REVIEW ARTICLE

Lysosomal Membrane Glycoproteins in Platelets

Marcel J. Metzelaar^{1, 2} and Hans C. Clevers¹From the Department of ¹Immunology and ²Haematology, University Hospital, Utrecht, The Netherlands

Introduction

Lysosomes have originally been defined in 1963 as organelles containing a variety of acid hydrolases (enzymes with an optimal activity in the pH range 3.5–5.5) that are involved in degradation of extracellular and intracellular material (1, 2). Due to variations in their content of acid hydrolases and internalized material, lysosomes are morphologically heterogeneous in different cell types.

Blood platelets contain three different types of granules: dense-granules, α -granules and primary lysosomes. These organelles harbor most biologically active platelet proteins. Primary lysosomes in platelets are morphologically distinct from α -granules and dense granules (3). Furthermore, they are the only platelet granules containing acid hydrolases. Platelet lysosomes differ from the two other platelet granules with respect to the stimulus necessary to induce secretion. Secretion of dense- and α -granule contents is observed after stimulation with all known platelet agonists, whereas lysosomal secretion requires a powerful stimulus and is only observed after stimulation with high concentrations of thrombin and collagen. Moreover, lysosomal secretion is slower and incomplete (maximally 60% of the granules); in contrast, dense- and α -granules secrete close to 100% of the granule contents within 1–2 min (4). The secreted lysosomal enzymes may be involved in clearing of platelet thrombi, or inactivation and degradation of heparin (5). During platelet secretion, granule membranes fuse with the surface-connected system and the plasma membrane. As a consequence, granule membrane proteins become expressed on the plasma membrane. This has been shown to occur for a 40 kDa membrane protein of dense-granules, the α -granule membrane proteins P-Selectin (PADGEM, GMP-140, CD62 antigen) and GMP-33 as well as for the lysosomal membrane glycoproteins LIMP-CD63, Lamp-1 and Lamp-2 (6–12).

In contrast to the knowledge of the platelet α -granule membrane proteins much less attention has been paid to platelet lysosomal membrane proteins. In recent years, several lysosomal membrane proteins have been characterized and their structures determined by cDNA cloning. This review will summarize current developments in this area.

Structure of Lysosomal Membrane Glycoproteins

In eukaryotic cells, the lysosomal membrane plays a pivotal role in a wide variety of specific lysosomal functions, including the maintenance of the acidic milieu of the lysosome and the translocation of amino acids, fatty acids and carbohydrates across the lysosomal membrane [for review see Kornfeld and Mellman (13)]. Moreover, the lysosomal membrane specifically recognizes and fuses with restricted subsets of other cytoplasmic organelles in the cell, including endosomes and the plasma membrane.

In order to understand the processes that involve the lysosomal membrane, several lysosomal membrane glycoproteins have been identified recently using polyclonal and monoclonal antibodies against purified lysosomal membranes (14, 15). The molecular weight of the identified glycoproteins ranges from 20 to 150 kDa. The major components of the lysosomal membrane, representing approximately 50% of total integral membrane protein are proteins with a molecular weight of 90–120 kDa (15). Among these, two distinct but highly homologous glycoproteins have been distinguished: the Lysosome-Associated Membrane Protein-1 (Lamp-1) and -2 (Lamp-2). Both proteins have been identified in various species. Lamp-1 is termed Igp 120 in rat and mouse (16, 17), LEP-100 in chicken (18), and Lamp-1 in mouse and human (19, 20). Lamp-2 is alternatively named Igp 110 in rat. cDNAs encoding these glycoproteins have been isolated. The deduced amino acid sequences revealed that Lamp-1 and -2 are highly related. They both contain a polypeptide core of approximately 40 kDa with a high number of N-linked glycosylation sites. Glycosylation accounts for approximately 60% of the total molecular mass. The Lamps are Type I-integral membrane proteins with large luminal domains of 380–396 amino acids, partitioned into two internally homologous domains separated by a 25–30 amino acid segment rich in proline, serine and threonine. Each domain contains 4 cysteines. The positions of the cysteine residues are well conserved between Lamp-1 and -2 and among the different species. Neighbouring cysteine residues are linked to each other, forming two disulfide loops of 36–38 amino acids in each domain. All Lamps contain a single transmembrane region and a short cytoplasmic tail of 10–11 amino acids (see Fig. 1B) (20). Both Lamp-1 and -2 have been described to be present in the platelet lysosomal membrane (11, 12).

