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Brief Communication

Structural and biosynthetic features of the Mo5 human myeloid differentiation antigen

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This report describes the basic structural and biosynthetic features of Mo5, a novel plasma membrane differentiation antigen (Ag) of the human myeloid lineage. As defined by the binding of anti-Mo5 monoclonal antibody (mAb), this Ag is expressed by virtually all myeloperoxidase-positive myeloid precursors beyond the CFU-GM in human bone marrow and is retained by circulating monocytes and polymorphonuclear neutrophils (PMNs) (1). Other hematopoietic elements of blood and bone marrow are Mo5-negative (1). Among myeloid cell lines, the monocytoid U-937 (2) and THP-1 (3) lines are Mo5-positive, while HL-60 (4) and KG-1 (5) are essentially negative (1). Mo5 expression by these myeloid lines (either positive or negative) exhibits no significant change as a result of their exposure to soluble stimuli inducing monocytic differentiation, which is in contrast to the acquired expression of Mo1 (CD11b/CD18) under the same conditions (1). Among leukemic cells of myeloid and lymphoid origin, Mo5 expression is restricted to acute myelogenous leukemia (FAB grp. M4, M5 > M1–3), providing an additional laboratory feature to distinguish the malignant cells of this lineage (6).

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The surface expression of Mo5 by PMNs has been shown to be modulated by factors that include degranulating stimuli, which produce a threefold increase in the number of antigenic sites, and anti-Mo5 mAb which induces antigenic down-modulation with the reversible loss of Mo5 expression (1). Although monocytes demonstrate a similar down-modulation of Mo5 surface expression after exposure to anti-Mo5 mAb, no analogous up-modulation of surface Mo5 by activating stimuli (e.g., PMA) has been demonstrated (1). Thus, Mo5 represents a unique myelomonocytic differentiation Ag whose surface expression is inducibly altered depending on the cell type and nature of the stimulus. To extend our characterization of the Mo5 Ag, we have pursued a series of immunoprecipitation experiments which serve to define its basic structural and biosynthetic features.

The anti-Mo5 mAb (clone 99, IgG2a) was purified from ascites by column chromatography using protein A-Sepharose (CL-4B, Pharmacia, Uppsala, Sweden) (7). Anti-Mo5-protein A-Sepharose conjugate was prepared according to the method of Schneider et al. (8). Control conjugates were made according to the same protocol using anti-Mo1 (9) and anti-BMM1 (10) mAb. Ag immunoprecipitation was accomplished by adding 10 μl aliquots of prewashed, packed mAb-protein A-Sepharose

conjugates to 150–200 µl of precleared radiolabeled lysate and tumbled at room temperature for 2 h (11). The immunoadsorbed conjugates were washed 4 times with 10 mM Tris-HCl, pH 8.6, 0.1% SDS, 0.05% NP-40, 300 mM NaCl. The washed conjugate pellets were eluted by boiling at 100°C for 5 min under either reducing or non-reducing conditions and subjected to SDS-PAGE (12).

As shown in Fig. 1, Mo5 appeared as a single band of 50 kD on SDS-PAGE after immunoprecipitation of Ag from NP-40 lysates of ^{125}I-labeled (13) U-937 cells (Fig. 1; lane 1; U-937, non-reducing not shown) and as a somewhat more heterogeneous species of similar m.w. from FMLP-stimulated PMNs (14, 15) (Fig. 1; lanes 3, 5). In both cell types, the electrophoretic mobility of Mo5 was unaltered by reducing conditions suggesting the absence of intrachain disulfide bonds (U-937, non-reducing; data not shown). ^{3}H-labeling of surface glycan residues (16) using sodium borohydride [NaB(^{3}H)$_4$] produced identical results (Fig. 1; lanes 7, 9), indicating that Mo5 is a glycoprotein Ag.

To assess the nature and extent of glycosylation of the surface molecule, glycosidase digestion (17) of the immunoprecipitated Mo5 Ag on U-937 cells and FMLP-stimulated PMNs was performed. As shown in Fig. 2, Mo5 is a highly glycosylated molecule comprised of predominantly N-linked complex carbohydrate as reflected by a nearly 40% reduction in apparent m.w. of the immunoprecipitated Ag from both cell types (Fig. 2; lanes 3, 4) after treatment with N-Glycanase, which cleaves nearly all N-linked oligosaccharides between the asparagine and terminal carbohydrate residues (18). Despite the apparent heterogeneity of native PMN-derived Ag, only a single homogeneous band was observed after N-Glycanase digestion (identical to the m.w. of N-Glycanase-digested U-937 Ag). Digestion with Endo-H, which cleaves high-mannose and some hybrid, but not complex N-linked oligosaccharides (19), resulted in an approximate 3 kD reduction in apparent m.w. (Fig. 2; lanes 5, 6 compared to 'sham' lanes 1, 2). This is consistent with the presence of a relatively small component of high-mannose or hybrid asparagine-linked structures in the surface glycoprotein. Neuraminidase treatment reduced the apparent m.w. of Mo5 by 1–3 kD, indicating the presence of sialic

