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Identification of the Lysosomal Membrane Glycoprotein Lamp-1 as a Receptor for Type-1-Fimbriated (Mannose-Specific) *Escherichia Coli*

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The presence of several glycosylated sites with high-mannose oligosaccharides on the lysosome-associated membrane glycoproteins (Lamps) combined with the fact that neutrophil Lamps are present in mobilizable organelles inspired us to investigate their ability to bind type-1 fimbriated (mannose-binding) *Escherichia coli* and subsequently define a potential function for the Lamps in human neutrophils. Bacterial binding to Lamps purified from chronic myeloic leukemia cells was investigated by separation of the proteins by sodium dodecyl sulfate polyacrylamide gel electrophoresis, transfer to a blotting membrane and overlay with type-1-fimbriated bacteria. The overlays were developed by growth. The bacteria bound readily to Lamp-1 while there was almost no binding to Lamp-2. Hence, we state that a possible function for neutrophil Lamp-1 is bacterial binding.

The outcome of an interaction between invading bacteria and mammalian phagocytic cells (neutrophil granulocytes and monocytes/macrophages) is of importance in the pathogenesis of infectious agents. Phagocytic cells form the front line in our defense against infection and to fulfill their role, the phagocytes depend on the capacity to engulf the intruder, to secrete inflammatory mediators and to produce large amounts of reactive oxygen species upon ingestion of the prey (1). The initial stage in phagocyte killing of invading microbes involves recognition and attachment of the prey by the phagocyte, events mediated by complementary cell surface structures. In the absence of opsonins, the ingestion process can be mediated through highly specific lectin-carbohydrate interactions, a process called lectinophagocytosis (2). This process most often involves the interaction between a neutrophil glycoconjugate receptor and a microbial lectin, as in the binding of type-1-fimbriated microbes, carrying mannose-specific lectins, to human neutrophils (3, 4, 5). Two different neutrophil receptors that mediate such binding of type-1-fimbriated *Escherichia coli (E. coli)* have been identified: the neutrophil complement receptor 3 (CR3, CD11b/CD18) (6) and the nonspecific cross-reacting antigen (NCA) (7). However, we have recently shown the presence of at least five (yet unidentified) additional neutrophil receptors (8), one of these in the 120 kD region.

A class of highly glycosylated proteins, the lysosome-associated membrane glycoproteins (Lamps), has been identified as a major glycoprotein constituent in lysosomal membranes in many cells (9, 10). In neutrophils, the Lamps are absent from the lysosomal granules, but present in other mobilizable organelles (11). Hence, these glycoproteins are exposed on the cell surface during mobilization of the neutrophil secretory vesicles and specific granules (11), and since the Lamps carry several different lectin specific determinants, they may be of special interest with respect to

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Abbreviations used: Lamp-1 and Lamp-2, lysosome-associated membrane glycoprotein-1 and −2; CML, chronic myelogenous leukemia; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; *E. coli*, *Escherichia coli*; CR3, complement receptor 3; NCA, nonspecific cross-reacting antigen; PVDF, polyvinylidene fluoride; PBS, phosphate buffered saline; BSA, bovine serum albumin; EMB, eosin-methylene blue.

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their bacterial binding capacity. We thus decided to investigate the possibility of the neutrophil Lamps as potential receptors for type-1-fimbriated bacteria.

MATERIALS AND METHODS

Isolation of neutrophil plasma membrane. Neutrophils isolated from buffy coats (12) obtained from apparently healthy adults were allowed to interact with ionomycin (a Ca^{2+} specific ionophore that mobilizes the secretory vesicles and the specific granules). The cells were disintegrated by nitrogen cavitation (Parr Instrument Company, Moline, IL) and fractionated on a two-layer Percoll gradient as described (11, 13). After centrifugation the cells were separated into one major band (γ) and two minor bands (α and β). The α, β and γ bands in order of decreasing density, contained the remaining azurophil granules, the remaining specific granules and the plasma membrane, respectively as determined by marker analysis (data not shown). The gradients were collected in 1.5 ml gradient fractions by aspiration from the bottom of the centrifuge tube and the γ band (plasma membrane enriched) was used for immunoblotting and bacterial binding.

Purification of Lamps from chronic myelogenous leukemia (CML) cells. The Lamps were purified from cells obtained from a patient with CML. Detergent solubilized CML cells were passed over a column of wheat germ agglutinin-agarose and sugar-eluted glycoproteins were applied to columns of immobilized monoclonal antibodies against Lamp-1 (BB6) and Lamp-2 (CD3), connected in series. The eluted Lamps were finally purified by preparative SDS-PAGE (14). ELISA tests using newly prepared rabbit polyclonal antibodies showed no cross reactivity between Lamp-1 and Lamp-2. The monoclonal antibodies were described earlier (14, 15).