Recently, a rat lysosomal membrane glycoprotein, LIMP II, was described (14, 21). LIMP II is a 74 kDa glycoprotein expressed in the membrane of lysosomes and secretory granules with lysosomal properties. The cDNA sequence revealed it to consist of 478 amino acids and to possess two transmembrane regions: an uncleavable signal peptide at the NH₂ terminus and a hydrophobic amino acid segment at the COOH terminus. The short NH₂ and COOH terminal ends of the protein probably represent cytoplasmic tails of this Type II-integral membrane glycoprotein. The major portion of the protein resides on the luminal face of the lysosome and contains 11 potential N-glycosylation sites and 5 cysteine residues (Fig. 1C). No homology between LIMP II and the lysosomal membrane proteins Lamp-1 and -2 was obvious. However, extensive homology was observed between LIMP II and human CD36 (platelet glycoprotein IV), a plasma membrane glycoprotein involved in cell adhesion (22). At present no data are available on LIMP II expression in the platelet lysosomal membrane.

A third major lysosomal membrane glycoprotein is the CD63 antigen. The CD63 antigen is a lysosomal integral membrane glycoprotein (LIMP-CD63) with a molecular weight of 30–60 kDa that was first described on platelets as an activation marker (10, 11). LIMP-CD63 is a protein of 238 amino acids with

Correspondence to: Dr. Marcel J. Metzelaar, Department of Immunology, University Hospital, P.O. Box 85500, 3508 GA Utrecht, The Netherlands

Table 1 Properties of the major lysosomal membrane glycoproteins

	Lamp-1	Lamp-2	LIMP II	LIMP-CD63
Synonyms				
Human	hlamp-1	hlamp-2		GP53, pltgp40, ME491, NK1/C3
Rat	LIMP III lgp120	LIMP IV lgp110	lgp85	LIMP I
Mouse	mLAMP-1	mLAMP-2		
Chicken	LEP100			
Molecular weight (kDa)	90–120	95–120	60–85	30–60
Number of aminoacids	382–396	380–389	478	238
Number of N-glycosylations	17–20	16–17	11	3
Gly-Tyr motif in cytoplasmic tail	yes	yes	no	yes

four putative hydrophobic transmembrane regions. Three N-linked glycosylation sites are present in a hydrophilic domain between the third and fourth transmembrane region. Biochemical observations indicate that these sites are indeed utilized and permit the conclusion that this region is exposed to the lumen of the endoplasmic reticulum during biosynthesis. Fig. 1A gives a schematic representation of the predicted orientation of the LIMP-CD63 in the lysosomal membrane assuming that each hydrophobic region traverses the membrane once. Database searches have revealed that LIMP-CD63 is identical to ME491, a protein originally described on the plasma membrane of malignant melanomas, nevocellular nevi, neuroendocrine tumors and adenocarcinomas (23–25). However, LIMP-CD63 expression has also been observed in the lysosomes of a wide variety of other cell types (11, 26), indicating that it should now be considered as a ubiquitous protein. Recently, Pltgp40, a 40 kDa platelet glycoprotein, was found to be identical to LIMP-CD63 and ME491 (27, 28).

The properties of the major lysosomal membrane glycoproteins are summarized in Table 1.

Fig. 1 Putative structure of LIMP-CD63, Lamp-1 and LIMP II. Cysteine residues are indicated by C. (●), N-glycosylation sites; (O), O-glycosylation sites. A. LIMP-CD63 adapted from reference (70). LIMP-CD63 contains four putative transmembrane domains. It is assumed that each hydrophobic domain traverses the membrane once. B. Lamp-1 adapted from reference (71). The majority of the Lamp-1 molecule resides in the lumen of the lysosome. It contains eight cysteines, neighbouring cysteine residues are linked to each other, forming four disulfide loops of 36–38 amino acids. Lamp-2 has an almost identical structure. C. LIMP II adapted from reference (21). LIMP II possesses two putative transmembrane regions. The major of the molecule resides on the luminal face of the lysosome

LIMP-CD63 Is a Member of a Novel Structurally Distinct Family of Glycoproteins

Recently, a group of proteins with homology to LIMP-CD63 has been identified. This group consists of p24/CD9, a surface marker present on a wide variety of hemopoietic and nonhemopoietic tissues (29, 30), the leukocyte antigens CD37 (31) and CD53 (32, 33), TAPA-1 (target of an antiproliferative antibody-1) (34), the R2 antigen (35), the tumor-associated antigen CO-029 (36), as well as the *Schistosoma mansoni* antigen Sm23 (37). The members of this family share a number of features in their overall structure. These include four putative transmembrane domains with identical spacing, similar distribution of cysteine residues and conservation of N-linked glycosylation sites (38).

