Sequential change of carbohydrate antigen associated with differentiation of murine leukemia cells: i–I antigenic conversion and shifting of glycolipid synthesis

(P^k antigen/ganglio-series glycolipid/lacto-series glycolipid/globo-series glycolipid/inducers)

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ABSTRACT Cell surface carbohydrate antigens and their metabolism were investigated during the course of differentiation of murine cultured leukemia cells (M1) into macrophage-like cells. The major glycolipids in undifferentiated M1 cells were of the ganglio series, with a small amount of lacto-series glycolipids. A novel branched structure was found as a tetraosylceramide of M1[−] cells. Upon differentiation, synthesis of lacto-series glycolipids was significantly enhanced and synthesis of globo-series glycolipids was newly induced but the ganglio-series synthesis was much reduced. Undifferentiated cells expressed only i antigen (i⁺I[−]P^{k−}); differentiated macrophage-like cells became I-antigen dominant and P^k-antigen positive (i⁺I⁺P^{k+}). The changes proceeded in two sequential steps: (i) an enhancement of lacto-series glycolipid synthesis associated with the conversion of i antigen to I antigen, and (ii) subsequent induction of globo-series glycolipid synthesis accompanied by the appearance of P^k antigen. The experimental system offers a clue for studies on the process of branching (i-to-I conversion) as well as the biological significance of three major glycolipids (globo-, lacto-, and ganglio-series) as markers of cell differentiation.

Development and differentiation are associated with a continuous change in cell surface carbohydrates (1). Certain glycolipids with defined chemical structures have been found to alter dramatically during the course of differentiation and development. Glycolipids are classified into three major categories, globo, lacto, and ganglio series, according to their carbohydrate structure and synthetic pathways (2). The change of glycolipid antigen during the course of development or differentiation involves several species of glycolipids (1, 3–6). The biological significance of the presence of three distinct species of glycolipid is unknown. The stage-dependent expression of each series of glycolipids in differentiation and ontogenesis is an attractive subject to study.

The murine myelogenous leukemia cell line M1, established from spontaneous leukemia in an SL/Am strain mouse, is capable of differentiating into cells that display various phenotypic characteristics of mature macrophages *in vitro* when cultured with various inducers (7, 8). This cell system has been utilized as a good experimental model for the study of normal myeloid cell differentiation (7–10).

We now find remarkable alterations in chemically well-defined carbohydrate antigens (Ii, P^k) during the course of differentiation, involving a sequential shift of glycolipid synthesis from ganglio- to lacto-series and, subsequently, to globo-series glycolipids.

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MATERIALS AND METHODS

Cells and Cell Cloning. M1 cells have an undifferentiated myeloblast-like morphology under usual culture conditions (7) and are referred to as "M1[−] cells" in this paper. For differentiation, M1[−] cells were incubated with conditioned medium (final, 10%) prepared from culture supernatants of BALB/c embryonic (18 days) fibroblasts as described (7–10). After 48-hr incubation with conditioned medium, M1 cells acquired phagocytic activity, locomotive activity, dish adhesiveness, and surface Fc receptors, with a significant suppression of cell proliferation. Differentiated cells showing those activities are termed "M1⁺ cells" in this paper. Cell cloning was carried out by limiting-dilution techniques with BALB/c thymocytes as feeder cells. A strongly I⁺ subclone (I-02) was isolated from Mm1 cells (11), a subclone of M1 cells that was I-dominant (68% of cells I⁺; 29% i⁺); an i⁺ clone (i-01) was from parental M1[−] cells; and i⁺ (i-D3) and Ii-negative (n-D6) clones were isolated from M1[−]D[−] clones (12), a subclone of M1[−] cells that showed i-antigen dominance (i⁺ cells, 41%; I[−]i[−] cells, 58%; negative with anti-I antibody). M1[−], Mm1, and M1[−]D[−] cells were obtained from T. Masuda (Institute of Immunology, Kyoto University, Kyoto, Japan). All cells and subclones are maintained in Dulbecco modified minimal essential medium with 10% horse serum.

