (Sigma) were dissolved to 100 mM in H_2O and stored at −20°C. BJA-B and HL60-I cells (3×10^5 per milliliter) were cultivated in the presence of the indicated concentrations of these sugars and then washed twice with phosphate-buffered saline (PBS). Where indicated, washed cells were treated with *Vibrio cholerae* sialidase (Behring, Marburg, Germany) (200 mU/ml for 60 min at 37°C).
13. Cells (1×10^7) were harvested, washed once in PBS, and lysed by hypotonic shock in 10 mM sodium phosphate (pH 7.5), 1 mM EDTA, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride (15 min, 4°C). The crude membrane fraction was pelleted by centrifugation at 100,000*g* (60 min, 4°C). The resulting supernatant was analyzed for UDP-GlcNAc 2-epimerase activity as described (*32*). The pellet was washed twice with H_2O and lyophilized. The content of membrane glycoconjugate-bound sialic acid relative to the protein content was determined by hydrolyzing the pellet for 1 hour with 2 M acetic acid [A. Varki and S. Diaz, *Anal. Biochem.* **137**, 236 (1984)], and released sialic acid was quantified by the thiobarbituric acid method [D. Aminoff, *Biochem. J.* **81**, 384 (1961)]. The protein concentration was measured by the method of M. M. Bradford [*Anal. Biochem.* **72**, 248 (1976)].
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24. Human hematopoietic cell lines were cultivated in RPMI 1640 or Dulbecco's minimum essential medium, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 2 mM glutamine, penicillin (100 U/ml), and streptomycin (100 μg/ml) in a humidified 5% CO_2 atmosphere at 37°C.

25. All BJA-B subclones are of clonal origin, because they display identical Ig heavy chain gene rearrangements.
26. HL60-I, HL60-II, BJA-B K20, and BJA-B K88 cells were cultivated for 7 days in medium containing either serum-free Nutridoma NS (Boehringer Mannheim) or 10% FBS (control). The sialic acid concentration (*13*) in HL60-I and BJA-B K20 cells cultivated in Nutridoma NS was reduced by ≥75% as compared with controls. In contrast, the sialylation status in HL60-II and BJA-B K88 cells was unaffected.
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31. Reverse transcription of 1 μg of polyadenylation [poly $(A)^+$]–selected mRNA (mRNA isolation kit, Boehringer Mannheim) was done with an avian myeloblastosis virus reverse transcriptase (AGS, Heidelberg, Germany) and DNA random hexamers as primers. The cDNA was subjected to RT-PCR analysis. The primers 5′-GAGATGGTTCGAGTGATGC and 5′-TAACTATTTCACCCTTCATGCT were suitable for both rat (*29*) and human (*33*) UDP-GlcNAc 2-epimerase mRNA sequences. Species-specific restriction sites were used to discriminate UDP-GlcNAc 2-epimerase RT-PCR products [516 base pairs (bp)] of human [Cla I (C); 354-bp and 162-bp fragments] and rat [Xmn I (X); 377-bp and 139-bp fragments] origin. PCR products were transferred onto nylon membranes (Gene Screen+, Dupont) and were detected by hybridization with a [α-^{32}P]-labeled primer 5′-GGGGAGAATGTTCTTCATGTCCG that binds to the larger restriction fragments. Primers specific for human porphobilinogen deaminase (PBGD) have been described [S. Chretien *et al.*, *Proc. Natl. Acad. Sci U.S.A.* **85**, 6 (1988)]. Northern blots were hybridized with a [α-^{32}P]-labeled 1.4-kb Pst1 fragment of rat glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA [P. Fort *et al.*, *Nucl. Acids. Res.* **13**, 1431 (1985)] to demonstrate the presence of similar amounts of mRNA in the samples.
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34. cDNA encoding the rat UDP-GlcNAc 2-epimerase (*31*) was cloned into vector pBK-CMV, which contains a G418 resistance gene. BJA-B K20 and K88 cells and HL60-I cells were transfected by electroporation with 20 μg DNA of pBK-CMV-rEpi-G418 with voltages of 180 V for BJA-B and 220 V for HL-60 cells. After 2 days in culture, transfected cells were grown under limiting dilution conditions in 96-well plates (Nunc, Wiesbaden, Germany) in medium containing G418 (Sigma) (2700 μg/ml for BJA-B; 1000 μg/ml for HL60-I) (*9*). Drug-resistant single clones were expanded, and individual clones were analyzed as indicated.
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40. The cDNA specific for a CD22-Ig fusion gene was inserted into the immunoglobulin expression vector pIg and was introduced into COS cells by the DEAE-dextran method (*35*). CD22-Ig contains the first three immunoglobulin-like domains of CD22 linked to the Fc portion of human IgG1. CD22-Ig was purified from COS transfectant culture supernatants by chromatography on protein A-Sepharose 4B. CD22-Ig chimera binding to cells was quantified by flow cytometry as described previously [M. Bergmann *et al.*, *Glycobiology* **8**, 963 (1998)].
41. Binding of human P-selectin-Ig (*2*) to cells was quantified by flow cytometry [M. Sammar *et al.*, *Int. Immunol.* **6**, 1027 (1994)].
42. We thank H. zur Hausen for support; R. Stäsche for rat UDP-GlcNAc 2-epimerase cDNA; W. Knapp and B. Kniep for mAb VIM-2; I. Stamenkovic for the CD22-Ig chimera cDNA; P. Altevogt and J. Friederichs for mAb CSLEX-1, P-selectin-Ig chimera, and advice; S. Funderud for mAb HH2 and ST6Gal I cDNA; T. Flohr for rat liver mRNA; A. Zakrzewicz for HL60-II cells; A. Merling for technical assistance with the CD22-Ig binding assay; T. Holz for assistance with the graphics; and D. Keppler for critical reading of the manuscript. Supported by grants from the Bundesministerium für Bildung und Forschung to W.R. and M.P., from the Sonnenfeld-Stiftung to W.R., and from the Wilhelm-Sander Stiftung to M.P.

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