At present, no definitive biological function is known for any of these molecules, although several functions have been suggested. p24/CD9 might participate in signal transduction processes of platelets and other cell types. Antibodies to the p24/CD9 antigen cause platelet activation and aggregation. Anti-CD37 monoclonal antibodies have been shown to modulate activation of B lymphocytes. The TAPA-1 antigen may be involved in cell growth (34). Antibodies to LIMP-CD63 (ME491) have been reported to inhibit monocyte-adherence to serum-coated surfaces, and aggregation of T and B lymphocytes (38). These data suggest that the members of this family may participate in signal transduction.

Intracellular Routing of Lysosomal Membrane Glycoproteins

In nucleated eukaryotic cells, lysosomal sorting of membrane proteins differs from that of soluble proteins. Most soluble proteins are transported to lysosomes by a mannose 6-phosphate (M6-P)-dependent mechanism. After synthesis in the endoplasmic reticulum, M6-P is linked to lysosomal enzymes in the medial Golgi apparatus. M6-P groups subsequently bind to the mannose 6-phosphate receptor (MPR) in the trans Golgi. Enzyme/receptor complexes are then transported to endosomes, where they are dissociated by low pH [for review see Kornfeld and Mellman (13)].

Lysosomal membrane glycoproteins do not contain M6-P and, in contrast to soluble proteins are routed from the Golgi complex to lysosomes independently of MPRs (14, 16). It is assumed that lysosomal membrane glycoproteins contain a positive signal in their peptide sequence that regulates the sorting from the Golgi complex to lysosomes. Comparison of the amino acid sequence of Lamp-1 and -2, lysosomal acid phosphatase (LAP) (39), an enzyme synthesized as an integral membrane protein precursor, and LIMP-CD63 does not display any overall similarity. However, alignment of the short cytoplasmic tail sequences of Lamp-1, -2, LAP, and LIMP-CD63 reveals the presence of a conserved glycine-tyrosine dipeptide (Fig. 2). Site-directed mutagenesis of human Lamp-1 and the LAP cytoplasmic tail sequences has shown that the tyrosine residue and its relative position to the membrane and to the carboxy terminus of the protein were necessary for efficient targeting of these lysosomal membrane glycoproteins to lysosomes (40, 41). Truncation of the cytoplasmic tail or substitution of the tyrosine residue in the human Lamp-1 cytoplasmic tail for an alanine or phenylalanine resulted in accumulation of the protein on the plasma membrane. Moreover, the construction of a chimeric reporter molecule bearing the human Lamp-1 cytoplasmic tail was sufficient to cause redistribution of the chimeric molecule to lysosomes (40). Similar results were obtained for LAP (41).

Two possible pathways for the sorting of lysosomal membrane glycoproteins have been suggested. In the first pathway,

Fig. 2 Comparison of cytoplasmic tail sequences of lysosomal membrane glycoproteins. Alignment of the short cytoplasmic tail sequences of Lamp-1, Lamp-2, LIMP-CD63 and lysosomal acid phosphatase (LAP), an enzyme synthesized as an integral membrane protein precursor, reveals the presence of a conserved Glycine-Tyrosine dipeptide

lysosomal membrane glycoproteins are sorted from plasma membrane molecules and soluble proteins in the Golgi complex, and are transported directly to late endosomes and lysosomes. Alternatively, transport of the lysosomal membrane glycoproteins might occur via the plasma membrane along with plasma membrane molecules. They are then sorted after transportation to the plasma membrane by internalization, followed by targeting to lysosomes. Evidence has been presented for both pathways. Various studies have described the expression of lysosomal membrane glycoproteins on the plasma membrane. In baby hamster kidney cells, LAP could be detected on the cell membrane before endocytosis and eventually transported to lysosomes (42). In another study, however, LAP was reportedly transported to lysosomes without surface expression (43). In addition, kinetic studies on the biosynthesis of Lamp molecules in macrophages and fibroblasts suggest that Lamp molecules are not transported to the plasma membrane before reaching the lysosomes, but are sorted directly from Golgi complex (44, 45). A study using CHO cells stably transfected with wildtype Lamp-1 or mutants affecting the glycine-tyrosine dipeptide in the cytoplasmic tail, also showed that the majority of newly synthesized Lamp-1 does not pass through the cell surface when routed to lysosomes (46). If both sorting pathways coexist, the same sorting signal (the tyrosine-containing cytoplasmic tail) may mediate intracellular sorting as well as endocytosis.