Immunological Detection of Carbohydrate Antigens. Monoclonal antibodies anti-I (Ma, human IgM) and anti-i (Dench, human IgM) were donated by E. R. Giblett (Puget Sound Blood Bank, Seattle); anti-P^k (38-13, rat IgM) was a gift from M. Lipinski, J. Wiels, and T. Tursz (Institute G. Roussy, Villejuif, France) (13). Monoclonal anti-Gg₃ and anti-N-acetylactosamine (both mouse IgM) were prepared as described (14, 15). Antigens at the cell surface were detected by indirect immunofluorescence staining, fluorescence microscopy observation, and by fluorescence-activated cell sorter analysis (FACS-II, Becton Dickinson) with a logarithmic data analyzer or by a com-

Abbreviations: HPTLC, high performance thin-layer chromatography; FACS, fluorescence-activated cell sorter. Shorthand designation of glycolipid is according to the IUPAC–IUC nomenclature (31): Gg₃, gangliotetraosylceramide (asialo GM₂) GalNAcβ1→4Glcβ1→1Cer; Gg₄, gangliotetraosylceramide (asialo GM₁) Galβ1→3GalNAcβ1→4Glcβ1→1Cer; NeuAcα2→3Gg₄, sialosylgangliotetraosylceramide (GM_{1b}) NeuAcα2→3Galβ1→3GalNAcβ1→4Glcβ1→1Cer; Gb₃, globotriaosylceramide (P^k antigen, CTH) Galα1→4Glcβ1→4Glcβ1→1Cer; Lc₃, lactotriaosylceramide GlcNAcβ1→3Galβ1→4Glcβ1→1Cer; nLc₄, neolactotetraosylceramide (paragloboside) Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer; nLc₆, nor-neolactohexaosylceramide (i antigen) Galβ1→4GlcNAcβ1→3Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer; iso-nLc₆, iso-neolactohexaosylceramide (I antigen) Galβ1→4GlcNAcβ1→3[Galβ1→4GlcNAcβ1→6]Galβ1→4GlcNAcβ1→3Galβ1→4Glcβ1→1Cer; Hex₁Cer, monohexaosylceramide (CMH) Glcβ1→1Cer; Hex₂Cer, dihexaosylceramide (CDH) Galβ1→4Glcβ1→1Cer.

plement-dependent cytotoxicity test. Immunological reactivities of isolated glycolipids were ascertained by TLC and immunostaining on high performance thin-layer chromatography (HPTLC) plates as described by Magnani *et al.* (16) modified as reported (5).

Glycolipid Purification and Analysis. Total glycolipids were extracted from 40 ml of packed M1⁻ or M1⁺ cells or 10 ml of subclones I-02, i-01, i-D3, and n-D6. The majority of glycolipids in these cells were neutral glycolipids and were purified by HPLC (17) and analyzed by total mass spectrometry, methylation analysis, and exo-glycosidase treatments as described (5). The amounts of acidic glycolipids (gangliosides) were very small to be chemically analyzed; only the major ganglioside was characterized. The amount of gangliosides was especially low in differentiated cells. For metabolic labeling, M1 cells were incubated with [³H]palmitic acid at 1 μ Ci/10⁶ cells (1 Ci = 3.7 $\times$ 10¹⁰ Bq). Aliquots were taken at indicated hours of culture after the addition of conditioned medium. Labeled glycolipid was analyzed by TLC, HPTLC, and HPLC.