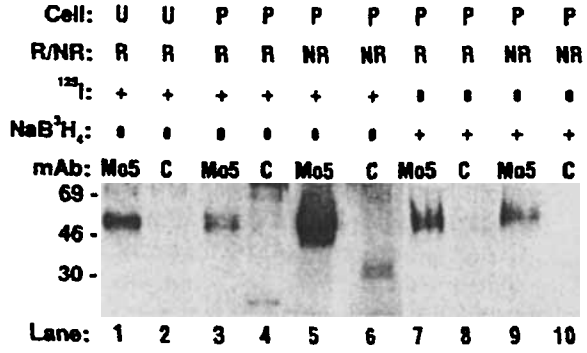

Figure 1. Composite SDS-PAGE of Mo5 immunoprecipitated from NP-40 lysates of surface radiolabeled U-937 cells (U) and FMLP-stimulated PMNs (P) under reducing (R) and nonreducing (NR) conditions (U-937, nonreducing; not shown). Unstimulated U-937 cells and PMNs exposed to 1 µM FMLP for 15 min were surface labeled with ^{125}I or NaB^{3}H$_4$, and lysed in NP-40-containing buffer. Mo5 Ag was immunoprecipitated from precleared NP-40 lysates, eluted in sample buffer in the presence (reducing) or absence (nonreducing) of 2-ME and subjected to SDS-PAGE (10% acrylamide). Even-numbered lanes represent isotype-identical control (C) immunoprecipitates (IgG2a; anti-CD11b 44 mAb or anti-BMM1 mAbs). In lanes 1–4, the Ag shown were "sham-treated" under the conditions of N-glycanase digestion described in Fig. 2 prior to SDS-PAGE. Numbers on left represent the m.w. of protein standards: BSA, 69 400; ovalbumin, 46 000; and carbonic anhydrase, 30 000. Similar results were obtained in 3 other experiments. No specific bands of a higher or lower m.w. than what is shown were observed.

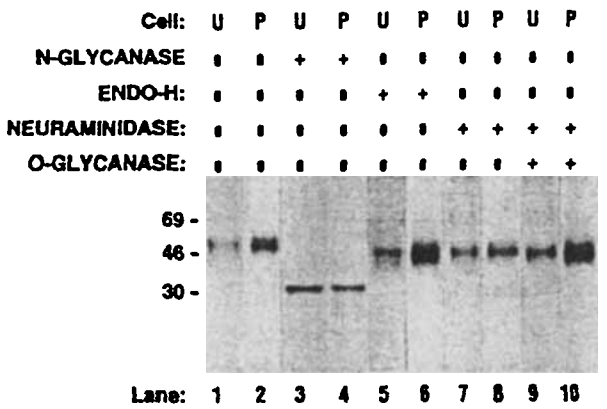

Figure 2. SDS-PAGE analysis of glycosidase sensitivity of Mo5 Ag immunoprecipitated from precleared NP-40 lysates of radioiodinated U-937 cells (U) and FMLP-stimulated PMNs (P) under reducing conditions. After immunoprecipitation, ^{125}I-labeled Mo5 Ag was eluted from the immunoconjugate and subjected to glycosidase treatment overnight a 37°C with N-glycanase (N-linked carbohydrate), lanes 3, 4; Endo-H (high-mannose), lanes 5, 6; neuraminidase (sialic acid), lanes 7, 8; and neuraminidase/O-glycanase (O-linked carbohydrate), lanes 9, 10, versus sham treatment, lanes 1, 2. Neuraminidase treatments, alone or preceding O-glycanase digestion, were performed at 37°C for 1 h. Digestions were performed in parallel with immunoprecipitates from isotype-identical control immunoconjugates which were devoid of radiolabeled material in the relevant m.w. range for Mo5 (not shown). Results shown are a composite of six separate gels in which enzyme-treated Ag were run in parallel with sham-treated Ag (under identical conditions) on the same gel in adjacent lanes allowing reliable detection of small differences in apparent m.w. For simplicity, only two (of six) representative sham-treated controls are shown under conditions of neuraminidase/O-glycanase digestion. Similar results were obtained in two additional experiments for each cell type.

