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Aminopeptidase N/CD13 Is Associated with Raft Membrane Microdomains in Monocytes

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Ectopeptidases play important roles in cell activation, proliferation, and communication. Human monocytic cells express considerable amounts of aminopeptidase N/CD13, a transmembrane protein previously proposed to play a role in the regulation of neuropeptides and chemotactic mediators as well as in adhesion and cell–cell interactions. Here, we report for the first time that aminopeptidase N/CD13 in monocytes is partially localized in detergent-insoluble membrane microdomains enriched in cholesterol, glycolipids, and glycosylphosphoinositol-anchored proteins, referred to as "rafts." Raft fractions of monocytes were characterized by the presence of GM1 ganglioside as raft marker molecule and by the high level of tyrosine-phosphorylated proteins. Furthermore, similar to polarized cells, rafts in monocytic cells lack Na^+, K^+-ATPase. Cholesterol depletion of monocytes by methyl-β-cyclodextrin greatly reduces raft localization of aminopeptidase N/CD13 without affecting ala-*p*-nitroanilide cleaving activity of cells. © 2000 Academic Press

Key Words: **aminopeptidase N/CD13; microdomains; raft; cyclodextrin; GM1 ganglioside.**

Ectopeptidases are a multifunctional group of enzymes implicated in the control of growth and differentiation of various cellular systems. Aminopeptidase N (APN/CD13) (EC 3.4.11.2) is a widely expressed zinc-dependent enzyme that preferentially catalyses the removal of neutral amino acids from small peptides. It is a homodimer of two 150 kDa polypeptides, each anchored to the cell membrane by a single transmembrane helical region near the N-terminus with a short cytoplasmic tail of only 8–10 amino acids (1).

Within the hematopoietic lineage, APN/CD13 is not only expressed on myeloid precursor cells, monocytes/macrophages and granulocytes, but also on T lymphocytes in inflamed joints or in renal cancer (2). It has been described that differentiation of the myeloid precursor cell line HL60 into monocytic cells leads to an increase in APN/CD13 expression (3). The functional significance of the high monocyte expression level of APN/CD13 is not fully understood at present, but it represents a cellular potential for inactivation of inflammatory mediators such as neuropeptide hormones, kinins and chemotactic substances. In addition, like dipeptidyl peptidase IV (DPIV)/CD26, APN/CD13 is considered as an auxiliary adhesion molecule localized at sites of cell-cell contact in melanoma cell colonies and tightly associated with extracellular matrix components (4). Furthermore, the ectopeptidase is thought to play a role in cell surface antigen processing by trimming peptides protruding out of the binding groove of MHC class II molecules to generate the final T cell epitopes (5).

The lipid bilayer of the plasma membrane of all cells is organized into microdomains rich of cholesterol and glycosphingolipids which are insoluble after treatment with non-ionic detergents such as Triton X-100 at low temperature. These microdomains, commonly referred to as rafts, are thought to provide a platform for the selective delivery of membrane proteins to specialized cell areas. They contain a number of different types of proteins, including various receptors, membrane transporters and signal transducing kinases with functions in protein transport, cell polarization and signal transduction (6, 7).

Here, we report for the first time that APN/CD13 is partially localized in rafts in monocytes. We furthermore discuss the possible role of the raft localization of

Abbreviations used: APN, aminopeptidase N; DPIV, dipeptidyl peptidase IV; mAb, monoclonal antibody.

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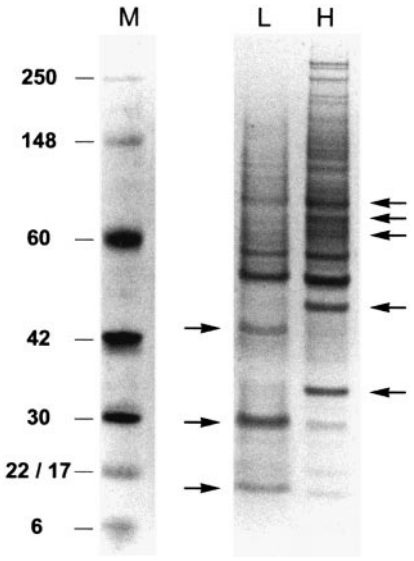

FIG. 1. Protein profile of the low-density (L, raft-containing) and the high-density (H, Triton-soluble) fractions in peripheral blood monocytes. The arrows mark the presence of differentially expressed proteins. M, molecular marker. Similar results were obtained for U937 and Mono-Mac-6 cells.