The lysosomal integral membrane protein LIMP II does not contain a tyrosine residue in its cytoplasmic tail. Construction of chimeric molecules of the plasma membrane molecules CD36 and CD8 with the cytoplasmic tail of LIMP II showed that this tail was necessary and sufficient for transport of the chimeric molecules from the Golgi complex to lysosomes. Time course studies of the cellular distribution revealed that the routing to lysosomes did not pass the plasma membrane, suggesting that the sorting occurred directly from the Golgi compartment to lysosomes. These data indicate that various targeting pathways may coexist that mediate sorting of lysosomal membrane glycoproteins from the Golgi complex to lysosomes, both dependent and independent of a tyrosine-containing cytoplasmic tail.

Plasma Membrane Expression of Lysosomal Membrane Glycoproteins

At present three membrane glycoproteins have been described in human platelet lysosomes: the homologous lysosome associated membrane proteins Lamp-1 and -2, and the lysosomal integral membrane glycoprotein LIMP-CD63. These molecules can be detected on the platelet plasma membrane after activation. Studies with radiolabeled monoclonal antibodies revealed that resting platelets express little or no Lamp-1 and -2 and LIMP-CD63 on their surface. Platelets activated with thrombin expressed approximately 1,000 Lamp molecules and 9,000–13,000 LIMP-CD63 molecules on their surface (10–12,

47). LIMP-CD63 was originally described as a platelet activation marker, however, surface expression has also been induced on other cell types: e. g. formyl-methionyl-leucyl-phenylalanine- and IgE-mediated activation of basophilic granulocytes increases the amount of surface expressed LIMP-CD63 (48). In cytotoxic T-lymphocytes (CTLs), comparable phenomena were observed. CTLs exocytose granules during specific interaction with target cells. In this process, the granule contents (including the lethal proteins perforin and serine-esterases) are secreted into the intercellular cleft formed upon CTL/target cell interaction. Immunoelectron microscopic studies revealed that Lamp-1, Lamp-2 and LIMP-CD63 are abundantly present on the granule-delimiting outer membrane, which becomes incorporated into the CTL plasma membrane (49). The surface expression of Lamp-1 and -2 and LIMP-CD63 on activated platelets, basophils and CTLs correlates with exocytosis of the lysosomal contents upon cell activation and is explained by fusion of the lysosomal membrane with the plasma membrane.

The Function of Lysosomal Membrane Glycoproteins

Although the biochemical structure of many lysosomal membrane glycoproteins is known, and their cDNAs have been cloned, no specific functions have been identified for these molecules thus far. All lysosomal membrane molecules are heavy glycosylated, and Lamp molecules have been identified to be rich in poly-N-acetylglucosamines (50). When synthesized in the presence of tunicamycin, which inhibits N-glycosylation, Lamp molecules free of N-linked carbohydrates displayed shorter half-lives than fully glycosylated molecules (14). Moreover, the half-life of Lamp-1 and -2 is increased during differentiation of HL 60 cells to granulocytes. This differentiation is accompanied by increased amounts of poly-N-acetylglucosamines present at N-glycosylation sites of Lamp-1 and -2 (51, 52). Since the N-glycosylation of the Lamp molecules protects them from degradation by various hydrolytic enzymes, the presence of a continuous coat of highly glycosylated lysosomal membrane proteins at the luminal site of the lysosome may possibly protect the delimiting membrane against degradation by lysosomal hydrolases.

In conjunction with this possible function, the expression of lysosomal membrane glycoproteins on the plasma membrane of activated platelets, cytotoxic T-cells and various other cell types during lysosome secretion may serve a function, e. g. the protection of the plasma membrane from degradation by released lysosomal hydrolases. Recently it was shown that highly metastatic tumor cells express more Lamp molecules and poly-N-acetylglucosamines on their cell surface than poorly metastatic cells (53, 54). Moreover, LIMP-CD63 (ME491) has been described as being abundantly present on the plasma membrane of cells in malignant melanomas, nevocellular nevi, neuroendocrine tumors and adenocarcinomas (23–25). One of the factors that enable tumor cells to become invasive is the secretion of lysosomal enzymes to degrade surrounding extracellular matrix (55). The presence of poly-N-glycosamines in highly metastatic tumor cells may protect the tumor cells during degradation of surrounding tissues. However, the surface expression of lysosomal membrane glycoproteins may just be a by-product of lysosome-plasma membrane fusion during lysosome secretion.

To establish metastasis, blood-borne tumor cells lodge in capillary beds between endothelial cells (56). This process can be mediated by interaction of adhesion molecules of the Selectin family on the surface of endothelial cells and tumorigenic cells (57–59). The adhesion molecules of the Selectin family, present on platelets and endothelial cells bind to sialyl Le^x-structures on neutrophils and monocytes at sites of inflammation (60–62).