RESULTS

Change in Carbohydrate Antigens During the M1 Cell Differentiation. The results of indirect immunofluorescence staining of M1 cells with monoclonal anti-carbohydrate antibodies are shown in Fig. 1. The majority of undifferentiated M1⁻ cells were i-positive (under a fluorescence microscope: I⁺, 26%; i⁺, 88%). In contrast, differentiated cells were strongly positive with anti-I and less active with anti-i (I⁺, 98%; i⁺, 17%). Differentiated M1 cells also expressed the P^k antigen, which undifferentiated cells lacked completely (52% of cells were P^k after differentiation). This remarkable alteration in cell surface antigens was further confirmed by the results of complement-dependent cytotoxicity tests. Based on the difference in Ii expression in M1 cell populations, pure I-expressor and i-expressor clones were isolated. The I-expressor (clone I-02) was strongly I-positive (100% positive under microscope), whereas the i-expressor (clone i-01 and i-D3) showed I⁻i⁺ specificity (I⁺ 7% and i⁺ 86% in i-01, and I⁺ 1% and i⁺ 99% in i-D3 clone; see Fig. 1 c-e). Subclone n-D6 cells were completely negative for

Table 1. Cytological properties of M1 cell series

Cells	Adhesive- ness, %	Latex phago- cytosis, %	EA rosette, %
I-expressor			
M1 (undifferentiated)	23	72	66
Clone I-02	76	90	82
i-expressor			
M1 (undifferentiated)	6	1	12
Clone i-01	1	1	4
Clone i-D3	1	0	4
No expressor			
Clone n-D6	2	1	2

Cell adhesiveness was assessed by counting cells in logarithmic growth stage (2.5×10^5 cells per ml) adherent to plastic culture dishes. Phagocytic activity was studied by incubating 1×10^6 cells with 0.01% latex particles (1 μ m in diameter) for 4 hr at 37°C. The data are expressed as percentage of cells internalizing more than five latex particles. Fc-receptor activity was studied by incubating 1×10^6 cells with 1% sensitized sheep erythrocyte (EA) suspension at 37°C for 30 min. The data are expressed as percentage of EA-rosetting cells that bound more than four erythrocytes.

Ii antigens. The change of Ii-antigen status correlated well with the acquisition of macrophage-like functions (Table 1).

The acquisition of P^k antigen during the course of differentiation was slower than i-I conversion. Significant i-I conversion was observed 12 hr after the addition of conditioned medium (data not shown), whereas the expression of P^k was significant only after 24 hr of culture. This suggested that the induction of P^k occurred at a later stage of differentiation than did the i-I conversion. This was further supported by the finding that I-02 clones, which show strong I antigen and significant cytological properties of mature macrophages, completely lacked P^k antigen under usual culture conditions but 100% of cells expressed P^k after culture with conditioned medium (Fig. 2). The arrest of cell growth was apparent in the presence of conditioned medium.

FIG. 1. FACS analysis of surface carbohydrate antigens in undifferentiated M1⁻ cells (a), differentiated M1⁺ cells (b), and clones I-02 (c), i-D3 (d), and n-D6 (e). In b, M1 cells were differentiated by 48-hr culture with 10% conditioned medium. The first-layer antibodies were diluted (1:100) anti-I (Ma), anti-i (Dench), and anti-P^k. The second-layer antibodies were diluted (1:50) fluorescein isothiocyanate-labeled rabbit anti-human IgM (μ -chain specific) for anti-Ii and anti-mouse IgM (μ -chain specific) for anti-P^k. Control shows endogenous fluorescence.

FIG. 2. FACS analysis of induction of P^k antigen in I-02 clones cultured in the presence of conditioned medium (final, 10%).