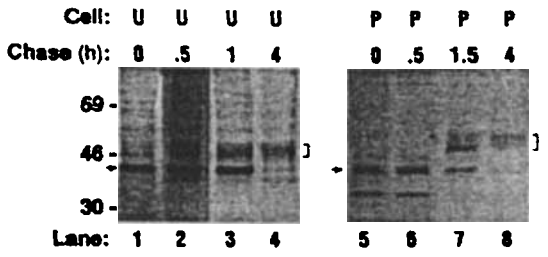

Figure 3. Pulse-chase analysis of ^{35}S-methionine-labeled Mo5 Ag from U-937 cells (U) and FMLP-stimulated PMNs (P) by SDS-PAGE under reducing conditions. U-937 cells (lanes 1–4) and FMLP-stimulated PMNs (lanes 5–8) were pulse-labeled for 15 min in medium containing ^{35}S-methionine, and then 'chased' with medium containing an excess of cold methionine for the times indicated prior to NP-40 cell lysis and immunoprecipitation. Arrows and brackets indicate the electrophoretic mobility of the precursor and mature form, respectively. In U-937 lanes, faint bands of approximately 47 kD were present in all immunoprecipitates, including isotype-identical controls (not shown), consistent with non-specific background. Similarly, bands of approximately 35 kD were present in all PMN lanes, including isotype-identical control immunoprecipitates (not shown), consistent with non-specific background. Lanes 7 and 8 were contrast-enhanced for purposes of reproduction. Similar results were obtained in two additional experiments for each cell type.

acid on carbohydrate branches (Fig. 2; lanes 7, 8), but subsequent O-Glycanase digestion of neuraminidase-treated Ag produced no further reduction in the m.w. of Mo5 (Fig. 2; lanes 9, 10), suggesting there are no O-linked oligosaccharides on the surface molecule.

The biosynthesis of Mo5 in two cell types was examined by ^{35}S pulse-chase experiments. U-937 cells and FMLP-stimulated PMNs were pulse-labeled for 15 min in medium containing ^{35}S-methionine, and then 'chased' with medium containing an excess of 'cold' methionine for the times indicated prior to NP-40 cell lysis and immunoprecipitation (20) (Fig. 3). In U-937 cells, analysis by SDS-PAGE revealed a single band of 41 kD at 0 min. By 30–60 min, a broader band of 47–55 kd was apparent in addition to the 41 kD component. By 4 h, the 41 kD 'precursor' molecule was superseded by the broader 47–55 kD band (Fig. 3; lanes 1–4), approximating the electrophoretic mobility of the 'mature' surface-labeled glycoprotein. Similarly, in PMNs, Mo5 appeared initially as a 43 kD band at 0 min. By 1 hour, a second broader band of 46–52 kD was seen which, by 4 h (Fig. 3; lanes 5–8), superseded the 'precursor' band, reflecting the 'mature' form of the surface glycoprotein (Fig. 1; lane 3 vs. Fig. 3; lane 8). Early glycosylation events concurrent with polypeptide synthesis (19) would account for the ~10 kD difference in m.w. between the N-Glycanase-treated surface Ag and the internally-labeled precursor form. No immunoprecipitated Ag was detected in lysates of biosynthetically labeled, tunicamycin-treated cells (data not shown). Our inability to demonstrate immunoprecipitable Ag after cells were incubated in the presence of tunicamycin, which selectively blocks the initiation of N-linked glycosylation (data not shown), may suggest either that the Mo5 antigenic epitope is in fact an oligosaccharide or that it is rapidly degraded under conditions which prevent N-linked glycosylation. Although surface expression of Mo5 on PMNs is immediately up-modulated several fold by exposure to FMLP, presumably as a consequence of its expression by granular membranes, FMLP had no discernible effect on the biosynthesis of Mo5 at 0 min or 4 h in stimulated PMNs as compared to unstimulated cells (data not shown).

To examine the possibility that Mo5 is among the varied group of surface macromolecules linked to the plasma membrane via glycosyl-phosphatidylinositol (GPI) (21), we analyzed surface expression of Mo5 on PMNs before and after exposure to PI- PLC via indirect immunofluorescence analysis (17). No change in Mo5 surface expression as detected by anti-Mo5 mAb binding was seen, in contrast to the marked reduction of a PI-PLC sensitive antigen, CD16, under the same conditions (data not shown).

Of particular interest is the finding that Mo5 is one of but a few glycoproteins which have been shown to be actively synthesized by the mature, differentiated PMN, traditionally thought to be metabolically inactive, incapable of significant *de novo* protein synthesis (22). Recent efforts to assess the synthetic activity of the neutrophil have established that the mature blood PMN retains the ability to synthesize, *de novo*, a broad spectrum of macromolecules such as the cytokines IL-1 (23), IL-6 (24), IL-8 (25), G-CSF (26), M-CSF (26), and IFNα (27); certain extracellular matrix proteins such as fibronectin and thrombospondin (28); and the protease inhibitor, alpha 1-antiproteinase (α1-PI) (29). Mo5 can be distinguished as a novel addition to this select group of macromolecules on the basis of its distinct m.w. of 50 kD, or, in the case of α1-PI (also 50 kD), by its membrane localization (no surface staining by anti-α1-PI [data not shown]).