Furthermore, several laboratories showed that on the cell surface of various tumors in situ the amount of sialyl Le^x- and sialyl Le^a-structures is increased. Since as much as 30% of the sialyl Le^x-structures can be carried by the cell surface Lamp molecules, it is possible that the increased expression of Lamp molecules on the surface of highly metastatic tumors may function as mediator of cell adhesion between endothelial cells and tumor cells at metastatic sites (54). In conjunction with this mechanism, the surface expression of lysosomal membrane glycoproteins on the surface of activated platelets may attribute to the binding of activated platelets to Selectin molecules expressed on endothelial cells.

The Use of Lysosomal Membrane Glycoproteins for the Detection of Activated Platelets

Clinical studies indicate that platelets are activated in a number of vascular disorders such as arteriosclerosis and thrombosis. Detection of activated platelets may facilitate the identification of thrombotic processes. The activation of platelets is associated with changes in platelet morphology, biochemistry and membrane composition. Exposure of granule membrane proteins during platelet secretion (as a result of the fusion of granule membranes with the plasma membrane) has been described for a 40 kDa membrane protein of dense-granules, the α -granule membrane proteins P-Selectin (PADGEM, GMP-140, CD62 antigen) and GMP-33, and the lysosomal membrane antigens LIMP-CD63 and Lamp-1 and -2. In contrast to other methods that measure platelet activation, flow cytometric analysis of the platelet-surface allows the detection of changes in specific epitopes induced by activation, and it provides a direct indication for the activation status in patients [for review see (47, 63)]. To date, flow cytometric analysis of the expression of lysosomal membrane proteins on platelets, as an indication for platelet activation, has been evaluated in a limited number of situations: during platelet storage, and during cardiopulmonary bypass surgery. Increased expression of LIMP-CD63 during storage could be observed in platelet concentrates stored under normal conditions, indicating that platelet degranulation occurs (64, 65). In patients undergoing cardiopulmonary bypass surgery, a model for in vivo platelet activation, increased numbers of platelets expressing LIMP-CD63 on their plasma membrane were observed after bypass as compared to blood samples drawn before extracorporeal perfusion, again indicative of platelet activation (10, 66).

In studies of clinical disorders, increased expression of LIMP-CD63 was observed in patients with deep venous thrombosis and in diabetes mellitus (10, 66, 67). Moreover, increased LIMP-CD63 expression could be detected in patients at risk for periprocedural occlusion after coronary percutaneous transluminal angioplasty (68). No increase of LIMP-CD63 expression was observed in patients with anti-phospholipid antibodies (69).

In summary, the data on the clinical utility of flow cytometric analysis of the expression of lysosomal membrane glycoproteins are scarce, and further studies will be necessary. It is anticipated that an important application will be the monitoring of platelet activation during preparation of platelet concentrates and during platelet filtration.

Acknowledgements

The authors wish to thank Drs. H. K. Nieuwenhuis, J. J. Sixma and G. Strous for critically reading the manuscript.