Chemical Characterization of $M1^-$ and $M1^+$ Cell Glycolipids. Glycolipids having the same mobility as standard Gg_3 , Gg_4 , and $NeuAc\alpha 2 \rightarrow 3Gg_4$ (GM_{1b}) were detected on TLC of undifferentiated $M1^-$ cells (Fig. 3a, lane 3). The glycolipids in $M1^-$ cells that comigrated with Gg_3 and Gg_4 were identified as $GalNAc\beta 1 \rightarrow 4Gal\beta 1 \rightarrow 4Glc1 \rightarrow 1Cer$ and $Gal\beta 1 \rightarrow 3GalNAc\beta 1 \rightarrow 4Gal\beta 1 \rightarrow 4Glc1 \rightarrow 1Cer$, respectively, by methylation analysis, total mass spectrometry, and exoglycosidase treatment. The details of chemical analysis will be described elsewhere. Gb_3 (P^k antigen) was completely absent from undifferentiated cells; essentially all triaolsylceramide was Gg_3 . This was further ascertained by immunostaining on TLC plates (Fig. 3c and d). A significant glycolipid band comigrating with nLc_4 standard and a faint band comigrating with nLc_6 (i antigen) standard were detected in $M1^-$ cells; however, methylation analysis of the glycolipid comigrating with nLc_4 showed the presence of 2,3,6-*O*- Me_3Glc , 2,6-*O*- Me_2Gal , 3,4,6-*O*- $Me_3GlcNAcMe$, and 3,4,6-*O*- $Me_3GalNAcMe$, and less than 1/10th of the glycolipid had a Gal terminus on methylation analysis, suggesting a novel carbohydrate structure $GalNAc1 \rightarrow 4$ /or $3[GlcNAc1 \rightarrow 4$ /or $3Gal1 \rightarrow 4Glc1 \rightarrow 1Cer$; further confirmation of this novel structure will be described elsewhere. Thus, the amounts of nLc_4 and nLc_6 must be small in these cells; however, their presence was ascertained by staining with a specific monoclonal anti-*N*-acetylglucosamine antibody (Fig. 3b, lane 1) which reacts specifically with the $Gal\beta 1 \rightarrow 4GlcNAc$ terminal structure (17). These

FIG. 4. Metabolic labeling of M1 cell glycolipids during the course of differentiation. M1 cells were incubated with [3H]palmitic acid in the presence or absence of conditioned medium (final, 10%). Triaolsylceramides were separated by HPLC with a shallow-gradient system of isopropyl alcohol/hexane/water, 55:43:2 to 55:40:5 (vol/vol) in 100 min. Elution positions were: Gb_3 , 37–40 min; Lc_3 , 41–44 min; and Gg_3 , 61–64 min. Gg_4 and nLc_4 were separated on TLC, and bands corresponding to standard glycolipids were scraped off and assayed for radioactivity. ●, Cultured with conditioned medium; ○, without conditioned medium.

results showed that the major glycolipids in undifferentiated cells belong to the ganglio series, comprising >60% of total glycolipids (Gg_3 , 41%; Gg_4 , 14%; $NeuAc\alpha 2 \rightarrow 3Gg_4$, 5%), with a small amount of lacto-series glycolipids (<5%). The other glycolipid was composed of Hex_1Cer (11%), Hex_2Cer (12%), and unidentified compounds (11%), mostly minor gangliosides.

On the other hand, the major glycolipid band in differentiated $M1^+$ cells, which migrated at the triaolsylceramide region, was a mixture of Gb_3 and Gg_3 . These were clearly separated on HPLC. The glycolipids that comigrated with Gb_3 on HPTLC and HPLC were identified as $Gal\alpha 1 \rightarrow 4Gal\beta 1 \rightarrow 4Glc1 \rightarrow 1Cer$ (P^k antigen) by methylation analysis and glycosidase treatments, and the amount of Gb_3 was about 4 times that of Gg_3 . This was also shown by the results of immunostaining on the TLC plate: strong staining of $M1^+$ cell triaolsylceramide with anti- P^k antibody and faint staining with anti- Gg_3 antibody (Fig. 3c and d, lane 2).