SDS-PAGE and protein transfer. Proteins from the neutrophil γ band (amount corresponding to 1×10^6 cells) or purified Lamps (1 μg) were separated on polyacrylamide gels (16) and transferred to polyvinylidene fluoride (PVDF) membranes using a Tris-Glycine buffer system (17). Immunoblotting was performed as previously described (11).

Overlay with type-1-fimbriated bacteria. The overlay technique used to identify fimbriae binding proteins was described earlier (8). In short, *E. coli* bacteria (strain PN7, previously characterized; (5, 18)) were cultured statically at 37°C for 48 + 48 h in nutrient broth to promote expression of type-1-fimbriae. The bacteria were washed twice in phosphate buffered saline (PBS, pH 7.3) and resuspended in PBS supplemented with bovine serum albumin (BSA; 2.5% w/v) to a concentration of 10^7 bacteria/ml. Protein loaded replicas (PVDF membranes) were incubated statically in 10 ml of the bacterial suspension over night at 4°C. To inhibit bacterial binding, α-methyl-mannoside (20 μg/ml) was added. The replicas were washed 6 × 5 min in PBS-Tween (0.05% v/v). Bound bacteria were visualized by growth, placing the replicas (protein side up) on EMB-agar plates and incubating at 37°C for approximately 6 h.

RESULTS

Bacterial binding to neutrophil glycoproteins. Neutrophil secretory vesicles and specific granules were mobilized through stimulation with ionomycin (19) and the plasma membranes were isolated on Percoll gradients. Membrane proteins were separated by SDS-PAGE, and transferred to PVDF replicas. The presence of type-1-fimbriae binding glycoproteins was visualized by a bacterial overlay technique: type-1-fimbriated *E. coli* were allowed to bind to the proteins on the PVDF replicas and binding was visualized by growth of bound bacteria into colonies, placing the blotting membrane on a solid substratum. The colonies appeared as dark-colored bands protruding from the replica surface (Fig 1). The binding was inhibited by α-methyl mannoside (not shown by figure). Analysis of the binding pattern revealed at least nine different proteins ranging in molecular weight from around 40 kDa to around 155 kDa (Fig 1). Two members of the lysosomal membrane glycoprotein class of molecules, Lamp-1 and Lamp-2, are present in the neutrophil plasma membrane fraction, and they appear in a region (around 120 kDa: Fig 1) in which bacterial binding was obvious.

Bacterial binding to purified Lamps. Lamp-1 and Lamp-2 purified from CML cells were run on SDS-PAGE and transferred to a PVDF membrane. The PVDF replica was immunoblotted as well as exposed to type-1-fimbriated bacteria as described above. Lamp-1 isolated from CML cells showed extensive receptor-activity for the type-1-fimbriated *E. coli* while Lamp-2 showed almost no binding (Fig 2). The difference in receptor activity between Lamp-1 and Lamp-2 was even more obvious when the Lamps were diluted 100 times and Lamp-1 showed remaining (but less) binding capacity while there was no binding to Lamp-2 (data not shown). The binding of the bacteria to Lamp-1 was totally inhibited by the addition of α-methyl mannoside (not shown by figure).

DISCUSSION

In neutrophils, the membrane glycoproteins Lamp-1 and Lamp-2 are present in mobilizable

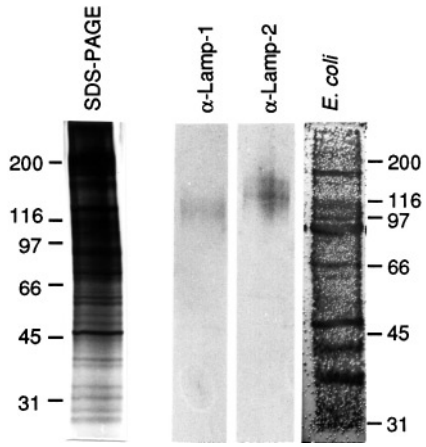

FIG. 1. Immunoblotting and binding of type-1-fimbriated *E. coli* to neutrophil membrane proteins. Proteins derived from the plasma membrane of ionomycin-stimulated neutrophils were separated by SDS-PAGE and silver stained in the gel or transferred to a PVDF membrane by Western blotting. The PVDF replicas were immunoblotted with specific antibodies to Lamp-1 (BB6) or Lamp-2 (CD3) or overlaid with type-1-fimbriated *E. coli* and developed by growth. Molecular sizes are given in kilodaltons.