REFERENCES

- de Duve C. The lysosomal concept. In: Lysosomes. de Reuck AVS, Cameron MP (eds). Ciba Found. Symp. Churchill, London 1963, p 113.
- de Duve C. Lysosomes revisited. *Eur J Biochem* 1983; 137: 391-7.
- Sixma JJ, van den Berg A, Hasilik A, et al. Immunoelectron microscopical demonstration of lysosomes in human blood platelets and megakaryocytes using anti-cathepsin D. *Blood* 1985; 65: 1287-91.
- Holmsen H, Day HJ. The selectivity of the thrombin induced release reaction: subcellular localization of released and retained constituents. *J Lab Clin Med* 1970; 75: 840-55.
- Oosta GM, Favreau LV, Beeler DL, et al. Purification and properties of human platelet heparinase. *J Biol Chem* 1982; 257: 11249-55.
- Gerrard JM, Lint D, Sims PJ, et al. Identification of a platelet dense granule membrane protein that is deficient in a patient with the Hermansky-Pudlak Syndrome. *Blood* 1991; 77: 101-12.
- Berman CL, Yeo EL, Wencel-Drake JD, et al. A platelet alpha granule membrane protein that is associated with the plasma membrane after activation. Characterization and subcellular localization of platelet activation-dependent granule-external membrane protein. *J Clin Invest* 1986; 78: 130-7.
- Stenberg PE, McEver RP, Shuman MA, et al. A platelet alpha-granule membrane protein (GMP-140) is expressed on the plasma membrane after activation. *J Cell Biol* 1985; 101: 880-6.
- Metzelaar MJ, Heijnen HFG, Sixma JJ, et al. Identification of a 33 kDa protein associated with the alpha-granule membrane (GMP-33), that is expressed on the surface of activated platelets. *Blood* 1992; 79: 372-9.
- Nieuwenhuis HK, van Oosterhout JJG, Rozemuller E, et al. Studies with a monoclonal antibody against activated platelets: evidence that a secreted 53,000-mw lysosome-like granule protein is exposed on the surface of activated platelets in the circulation. *Blood* 1987; 70: 838-45.
- Metzelaar MJ, Wijngaard PL, Peters PJ, et al. CD63 antigen. A novel lysosomal membrane glycoprotein, cloned by a screening procedure for intracellular antigens in eukaryotic cells. *J Biol Chem* 1991; 266: 3239-45.
- Febbraio M, Silverstein RL. Identification and characterization of LAMP-1 as an activation-dependent platelet surface glycoprotein. *J Biol Chem* 1990; 265: 18531-7.
- Kornfeld S, Mellman I. The biogenesis of lysosomes. *Annu Rev Cell Biol* 1989; 5: 483-525.
- Barriocanal JG, Bonifacino JS, Yuan L, et al. Biosynthesis glycosylation, movement through the Golgi system and transport to lysosomes by a N-linked carbohydrate-independent mechanism of three lysosomal integral membrane proteins. *J Biol Chem* 1986; 261: 1604-7.
- Marsh M, Schmid S, Kern H, et al. Rapid analytical and preparative isolation of functional endosomes by free-flow electrophoresis. *J Cell Biol* 1987; 104: 875-86.
- Howe CL, Granger BL, Hull M, et al. Derived protein sequence, oligosaccharides, and membrane insertion of the 120-kDa lysosomal membrane glycoprotein (Igp 120): identification of a highly conserved family of lysosomal membrane glycoproteins. *Proc Natl Acad Sci USA* 1988; 85: 7577-81.
- Granger BL, Green SA, Gabel CA, et al. Characterization and cloning of Igp 110, a lysosomal membrane glycoprotein from mouse and rat cells. *J Biol Chem* 1990; 265: 12036-43.
- Fambrough DM, Takeyasu K, Lippincott-Schwartz J, et al. Structure of LEP100, a glycoprotein that shuttles between lysosomes and the plasma membrane, deduced from the nucleotide sequence of the encoding cDNA. *J Cell Biol* 1988; 196: 61-7.
- Chen JW, Cha Y, Yuksel KU, et al. Isolation and sequencing of a cDNA clone encoding lysosomal membrane glycoprotein mouse LAMP-1. Sequence similarity to proteins bearing onco-differentiation antigens. *J Biol Chem* 1988; 263: 8754-8.
- Fukuda M, Viitala J, Matteson J, et al. Cloning of cDNAs encoding human lysosomal membrane glycoproteins, h-Lamp-1 and h-Lamp-2. Comparison of their deduced amino acid sequences. *J Biol Chem* 1988; 263: 18920-8.
- Vega MA, Segui-Real B, Garcia AJ, et al. Cloning, sequencing and expression of a cDNA encoding rat LIMP II, a novel 74 kDa lysosomal membrane protein related to the surface adhesion protein CD36. *J Biol Chem* 1991; 266: 16818-24.
- Oquendo P, Hundt E, Lawler J, et al. CD36 directly mediates cytoadherence of plasmodium falciparum parasitized erythrocytes. *Cell* 1990; 58: 95-101.
- Atkinson B, Ernst CS, Ghrist BFD, et al. Identification of melanoma associated antigens. *Cancer Res* 1984; 44: 2577-81.
- Atkinson B, Ernst CS, Ghrist BFD, et al. Monoclonal antibody to a high glycosylated protein reacts in fixed tissue with melanoma and other tumors. *Hybridoma* 1985; 4: 243-55.
- Ernst CS, Shen JW, Litwen S, et al. Multiparameter evaluation of the expression in situ of normal and tumor associated antigens. *J Natl Cancer Inst* 1986; 77: 387-95.