Glycolipids having the same mobility as standard nLc_4 and

FIG. 3. TLC (a) and immunostaining pattern of M1 cell glycolipids with monoclonal anti-*N*-acetylglucosamine (b), anti- Gg_3 (c), and anti- P^k (d) antibodies. (a) Glycolipids were prepared from n-D6 clone (lane 1), i-01 clone (lane 2), undifferentiated M1 cells (lane 3), I-02 clone (lane 4), and differentiated M1 cells after 72-hr culture with 10% conditioned medium (lane 5). Bands were visualized with orcinol reagent. (b–d) For immunostaining with monoclonal antibodies M1 cell glycolipids were chromatographed on HPTLC plates and treated with monoclonal anti-carbohydrate antibodies followed by secondary antibodies and ^{125}I -labeled protein A solution (5, 16). Autoradiography was carried out with Kodak x-ray film. Lanes: 1, control glycolipids; 2, neutral glycolipids prepared from $M1^-$ cells; 3, neutral glycolipids from differentiated $M1^+$ cells (72 hr with 10% conditioned medium). In b, lane 1 is the mixture of human erythrocyte neutral glycolipids, serving as a mobility control on TLC of nLc_4 , nLc_6 , and iso- nLc_8 . The positive spots in lane 2 appearing above nLc_4 and below nLc_8 standards remain uncharacterized. In c, lane 1 is the gangliotriaolsylceramide (Gg_3) purified from guinea pig erythrocytes. In d, lane 1 is a mixture of human erythrocyte neutral glycolipids showing positive staining only in Gb_3 . Extracted glycolipids equivalent to 8×10^7 cells (undifferentiated cells) and 3×10^7 cells (differentiated cells) were applied to each spot. Solvent systems used in TLC: a and b, chloroform/methanol/water, 60:35:8 (vol/vol); c and d, same at 100:40:6.

nLc₆ and a small amount of glycolipid comigrating with iso-nLc₈ (I antigen) were detected on TLC of glycolipids from M1⁺ cells (Fig. 3a, lane 5). TLC immunostaining with anti-*N*-acetylactosamine antibody revealed the presence of these structures (Fig. 3b). The staining of nLc₄ and nLc₆ bands was stronger than in undifferentiated cells, and a faint staining at the iso-nLc₈ region was observed. Glycolipid comigrating with nLc₄ in M1⁺ cells was completely cleaved by exo- β -galactosidase from jack bean, showing that most of the glycolipid had a β -Gal terminus. The amount of glycolipids comigrating with Gg₄ and NeuAca2 $\rightarrow$ 3Gg₄ was almost negligible in M1⁺ cells. These results of M1⁺ cell glycolipid analysis indicate that the major glycolipid species in differentiated cells are of the globo series and that the amount of lacto-series glycolipids is increased 3- to 4-fold compared to the undifferentiated type cells: globo series (Gb₃), 47% of total cellular glycolipids; lacto series, 17%; ganglio series, 14%; Hex₁Cer, 5%; Hex₂Cer, 16%; unidentified, 1%.

Glycolipids in Subcloned Cells. I-02 clones showed an intermediate glycolipid composition—i.e., Gg₃ was the major triaosylceramide, and no Gb₃ was detected on methylation analysis, TLC/immunostaining, and exoglycosidase treatment. However, the amount of glycolipids comigrating with Gg₄ and NeuAca2 $\rightarrow$ 3Gg₄ standards was almost negligible, but significant amounts of nLc₄, nLc₆, and iso-nLc₈ were detected on TLC by the orcinol reaction (Fig. 3a, lane 4) and immunostaining with anti-*N*-acetylactosamine antibody (data not shown). Thus,

the I-02 clone contains only Gg₃ as ganglio-series glycolipids; the rest of the glycolipids were mostly lacto-series glycolipids. The glycolipid pattern of i-01 clone was almost the same as in M1⁺ cells. In n-D6 clone, the amount of Gg₄ exceeded that of Gg₃ (Fig. 3a, lane 1), and nLc₆ was hardly detected by immunostaining. This indicates that the ganglio-series glycolipids are more predominant in the n-D6 clone than in M1⁺ cells.

Glycolipid Metabolism During the Differentiation of M1 Cells. In a metabolic study using radioactive fatty acid precursor (Fig. 4), a remarkable induction of Gb₃ synthesis was observed 12 hr after the addition of conditioned medium. An enhancement of Lc₃ synthesis was also detected and was significant at 6 hr of culture, prior to the induction of Gb₃ synthesis. Synthesis of Gg₃ showed no appreciable change, and the synthesis of Gg₄ was significantly suppressed. These findings were generally in good agreement with the results of chemical analysis.