organelles, but absent in the classical lysosomes (the azurophil granules; 11). The fact that the Lamps are localized in secretory organelles, suggests that these glycoproteins may have potentially important roles on the surface of activated/extravasating phagocytes. Since recognition of micro-organisms often involves microbial lectins that bind to neutrophil glycoconjugate receptors (20, 21) the Lamps could be part of a mobilizable system designed to recognize and eliminate microbial intruders. The type-1-fimbriated bacteria used in this study were found to bind at least nine different neutrophil membrane proteins. The 155-, 100- and 50 kDa proteins recognized by the bacteria may correspond to the CR3 (CD11b, 155 kDa; CD 18, 100 kDa) and the nonspecific

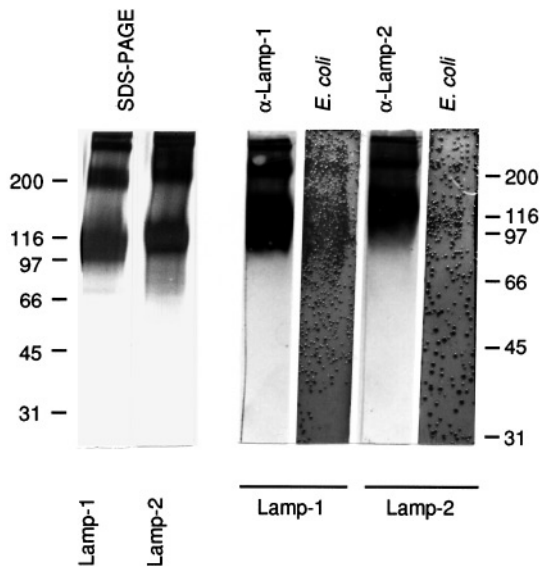

FIG. 2. Immunoblotting and binding of type-1-fimbriated *E. coli* to Lamps from CML cells. Lamp-1 and Lamp-2 purified from CML cells were run on SDS-PAGE and silver stained in the gel or transferred to a PVDF membrane. The PVDF replica was immunoblotted with specific antibodies to Lamp-1 (BB6) or Lamp-2 (CD3) or overlaid with type-1-fimbriated *E. coli* and developed by growth. Molecular sizes are given in kilodaltons.

cross-reacting antigen (NCA; 50 kDa) previously reported to act as receptors for type-1-fimbriated *E. coli* (6, 7). The findings of this paper suggest that the protein in the 120 kDa region may correspond to Lamp-1. The study also shows that type-1-fimbriated bacteria readily bind to Lamp-1, but barely at all to Lamp-2.

The Lamps are extensively glycosylated proteins with both N-linked and O-linked carbohydrate structures. There is no difference between Lamp-1 and Lamp-2 with respect to the basic protein structure. The amino terminal parts of the molecules are located at the extracytoplasmic or extracellular side of the membrane and consist of two similar domains separated by a hinge region. The proteins are anchored in the membrane by a single transmembrane domain. The O-linked carbohydrate chains are attached to the hinge region, whereas a number of N-linked carbohydrate chains are attached to both domains enclosing the hinge (22). The glycosylation patterns of the Lamps are quite complex. Our knowledge about the difference between the two glycoproteins with respect to glycosylation is restricted to quantitative determinations of different saccharides. Lamp-1 carries more oligosaccharides of the high mannose-type (23) and since mannose is the sugar residue involved in type-1-fimbriae binding, this difference may be of importance in defining receptor-activity. It should be pointed out, however, that not only quantitative but also qualitative differences in glycosylation is of importance in the context of binding. This is clearly illustrated by the fact that con A (a mannose-specific lectin) and type-1-fimbriated bacteria show unique (but partially overlapping) binding patterns to neutrophil membrane proteins (8). Further, different mannose-binding lectins (Con A, GNA, PSA and LcH), have completely different binding patterns when allowed to interact with neutrophil membrane proteins, despite the fact that the sugar specificity is similar (Karlsson, unpublished observation).

Although the Lamps are relatively well characterized, their biological functions are not known. From the fact that the molecules in most cells are found in an hydrolase-rich acidic lysosomal milieu, a major function has been suggested to be the protection of the lysosomal membrane from degradation by hydrolases. Since granulocytes carry Lamps in mobilizable organelles other than the lysosomes, the molecules may be of importance in recognition processes related to inflammation. The Lamps are the major carriers of polylactosaminoglycans, and these high-molecular-mass carbohydrates may carry specific determinants such as ABO and I/i blood groups as well as Lewis^x and sialylLewis^x which serve as ligand for selectins (24). Further, the Lamps can constitute a part of a mobilizable system designed to recognize and eliminate microbial intruders. Future studies will hopefully disclose whether the short C terminal cytoplasmic tail (10–11 amino acids) of Lamps, known to be of importance for sorting of the molecule, is able to mediate also signals of importance for engulfment and/or killing of invading microbes.

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