26. Metzelaar MJ, Schuurman HJ, Sixma JJ, et al. Biochemical and immunohistochemical characterisation of CD62 and CD63 monoclonal antibodies. Expression of GMP-140 and LIMP-CD63 (CD63 antigen) in human lymphoid tissue. *Virchows Archiv B, Cell Pathol* 1991; 61: 269-77.
27. Azorsa DO, Hyman JA, Hildreth JE. CD63/Pltgp40: a platelet activation antigen identical to the stage-specific, melanoma-associated antigen ME491. *Blood* 1991; 78: 280-4.
28. Metzelaar MJ, Nieuwenhuis HK. Identity of Pltgp40 and lysosomal integral membrane protein-CD63 [letter]. *Blood* 1991; 78: 534-5.
29. Bourcheix C, Benoit P, Frachet P, et al. Molecular cloning of the CD9 antigen. A new family of cell surface proteins. *J Biol Chem* 1991; 266: 117-22.
30. Lanza F, Wolf D, Fox CF, et al. cDNA cloning and expression of platelet p24/CD9. Evidence for a new family of multiple membrane spanning proteins. *J Biol Chem* 1991; 266: 10638-45.
31. Classon BJ, Williams AF, Willis AC, et al. The primary structure of the human leukocyte antigen CD37, a species homologue of the rat MCR OX-44 antigen. *J Exp Med* 1989; 169: 1497-502.
32. Amiot M. Identification and analysis of cDNA clones encoding CD53, a Pan Leukocyte antigen related to membrane transport proteins. *J Immunol* 1990; 145: 4322-5.
33. Angelisová P, Vitek C, Stefanova I, et al. The human leukocyte surface antigen CD53 is a protein structurally similar to the CD37 and MRC OX-44 antigens. *Immunogenetics* 1990; 32: 281-5.
34. Oren R, Takashi S, Doss C, et al. TAPA-1, the target of an anti-proliferative antibody defines a new family of transmembrane proteins. *Mol Cell Biol* 1990; 10: 4007-15.
35. Gaugitsch HW, Hofer E, Huber NE, et al. A new superfamily of lymphoid and melanoma cell proteins with extensive homology to *Schistosoma mansoni* antigen SM23. *Eur J Immunol* 1991; 21: 377-84.
36. Szala S, Kasai Y, Steplewski S, et al. Molecular cloning of cDNA for the human tumor-associated antigen CO-029 and identification of related transmembrane antigens. *Proc Natl Acad Sci USA* 1990; 87: 6833-7.
37. Wright MD, Henkle KJ, Mitchell GE. An immunogenic mw 23000 integral membrane protein of *Schistosoma mansoni* worms that closely resembles a human tumor-associated antigen. *J Immunol* 1990; 144: 3195-200.
38. Horejsi V, Vitek C. Novel structurally distinct family of leukocyte surface glycoproteins including CD9, CD37, CD53 and CD63. *FEBS Lett* 1991; 288: 1-4.
39. Pohlman G, Krentler C, Schmidt B, et al. Human lysosomal acid phosphatase: cloning, expression and chromosomal assignment. *EMBO J* 1988; 7: 2343-50.
40. Williams MA, Fukuda M. Accumulation of membrane glycoproteins in lysosomes requires a tyrosine residue at a particular position in the cytoplasmic tail. *J Cell Biol* 1990; 111: 955-66.
41. Peters C, Braun M, Weber B, et al. Targeting of a lysosomal membrane protein: a tyrosine containing endocytosis signal in the cytoplasmic tail of a lysosomal acid phosphatase is necessary and sufficient for targeting to lysosomes. *EMBO J* 1990; 9: 3497-506.
42. Braun M, Waheed A, von Figura K. Lysosomal acid phosphatase is transported to lysosomes via the cell surface. *EMBO J* 1991; 8: 3633-40.
43. Tanaka I, Yano S, Furuno K, et al. Transport of acid phosphatase to lysosomes does not involve passage through the cell surface. *Biochem Biophys Res Commun* 1990; 170: 1067-73.
44. Green SA, Zimmer KP, Grilliths G, et al. Kinetics of intracellular transport and sorting of lysosomal membrane and plasma membrane proteins. *J Cell Biol* 1987; 105: 1227-40.
45. D'Souza MP, August JT. A kinetic analysis of biosynthesis and localization of a lysosome associated membrane protein. *Arch Biochem Biophys* 1986; 249: 522-32.
46. Harter C, Mellman I. Transport of the lysosomal membrane glycoprotein Igp120 (Igp-A) to lysosomes does not require appearance on the plasma membrane. *J Cell Biol* 1992; 117: 311-25.
47. Metzelaar MJ, Sixma JJ, Nieuwenhuis HK. Detection of platelet activation using activation specific monoclonal antibodies. *Blood Cells* 1990; 16: 85-93.
48. Knol EF, Mul FJ, Jansen H, et al. Monitoring human basophil activation via CD63 monoclonal antibody 435. *J Allergy Clin Immunol* 1991; 88: 328-38.