Likewise, whereas Mo5 shares certain characteristics with other previously identified myeloid surface markers including its relative lineage specificity (30), up-modulation of surface expression on PMNs after exposure to soluble activating stimuli (31), and down-modulation caused by exposure to divalent mAb (32), it demonstrates unique features

Table 1.
Distinction between Mo5 and other myeloid surface antigens

Antigen[a]	Predominant Hematopoietic Cellular Reactivity	m.w. (kD)	PI-Linked[b]	Up-Modulation[c]	References[d]
Mo5	M, PMN[e]	50	No	Yes	1, this report
Mo3	M, (PMN)	42–71	Yes	No	17
CD11b	M, PMN, LGL	165	No	Yes	
CDw12	M, PMN	90–120	UK	UK	
CD13	CFU-GM, M, PMN	150	No	Yes	
CD14	M, MØ, (PMN)	55	Yes	Yes	
CD15	CFU-GM, PMN	(3-FAL, x-hapten)	NA	NA	
CD16	MØ, PMN, LGL	50–65	Yes[f]	Yes[g]	31, 33
CDw17	M, PMN, P	Lactosylceramide	NA	UK	
CDw32	MØ, PMN, B, E	40	No	UK	
CD33	CFU-GM, M	67	No	No	
CD35	M, PMN, B	160, 190, 220, 250	No	Yes	
CD48	(PMN)	41 (?)	Yes	No	
CD64	M, (PMN)	75	UK	No	
CDw65	M, PMN	Ceramide-Dodeca-saccharide 4c	NA	NA	
CD66	CFU-GM, PMN	180–200	No	Yes	
CD67	PMN	100	Yes	Yes	
CD68	MØ	110	UK	UK	

[a] As defined by the Fourth International Workshop and Conference on Human Leukocyte Differentiation Antigens (30).
[b] Sensitivity to PI-PLC.
[c] "Up-modulation" defined here as a *rapid* (within minutes) increase in surface expression after exposure of PMNs or monocytes to soluble activating stimuli (e.g., FMLP, PMA).
[d] The major source for the data shown is Leukocyte Typing IV – White Cell Differentiation Antigens (30). Citations indicate supplementary references.
[e] Abbreviations used: CFU-GM: colony forming unit-granulocyte, macrophages; M: monocyte; PMN: polymorphonuclear neutrophil; LGL: large granular lymphocyte (natural killer cell); MØ: macrophage; P: platelet; B: B cell; E: eosinophil; UK: unknown; PI: phosphatidylinositol; NA: not applicable; cell designation in parentheses ( ) indicates weak expression.
[f] PMN only (transmembrane form on M, LGL).
[g] CD16 on PMNs is up-modulated by purification procedures (31); subsequent exposure of PMNs to soluble activating stimuli results in release or shedding of surface Ag (33).

that distinguish it from other antigens recognized by the Fourth International Workshop on Human Leukocyte Differentiation Antigens (30), as seen in Table 1.

The specific function of the Mo5 plasma membrane glycoprotein remains to be elucidated. Since anti-Mo5 mAb has no inhibitory effect on neutrophil phagocytosis (34), enzyme release (34), aggregation (35), chemotaxis (1), or neutrophil-mediated tissue injury (36), the possibility exists that the epitope recognized by this reagent is functionally inert. Current efforts to raise a polyclonal antibody against affinity-purified Mo5 antigen may allow a better opportunity to screen for functional blocking activity. The results of recent preliminary experiments employing anti-Mo5 mAb (in the form of $F[ab]_2$ fragments) do, however, suggest the possibility that Mo5 has receptor-like activity: PMNs exposed to anti-Mo5 $F[ab]_2$ fragments demonstrate a specific, transient increase in intracellular calcium, which, as observed for other receptors, may be analogous to the effect of natural ligand in generating calcium transients and other second messengers (37).

In summary, Mo5 is a novel 50 kD glycosylated surface macromolecule unique to human myelomonocytic cells comprised of approximately 40% complex carbohydrate. It is synthetized in both U-937 cells and PMNs as a 41–43 kD precursor which is converted to the 'mature' 50 kD glycoprotein within 1–4 h. On comparison of its profile of lineage specificity, apparent molecular weight and carbohydrate composition, membrane linkage and modulated surface expression, it is unique among presently defined myelomonocytic differentiation antigens (30). Efforts are underway to elucidate the function of this molecule and to isolate the Mo5 gene utilizing expression cloning techniques.

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