APN/CD13 with respect to a function of the enzyme in signal transduction.

MATERIALS AND METHODS

Cell isolation and culture. The monocytoid cell line U937 was kindly provided by O. Werdelin (Panum Institute, Kopenhagen, Denmark) and cultured in RPMI 1640 with 10% fetal calf serum, antibiotics and 50 μmol 2-ME. Mono-Mac-6 cells as a more mature monocytic cell line were purchased from DSM (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany). Peripheral blood monocytes from normal, healthy donors were prepared from buffy coats by Ficoll-Hypaque density-gradient centrifugation. T lymphocytes were depleted by rosetting with sheep red blood cells. Monocytes were further enriched by plastic adherence (1 h, 37°C) and demonstrated a purity of >95%, detected by FACS analysis with anti CD14 antibodies. Monocytes were cultured at 37°C at a density of 1–2 $\times$ 10^6/ml in RPMI 1640 supplemented with antibiotics and 10% fetal calf serum.

Sucrose gradient fractionation of cell extracts. Rafts were isolated according to a procedure described by Kabouridis *et al.*, which is based on resistance to solubilization by Triton X-100 at 4°C and buoyancy at specific density in a linear sucrose gradient (8). Monocytes (2.5×10^7) were washed once with ice cold PBS (pH 7.2) and then lysed in 1 ml of MNE buffer (150 mM NaCl, 2 mM EDTA, 25 mM MES pH 6.5) containing 1% Triton X-100 and protease inhibitor mixture (Sigma, Deisenhofen, Germany), on ice for 20 min. An equal volume of an 80% sucrose solution in MNE buffer was mixed with the lysates to form a 40% suspension, and a step sucrose gradient was formed by overlaying with 2 ml of 30% sucrose-MNE and 1 ml of 5% sucrose-MNE. Isopycnic equilibration was achieved by centrifugation at 200,000*g* in an SW55 rotor (Beckmann Instruments, Palo Alto, CA) at 4°C for 15 h. Two 2-ml fractions were collected; one fraction, from the top of the tube that contained the 5/30% sucrose interface, and the other, which contained the 40% sucrose fraction from the bottom. Proteins in the fractions were precipitated with 10% trichloroacetic acid on ice for 30 min, and pellets were resuspended in 100 μl of 0.2 N NaOH. The protein concentration in the fractions was determined with the BCA protein assay reagent kit (Pierce, Rockford, IL).

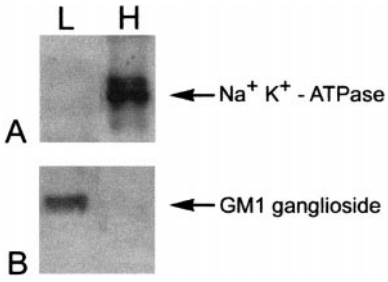

FIG. 2. Characterization of the low-density (L) and high-density (H) fractions in monocytes by the detection of differentiating markers. (A) Na^+K^+-ATPase was only detectable in the high-density fraction while GM1 ganglioside (B) could be observed only in the low-density fraction.

Western blot analysis. Samples were electrophoresed on NuPAGE gels as recommended by the manufacturer (NOVEX Electrophoresis GmBH, Frankfurt/Main, Germany). Cell proteins were transferred to nitrocellulose (ECL, Amersham Pharmacia Biotech, Braunschweig, Germany) using a semidry Fastblot apparatus (Biometra, Goettingen, Germany) according to the manufacturer's protocol. The nitrocellulose was blocked with 5% of dry milk powder in TBS-T buffer (10 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1% Tween 20) for one hour, and then incubated with the first antibody in a blocking solution containing 1% of dry milk powder at room temperature for one hour. The blots were washed three times with TBS-T buffer. Following incubation with the appropriate horseradish peroxidase-conjugated secondary antibody for one hour, blots were developed using an ECL-based system (Amersham Pharmacia Biotech, Braunschweig, Germany). Densitometry was performed using the SCANPACK II software (Biometra, Göttingen, Germany). For CD13 detection in western blots a mixture of the mAb clones SJ1D1,