49. Peters PJ, Borst J, Oorschot V, et al. Cytotoxic T lymphocyte granules are secretory lysosomes containing both perforin and granzymes. *J Exp Med* 1991; 173: 1099-109.
50. Carlsson SR, Roth J, Piller F, et al. Isolation and characterization of human lysosomal membrane glycoproteins, h-lamp-1 and h-lamp-2. Major sialoglycoproteins carrying polylactosaminoglycan. *J Biol Chem* 1988; 263: 18911-9.
51. Carlsson SR, Fukuda M. The polylactosaminoglycans of human lysosomal membrane glycoproteins lamp-1 and lamp-2. Localization on the peptide backbones. *J Biol Chem* 1990; 265: 20488-95.
52. Lee N, Wang WC, Fukuda M. Granulocytic differentiation of HL-60 cells is associated with increase of poly-N-acetylactosamine in Asn-linked oligosaccharides attached to human lysosomal membrane glycoproteins. *J Biol Chem* 1990; 265: 20476-87.
53. Hubbard SC. Differential effects of oncogenic transformation on N-linked oligosaccharide processing at individual glycosylation sites of viral glycoproteins. *J Biol Chem* 1987; 262: 16403-11.
54. Saitoh O, Wang WC, Lotan R, et al. Differential glycosylation and cell surface expression of lysosomal membrane glycoproteins in sublines of a human colon cancer exhibiting distinct metastatic potentials. *J Biol Chem* 1992; 267: 5700-11.
55. Sloane BF, Rozhin J, Johnson K, et al. Cathepsin B: association with plasma membrane in metastatic tumors. *Proc Natl Acad Sci USA* 1986; 83: 2483-7.
56. Nicolson GL. Metastatic tumor cell interactions with endothelium, basement membrane and tissue. *Curr Opin Cell Biol* 1989; 1: 1009-19.
57. Rice GE, Bevilacqua MP. An inducible endothelial cell surface glycoprotein mediates melanoma adhesion. *Science* 1989; 246: 1303-6.
58. Hession C, Osborn L, Goff D, et al. Endothelial leukocyte adhesion molecule 1: direct expression cloning and functional interactions. *Proc Natl Acad Sci USA* 1990; 87: 1673-7.
59. Aruffo A, Kolanus W, Walz G, et al. CD62/P-Selectin recognition of myeloid and tumor cell sulphatides. *Cell* 1991; 67: 35-44.
60. Lowe JB, Stoolman RP, Nair RD, et al. ELAM-1-dependent cell adhesion to vascular endothelium determined by a transfected human fucosyltransferase cDNA. *Cell* 1990; 63: 475-84.
61. Larsen E, Palabrica T, Sajer S, et al. PADGEM-dependent adhesion of platelets to monocytes and neutrophils is mediated by a lineage specific carbohydrate, LNFIH (CD15). *Cell* 1990; 63: 467-74.
62. Foxall C, Watson SR, Dowbenko D, et al. The three members of the selectin receptor family recognize a common carbohydrate epitope, the sialyl Lewis x oligosaccharide. *J Cell Biol* 1992; 117: 895-902.
63. Abrams C, Shattil SJ. Immunological detection of activated platelets in clinical disorders. *Thromb Haemostas* 1991; 65: 467-73.
64. Fijnheer R, Modderman PW, Veldman H, et al. Detection of platelet activation with monoclonal antibodies and flow cytometry. Changes during platelet storage. *Transfusion* 1990; 30: 20-5.
65. Fijnheer R, Pietersz RN, de-Korte D, et al. Platelet activation during preparation of platelet concentrates: a comparison of the platelet-rich plasma and the buffy coat methods. *Transfusion* 1990; 30: 634-8.
66. Metzelaar MJ, Korteweg J, Sixma JJ, et al. A comparative study on the use of platelet membrane markers for the detection of platelet activation in vitro and clinical disorders. *Thromb Haemostas* 1991; 65: 680 (Abstract).
67. Tschöpe D, Roesen P, Esser J, et al. Large platelets circulate in an activated state in Diabetes Mellitus. *Semin Thromb Hemostas* 1991; 17: 433-8.
68. Tschöpe D, Schulthess HP, Kolarov P, et al. Platelet activation markers are predictive for an increased PTCA risk. *Thromb Haemostas* 1991; 65: 772 (Abstract).
69. Out HJ, de Groot PG, van Vliet M, et al. Antibodies to platelets in patients with anti-phospholipid antibodies. *Blood* 1991; 77: 2655-9.
70. Metzelaar MJ, Wijngaard P, Peters PJ, et al. CD63 antigen: a novel lysosomal membrane glycoprotein, cloned by a screening procedure for intracellular antigens in eukaryotic cells. *J Biol Chem* 1991; 266: 3239-45.
71. Fukuda M. Lysosomal membrane glycoproteins: structure, biosynthesis, and trafficking to lysosome. *J Biol Chem* 1991; 266: 21327-30.

Received February 26, 1992 Accepted after revision June 26, 1992