DISCUSSION

We have described a change of cell surface glycolipids and their metabolism during the course of differentiation of mouse leukemia cells into macrophage-like cells. The major glycolipids in undifferentiated cells were of the ganglio series such as Gg₃, Gg₄, and NeuAca2 $\rightarrow$ 3Gg₄. Upon differentiation, the synthesis of ganglio-series glycolipids was significantly suppressed and lacto-series glycolipid synthesis was enhanced. The synthesis of

Table 2. Summary of sequential changes in glycolipid metabolism and surface antigen during the course of M1 cell differentiation

	<i>a</i>		<i>b</i>		<i>c</i>		<i>d</i>		
	Hex ₁ -Cer (CMH) ↓ Hex ₂ -Cer (CDH) ↓ Gg ₃ (GA ₂) Lc ₃ ↓ ↓ Gg ₄ (GA ₁) nLc ₄ ↓ ↓ IV ³ NeuAcGg ₄ ↓ (GM _{1b}) nLc ₆		Hex ₁ -Cer (CMH) ↓ Hex ₂ -Cer (CDH) ↓ Gg ₃ (GA ₂) Lc ₃ ↓ ↓ Gg ₄ (GA ₁) nLc ₄ ↓ ↓ IV ³ NeuAcGg ₄ ↓ (GM _{1b}) nLc ₆		Hex ₁ -Cer (CMH) ↓ Hex ₂ -Cer (CDH) ↓ Gg ₃ (GA ₂) Lc ₃ ↓ ↓ Gg ₄ (GA ₁) nLc ₄ ↓ ↓ IV ³ NeuAcGg ₄ ↓ (GM _{1b}) nLc ₆ ↓ nLc ₈		Hex ₁ -Cer (CMH) ↓ Hex ₂ -Cer (CDH) ↓ Gg ₃ (GA ₂) Lc ₃ Gb ₃ ↓ ↓ ↓ Gg ₄ (GA ₁) nLc ₄ ↓ ↓ ↓ ↓ IV ³ NeuAcGg ₄ ↓ ↓ (GM _{1b}) nLc ₆ ↓ ↓ ↓ nLc ₈ ↓ nLc ₈		
Major Glycolipid Species	Ganglio-Series		Ganglio-Series		Ganglio-Series		Ganglio-Series Lacto-Series Globo-Series		
Representative Cells and Clones	n-O6 Clone		M1 ⁺ Cells i-O1 Clone		I-02 Clone		M1 ⁺ Cells Differentiated I-02 Clone		
Surface Antigens Detected			i-antigen		I-antigen		p ^k -antigen I-antigen		

Cells having undifferentiated morphologic and functional characteristics are divided in two stages. In *a*, synthesis of ganglio-series glycolipid is predominant and the synthesis of lacto-series glycolipids is almost negligible. These cells show i⁺I⁺ antigenicity. In *b*, synthesis of lacto-series glycolipid is significant, but synthesis of ganglio-series glycolipid still is predominant. Cells in this stage show i⁺I⁺ specificity. Stage *a* is represented by n-D6 clone; stage *b* is represented by M1⁺ cells and i-01 and i-D3 clones. Cells having differentiated macrophage-like morphology and function are also divided in two stages. In *c*, synthesis of ganglio-series glycolipids is almost stopped at triaosylceramide, lacto-series synthesis is considerably enhanced, and synthesis of globo-series glycolipid is not yet induced. These cells show i⁺I⁺P⁺ antigenicity. In *d*, synthesis of globo-series glycolipid is induced and becomes the predominant glycolipid in the cells, in addition to the changes observed in stage *c*. These cells now show i⁺I⁺P⁺ antigenicity. Stage *c* is represented by I-02 clone, and stage *d* is represented by fully differentiated M1⁺ cells and I-02 cells cultured with conditioned medium. M1⁺ cells undergo differentiation from stage *b* to *d*; for I-02 clone it is from stage *c* to *d*. Clones n-D6, i-D3, and i-01 were not inducible to differentiate by conditioned medium.