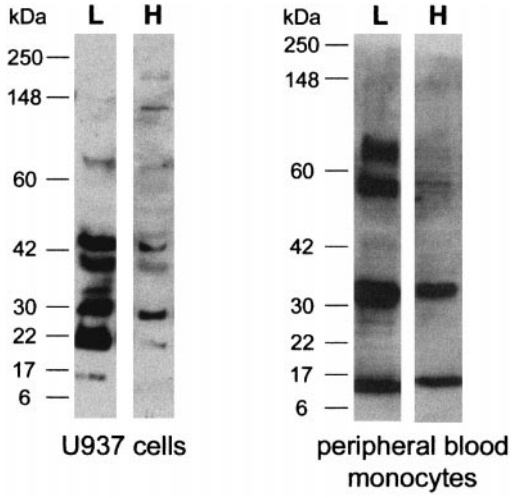

FIG. 3. Antiphosphotyrosine immunoblotting of monocytes. 10 μg of protein from the low-density (L) and high-density (H) fractions were separated on SDS-PAGE as described under Materials and Methods followed by immunoblotting using the phosphotyrosine antibody 4G10.

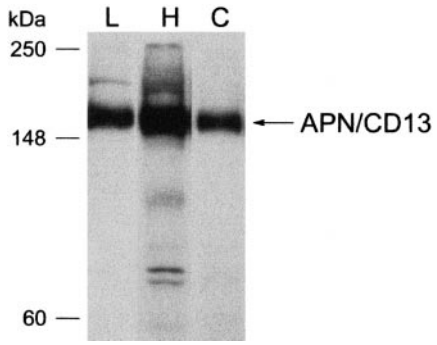

FIG. 4. CD13/APN immunoblotting of monocytes. Samples of membrane proteins (10 μg) were electrophoretically separated and blotted, the nitrocellulose membrane was detected with an anti-APN/CD13 antibody mixture. (L) Low-density fraction; (H) high-density fraction; (C) soluble APN/CD13 as a control.

MY7 (Coulter, Hamburg, Germany), Leu-M7 (Becton Dickinson, Heidelberg, Germany) and WM15 (Dianova, Hamburg, Germany) [each 1:1 000] was used. For tyrosine-phosphorylation the membrane was detected with the antibody 4G10 [1:1 500] (Calbiochem, Bad Soden, Germany). For detection of Na^+, K^+-ATPase (α-subunit), the mAb clone M7-PB-E9 [1:250] (Affinity Bioreagents Inc., Golden, CO) was used. Horseradish peroxidase-conjugated cholera toxin was purchased from Sigma (Deisenhofen, Germany).

Confocal laser scanning microscopy. Mono-Mac-6 cells were stained with anti CD13 mAb (clone Leu-M7, IgG1) or an isotype control at 4°C for 40 min followed by three cold washes and incubation with a Cy3-conjugated secondary anti-mouse antibody (Dianova, Hamburg, Germany). After antibody staining, cells were treated with FITC-conjugated cholera toxin (Sigma, Deisenhofen, Germany) at 4°C for 40 min for specific staining of GM1 ganglioside as raft marker molecule, washed, fixed with 2% paraformaldehyde in PBS (pH 7.2), washed and mounted. Laser scanning confocal microscopy was performed using a Zeiss LSM 410 microscopic system (Carl Zeiss, Jena, Germany). Image processing was done by using the co-colocalization program of the LSM 410 by selecting pixels of the same intensity higher than the relative brightness of 20%.

Methyl-β-cyclodextrin-treatment of monocytes. β-Cyclodextrins are cyclic oligosaccharides consisting of 7 α(1-4)-glucopyranose units. These agents possess a polar surface and a hydrophobic cavity, which enables them to enhance the solubility of hydrophobic compounds by forming conclusion complexes with them (9). β-Cyclodextrins have a very high affinity for cholesterol (10). For depletion of cholesterol from monocytes, the cells were incubated with 2% of methyl-β-cyclodextrin (Sigma, Deisenhofen, Germany) at 37°C for 60 min.