globo-series glycolipids was induced at the later stage of differentiation. This alteration of glycolipid synthesis is associated with two clear sequential shifts of cellular antigenicity; one is the conversion of i to I antigen, which is carried by a set of polylactosamines belonging to the lacto-series glycolipids (18, 19), and the other is the appearance of P^k antigen, which is carried by the globo-series glycolipid Gb₃ (20). The sequential changes in glycolipid metabolism and antigenicity are summarized in Table 2.

Replacement of i-positive fetal erythrocytes with I-positive adult erythrocytes in the neonatal period has been well documented (21); however, i-I conversion has not been experimentally demonstrated in *in vitro* differentiation from erythroblasts to erythrocytes (22). The I-i conversion is related to the process of GlcNAc β 1 $\rightarrow$ 6Gal branching (18, 19), and the glycosyltransferase (branching enzyme) is the key enzyme that determines the stage of differentiation. The M1 cell system offers a useful experimental model for the further study of this issue; the I-02 clone must have high enzymatic activity whereas I-negative clones should lack it. It is also noteworthy that the soluble factor that induces I-i conversion is secreted by fibroblasts of a fetus taken at the third trimester of pregnancy.

Unlike I antigen, P^k was not detectable on all of the differentiated M1 cells; only 40–60% of cells were positive. Differentiated M1 cells showed two clear peaks of P^k antigen expression on FACS analysis, and cells having a “dendritic cell-like” appearance (23) tended to be more strongly stained (data not shown). The subclone I-02 completely lacked P^k antigen but still was active in phagocytosis and adhesiveness. The clone can be further induced to differentiate into P^k-positive cells by conditioned medium with no further increase in phagocytic activity. This suggests that P^k antigen can be a marker of a macrophage subpopulation other than merely phagocytic cells. It is curious that the typical human erythrocyte alloantigens (blood type antigens) such as Ii and P^k are present in murine cells and display a clear differentiation dependency. It is not known whether or not these are also alloantigens in mice. Essentially no carbohydrate alloantigens have been established in mice, except a few suggestive data (24). In humans, some of the antigens in the P blood type system are suggested to be linked to HLA in chromosome 6 (25). M1 cells acquire helping activity to T and B lymphocytes in antibody production upon differentiation (26) and express some of the well-known murine alloantigens; Ia, H-2, and Ly-5 (T. Masuda, personal communication). It would be of interest to study how the change in cell surface carbohydrate antigens described in this paper is related to the expression of those reportedly proteinous mouse alloantigens.

The orderly changes in carbohydrate structure associated with cell differentiation are clearly defined in these studies by specific monoclonal antibodies directed to known carbohydrate sequences, and the changes are qualitative. This is in contrast to a few quantitative changes in membrane glycolipids reported previously (27, 28). It is surprising that such a drastic change in cellular glycolipid metabolism and the entire replacement of the major glycolipid species are essentially completed within as little as 48–72 hr. Normally, the turnover rate of glycolipid is considered to be slower than that of other membrane constituents, but our results indicate that an unexpectedly rapid change of glycolipid composition can occur at certain stages of differentiation. Similar rapid changes may occur during the course of differentiation of normal hematopoietic cells. Ganglio-series glycolipid is a minor component, and globo and lacto series are

dominant in most mature hematopoietic cells. But in some leukemia cells, ganglio-series glycolipids have been detected chemically or immunologically (29, 30). It is possible that each series represents specific stages of differentiation. An unknown soluble factor (or factors) secreted by embryonic fibroblasts triggered the shift of glycolipid synthesis from ganglio to lacto and globo series and initiation of branch formation in lacto-series glycolipid in M1 cells. This suggests that the stage-dependent expression of glycolipid antigens is partly mediated or regulated by humoral factors as well.

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