Functional assay of aminopeptidase activity. Enzyme activity was assayed using 1.5 mM alanine *p*-nitroanilide (Sigma, Deisenhofen, Germany) as substrate for APN/CD13. Monocytes, cultured both without and with addition of 2% of β-cyclodextrin for 60 min, were rinsed three times, counted, and incubated at 37°C in 67 mM Na_2HPO_4/KH_2PO_4 buffer pH 7.2 with the substrate for 30 to 60 min. The amount of *p*-nitroaniline formed was measured in the supernatant by reading the OD at 390 nm. Tests were run in quadruplicate; cell-free and substrate-free blanks were run in parallel. Enzyme activities were expressed as pkat/10^6 cells.

RESULTS

Isolation and characterization of rafts. The protein profile in the low-density (raft-containing) fraction and the high-density (Triton-soluble) fraction was analyzed by electrophoretic separation of 10 μg of protein lysates in NuPAGE gels followed by silver staining. Whereas the amount of total protein in the raft-fraction was approximately 50% of the amount of the high-density fraction, a clearly different pattern with respect to the protein distribution for the low and high-density fractions was found (Fig. 1).

To further characterize the low-density fraction, we analyzed the expression of an integral plasma membrane protein, Na^+, K^+-ATPase (110 kDa) for which a lack of expression has been demonstrated in the raft fraction of intestinal epithelial cells (11). Figure 2A shows that, similar as in polarized cells, in monocytes Na^+, K^+-ATPase was only detectable in the high-density fraction. Furthermore, we found that horse peroxidase-conjugated cholera toxin, which is known to bind the raft marker molecule GM1 ganglioside in the

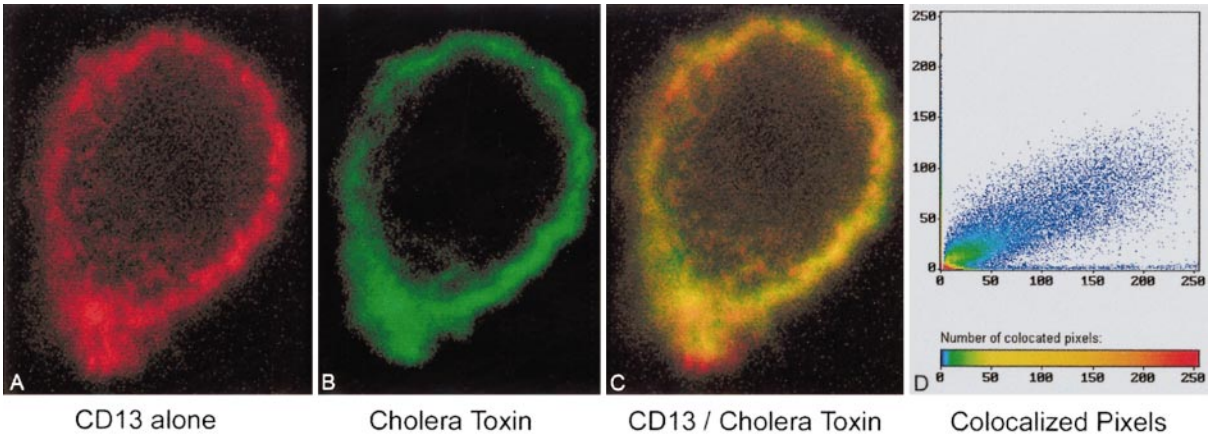

FIG. 5. APN/CD13 is colocalized with GM1 ganglioside on monocytes. Mono-Mac-6 cells were stained with anti-CD13 mAbs (A, visualized as red) followed by treatment with cholera toxin for staining of GM1 ganglioside (B, visualized as green). Colocalization of APN/CD13 and GM1 ganglioside is illustrated in C (visualized as yellow/gold). Quantification of pixel colocalization is represented in D.

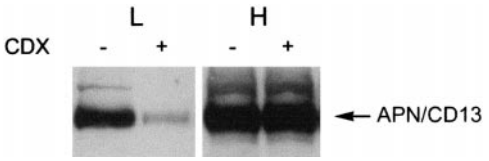

FIG. 6. Depletion of monocytic cholesterol by cyclodextrin abrogates APN/CD13 from rafts. Monocytes from peripheral blood were incubated without (−) or with (+) 2% cyclodextrin (CDX) at 37°C for 60 min. 10 μg of membrane protein of the low-density (L) and high-density (H) fractions was analyzed by Western blotting for the presence of APN/CD13. Similar results were obtained for U937 and Mono-Mac-6 cells.

low-density fraction, labels a <10 kDa protein in the low-density fraction only (Fig. 2B).

Antiphosphotyrosine immunoblotting analysis of monocytes revealed that the low-density fraction is rich in several tyrosine-phosphorylated proteins in the range of 17–60 kDa (Fig. 3). A clear difference with respect to phosphorylated proteins could be observed between the low-density fraction of U937 cells and of peripheral blood monocytes: as an example, the raft fraction of blood monocytes showed a high level of two phosphorylated proteins of approximately 55 and 80 kDa, whereas these proteins were not detected in U937 cells. On the other hand, in the raft fraction of U937 cells we found high levels of phosphorylated proteins with approximately 22, 38, and 45 kDa (Fig. 3).

Identification of APN/CD13 as a component of rafts. The isolated raft membrane fractions from monocytes were analyzed by immunoblotting with antibodies against APN/CD13. We detected APN/CD13 in the raft fraction and in the Triton-soluble high-density fraction (Fig. 4). By densitometric analysis we determined the APN/CD13 content in the low-density fractions to 35–40%, whereas 65–70% of APN/CD13 was detectable in the high-density Triton-soluble fraction.

Confocal laser scanning microscopy. The distribution of APN/CD13 (Cy3, red) and GM1 ganglioside as a raft-marker (FITC, green) was investigated using confocal microscopy. Colocalization of both the molecules (visualized as yellow/gold) was found in several areas of a monocyte as determined by pixel quantification software (Fig. 5).

Abrogation of APN/CD13 from rafts by cyclodextrin. Depletion of cholesterol from rafts by treatment of monocytes with 2% of cyclodextrin for 60 min abrogated APN/CD13 association in the raft fraction without any obvious effect on the APN/CD13 content of the high-density fraction (Fig. 6). Cyclodextrin-treatment did not affect the ala-*p*-nitroanilide hydrolyzing activity of monocytes. Using this substrate, U937 cells had an activity of 47 ± 5 pkat/10^6 cells (mean ± SD), whereas the corresponding activity of cholesterol-depleted cells was 112 ± 16% of the control (n = 4).

DISCUSSION

Previous studies from several laboratories have implicated APN/CD13 in various types of inflammatory processes including the interaction between synoviocytes, monocytes and lymphocytes (for review see 2). In the present work, we studied in closer detail the subcellular distribution of this membrane protein and observed that it partially resides in the low-temperature detergent-insoluble membrane fraction, representing the cholesterol- and glycolipid-rich membrane microdomains commonly referred to as rafts. Our results extend previews observations, which have shown APN/CD13 to be a raft component in the brush border membrane of intestinal enterocytes (11). Rafts provide a platform for a number of different types of proteins including the IP3-receptor for Ca^{2+}-signaling and signal transducing kinases (for review see 6, 7). Transmembrane proteins are excluded from rafts with only a few exceptions, such as CD4 in T cells, CD44 and integrins in myeloid cells (for review see 12). However, for the transmembrane ectoenzyme dipeptidyl peptidase IV (DPIV)/CD26 a raft association has been demonstrated in T cells (13). Additionally, neprilysin/CD10 (neutral endopeptidase 24.11) has been discussed to be a constituent of membrane microdomains in pre B cells (14), and proteins associated with neprilysin/CD10, such as the 56 kDa src-related kinase lyn, become tyrosine phosphorylated in vitro when co-immunoprecipitated with the ectopeptidase (14, 15).

Biologically active peptide substrates split by APN/CD13 include neuropeptides, such as enkephalins and endorphins (16), or chemotactic peptides, such as monocyte chemotactic protein MCP-1 (17). Interestingly, most of these substrates signal with G-protein-coupled heptahelical receptors. Signal transduction via these receptors involves kinase cascades commonly used by growth factors, or during adhesion via integrins. By activating or inactivating biologically active peptides, APN/CD13 could indirectly affect these signaling pathways. However, some observations point to a more complex picture with APN/CD13 being directly involved in signal transduction: CD13-specific mAbs not only inhibit cell proliferation but are able to trigger an increase in the concentration of free cytoplasmic Ca^{2+} in monocytic cell lines (18, 19). A similar effect is seen with DPIV/CD26, where cross-linking with mAbs can trigger Ca^{2+} signals and T cell activation (for review see 20). Raft association of APN/CD13 in monocytes could be a prerequisite for the observed calcium signals after ligation of the membrane enzyme. So far, the in vivo APN/CD13 ligands remain unknown. Since extracellular matrix components, such as collagen I, have been shown to bind to DPIV/CD26 (21), and since also APN/CD13 has been found in close contact to collagen (2, 4), extracellular matrix could be a potential

ligand for APN/CD13. Otherwise, substrates or natural inhibitors might act as ligands, e.g. bradykinin and substance P are natural peptides known to inhibit the enzyme in micromolar concentrations (22).

Our knowledge on the importance of rafts in monocytes is still in its early stages. A molecule known to be raft-associated in monocytes is the glycosylphosphoinositol-anchored membrane protein urokinase plasminogen activator receptor/CD87 as well as its ligand urokinase (23). In addition to its function in the fibrinolytic system (urokinase activation after receptor binding), receptor ligation results in monocytic adhesion induction and chemotactic movements coupled with changes in tyrosine phosphorylation and diacylglycerol formation (for review see 24). Several investigations demonstrated the presence of CD87 at the leading edge of migrating cells (25) as well as at sites of cell-cell contact (23, 25, 26). Interestingly, a similar accumulation at sites of cell-cell contacts has been described for APN/CD13 in melanoma cells (4).

Since membrane compartmentalization in rafts is required for many different cellular functions, further studies will be necessary to elucidate the role of APN/CD13 as raft-associated molecule in monocytes. We suggest that localization of APN/CD13 in rafts could point to a role of the enzyme as a signal transduction molecule in monocytes.

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REFERENCES

1. Olsen, J., Cowell, G. M., Konigshofer, E., Danielsen, E. M., Moller, J., Laustsen, L., Hansen, O. C., Welinder, K. G., Engberg, J., Hunziker, W., Spies, M., Sjöström, H., and Noren, O. (1988) Complete amino acid sequence of human intestinal aminopeptidase N as deduced from cloned cDNA. *FEBS Lett.* **238,** 307–314.
2. Riemann, D., Kehlen, A., and Langner, J. (1999) CD13—Not just a marker in leukemia typing. *Immunol. Today* **20,** 83–88.
3. Laouar, A., Wietzerbin, J., and Bauvois, B. (1993) Divergent regulation of cell surface protease expression in HL-60 cells differentiated into macrophages with granulocyte macrophage colony stimulating factor or neutrophils with retinoic acid. *Int. Immunol.* **5,** 965–973.
4. Menrad, A. D., Speicher, D., Wacker, J., and Herlyn, H. (1993) Biochemical and functional characterization of aminopeptidase N expressed by human melanoma cells. *Cancer Res.* **53,** 1450–1455.
5. Larsen, S. L., Pedersen, L. O., Buus, S., and Stryhn, A. (1996) T cell responses affected by aminopeptidase N (CD13)-mediated trimming of major histocompatibility complex class II-bound peptides. *J. Exp. Med.* **184,** 183–189.
6. Simons, K., and Ikonen, E. (1997) Functional rafts in cell membranes. *Nature* **387,** 569–572.
7. Brown, D. A., and London, E. (1998) Function of lipid rafts in biological membranes. *Annu. Rev. Cell Dev. Biol.* **14,** 111–136.
8. Kabouridis, P. S., Magee, A. I., and Ley, S. C. (1997) S-acylation of LCK protein tyrosine kinase is essential for its signalling function in T lymphocytes. *EMBO J.* **16,** 4983–4998.
9. Pitha, J., Irie, T., Sklar, P. B., and Nye, J. S. (1988) Drug solubilizers to aid pharmacologists: Amorphous cyclodextrin derivatives. *Life Sci.* **43,** 493–502.
10. Yancey, P. G., Rodriguez, W. V., Kilsdonk, E. P. C., Stoudt, G. W., Johnson, W. J., Phillips, M. C., and Rothblat, G. H. (1996) Cellular cholesterol efflux mediated by cyclodextrins. Demonstration of kinetic pools and mechanism of efflux. *J. Biol. Chem.* **271,** 16026–16034.
11. Hansen, G. H., Niels-Christiansen, L. L., Thorsen, E., Immerdal, L., and Danielsen, E. M. (2000) Cholesterol depletion of enterocytes. Effect on the Golgi complex and apical membrane trafficking. *J. Biol. Chem.* **275,** in press.
12. Horejsi, V., Drbal, K., Cebecauer, M., Cerny, J., Brdicka, T., Angelisova, P., and Stockinger, H. (1999) GPI-microdomains: A role in signaling via immunoreceptors. *Immunol. Today* **20,** 356–361.
13. Ilangumaran, S., Arni, S., Chicheportiche, Y., Briol, A., and Hoessli, D. C. (1996) Evaluation by dot-immunoassay of the differential distribution of cell surface and intracellular proteins in glycosylphosphatidylinositol-rich plasma membrane domains. *Anal. Biochem.* **235,** 49–56.
14. Angelisova, P., Drbal, K., Horejsi, V., and Cerny, J. (1999) Association of CD10/neutral endopeptidase 24.11 with membrane microdomains rich in glycosylphosphatidylinositol-anchored proteins and lyn kinase. *Blood* **93,** 1437–1439.
15. Ganju, R. K., Shpektor, R. G., Brenner, D. G., and Shipp, M. A. (1996) CD10/neutral endopeptidase 24.11 is phosphorylated by casein kinase II and coassociates with other phosphoproteins including the lyn src-related kinase. *Blood* **88,** 4159–4165.
16. Matsas, R., Stephenson, S. L., Hryszko, J., Kenny, A. J., and Turner, A. J. (1985) The metabolism of neuropeptides. Phase separation of synaptic membrane preparations with Triton X-114 reveals the presence of aminopeptidase N. *Biochem. J.* **231,** 445–449.
17. Weber, M., Uguccioni, M., Baggiolini, M., Clark-Lewis, I., and Dahinden, C. A. (1996) Deletion of the NH2-terminal residue converts monocyte chemotactic protein 1 from an activator of basophil mediator release to an eosinophil chemoattractant. *J. Exp. Med.* **183,** 681–685.
18. McIntyre, E. A., Jones, H. M., Roberts, P. J., Tidman, N., and Linch, D. C. (1987) Identification of signal transduction molecules on monocytic cells, *in* Leucocyte Typing. III. White Cell Differentiation Antigens (McMichael, A. J., Beverly, P. C. L., Cobbold, S., and Crumpton, M. J., Eds.), pp. 685–688, Oxford University Press.
19. Löhn, M., Mueller, C., Thiele, K., Kähne, T., Riemann, D., and Langner, J. (1997) Aminopeptidase N-mediated signal transduction and inhibition of proliferation of human myeloid cells. *Adv. Exp. Med. Biol.* **421,** 85–91.
20. von Bonin, A., Huhn, J., and Fleischer, B. (1998) Dipeptidylpeptidase IV/CD26 on T cells: Analysis of an alternative T-cell activation pathway. *Immunol. Rev.* **161,** 43–53.
21. Loster, K., Zeilinger, K., Schuppan, D., and Reutter, W. (1995) The cysteine-rich region of dipeptidyl peptidase IV (CD 26) is the collagen-binding site. *Biochem. Biophys. Res. Commun.* **217,** 341–348.
22. Xu, Y., Wellner, D., and Scheinberg, D. A. (1995) Substance P and bradykinin are natural inhibitors of CD13/aminopeptidase N. *Biochem. Biophys. Res. Commun.* **208,** 664–674.

23. Stahl, A., and Mueller, B. M. (1995) The urokinase-type plasminogen activator receptor, a GPI-linked protein, is localized in caveolae. *J. Cell Biol.* **129,** 335–344.

24. Preissner, K. T., Kanse, S. M., Chavakis, T., and May, A. E. (1999) The dual role of the urokinase receptor system in pericellular proteolysis and cell adhesion: Implications for cardiovascular function. *Basic Res. Cardiol.* **94,** 315–321.

25. Okada, S. S., Tomaszewski, J. E., and Barnathan, E. S. (1995) Migrating vascular smooth muscle cells polarize cell surface urokinase receptors after injury in vitro. *Exp. Cell Res.* **217,** 180–187.

26. Pollanen, J., Hedman, K., Nielsen, L. S., Dano, K., and Vaheri, A. (1988) Ultrastructural localization of plasma membrane-associated urokinase-type plasminogen activator at focal contacts. *J. Cell Biol.* **106,** 87–95.