myotubes often were not affected by stretch. To test directly the hypothesis that stretch will inactivate the active channels in *mdx* mouse, we stretched and measured $[Ca^{2+}]_i$ in individual fibers from the flexor digitorum brevis muscle from normal and *mdx* mice. Stretch increased $[Ca^{2+}]_i$ slightly in normal fibers and decreased $[Ca^{2+}]_i$ slightly in *mdx* fibers. However, a substantial difference remains. $[Ca^{2+}]_i$ at different sarcomere lengths was measured with fura 2 loaded as the acetomethoxy ester. Normal muscle had 133 ± 4 nM $[Ca^{2+}]_i$ in the relaxed state, and $[Ca^{2+}]_i$ in the same fibers increased slightly to 146 ± 3 when stretched (n = 14); *mdx* fibers had 174 ± 5 nM $[Ca^{2+}]_i$ when relaxed, and this value in the same fibers decreased slightly to 165 ± 1 nM when the fibers were stretched (n = 13). Normal relaxed sarcomere length was 2.20 ± 0.13 μm; normal stretched length was 3.04 ± 0.08 μm; *mdx* relaxed length was 2.12 ± 0.10 μm and *mdx* stretched length was 3.15 ± 0.11 μm.

21. Free Ca^{2+} was calculated from the fluorescence ratio obtained by dividing the fluorescence (500 to 530 nm) values from excitation at 350 nm by those at 385 nm [G. Grynkiewicz, M. Poenie, R. Y. Tsien, *J. Biol. Chem.* **260** (no. 6), 3440 (1985)]. It was necessary to correct the ratios obtained in calibration solutions because of increased dye fluorescence inside the myotubes at 385-nm excitation, resulting from differences in intracellular viscosity, ionic strength, or dye binding [(*1*); R. Y. Tsien and M. Poenie, *Cell Calcium* **6**, 145 (1985)]. A correction factor of 0.70 was determined by permeabilizing myotubes with 2500 units of *Staphylococcus aureus* alpha toxin (Gibco-BRL) per milliliter in Ringer solutions containing concentrations of free Ca^{2+} in the 60- to 600-nM range. Primary myoblasts were obtained from mouse hindlimb muscle as previously described [J. Dimario and R. C. Strohman, *Differentiation* **39**, 42 (1988)]. Normal human and Duchenne clonal myoblasts were from frozen stocks. Myoblast fusion was induced by culturing in Dulbecco's modified Eagles medium (DMEM) with 10% horse serum. In mouse and human cultures, myotubes were present 2 and 4 days after differentiation, respectively. Cultures were loaded with dye by exposure to 10 μM fura 2-AM (Molecular Probes, Eugene, OR) for 60 min at 25°C in DMEM under 5% CO_2/95% air. Measurements were at 37°C in the Hepes-buffered Ringer.

22. The normal human leak channel closed-time distribution could be fitted with three exponentials with time constant (τ) values of 0.678, 6.68, 11.42 ms. The analogous values for Duchenne channels were 0.335, 1.86, and 3.13 ms. Thus, although the number of closed states appears to be unaffected, the time constants are dramatically altered. In a similar fashion, the normal mouse leak channels closed-time distribution could be fitted with τ values of 0.646, 4.869, and 8.019 ms; the *mdx* τ values were 0.368, 2.199, and 3.381 ms. Open channel time distributions could be fitted with two exponentials and did not differ greatly with the dystrophic condition. Normal human open τ values were 0.433 and 2.04 ms. Normal mouse open τ values were 0.314 and 1.919 ms.

23. We thank W. G. Owen for critical reading of the manuscript and T. Heiman-Patterson and R. Ham for supplying clonal myoblasts from normal human and Duchenne patients. Supported by the NIH Institute of General Medical Sciences, the Muscular Dystrophy Association, and a generous gift from W. T. Baker.

18 April 1990; accepted 11 July 1990

An Unusual Form of Lipid Linkage to the CD45 Peptide

AKIKO TAKEDA* AND ABBY L. MAIZEL

Some protein kinases and phosphatases are myristoylated on their amino terminus, which perhaps contributes to subcellular localization or regulation. Glycoprotein CD45, a hematopoietic tyrosine phosphatase, was examined for fatty acid content. The CD45 protein incorporated [^{3}H]myristate, but little [^{3}H]palmitate. The label was not metabolized and reincorporated into amino acids or saccharides, as revealed by peptide maps of CD45 labeled with [^{3}H]myristate, ^{14}C-labeled amino acids, [^{35}S]methionine, or ^{125}I, and glycosidase treatments, respectively. The myristate label was resistant to mild alkaline methanolysis and was found in fatty acid and sphingosine, indicating an unusual form of lipid attachment to CD45.

CD45 [ALSO DESIGNATED T200, B220, Ly5, and the leukocyte common antigen (LCA)] is a transmembrane glycoprotein of most hematopoietic cells (*1*). Several isotypes of CD45 may be variably expressed in a lineage- and developmental stage–specific manner as a result of differential usage of extracellular, NH_2-side exons (*2*). The intracellular portion contains phosphorylated serine residues (*3*) and tyrosine phosphatase activity (*4*) that, in turn, activates T cell tyrosine kinase p56^{lck} (*5*). CD45 is also involved in signal transduction (*6*).

Because some NH_2-terminal myristoylated proteins are protein kinases or phosphatases (*7*), we examined myristic acid incorporation into CD45. It has been suggested that myristoylation exerted regulatory roles by directing the juxtaposition of acylated proteins with other cellular components such as membranes and other peptides. A Moloney leukemia virus–induced murine (A/Sn), CD4$^+$8$^-$ T lymphoma line, YAC-1 (*8*), was cultured overnight in the presence of [^{35}S]methionine, [^{3}H]myristic acid, or [^{3}H]palmitic acid, and the cell lysates were immunoprecipitated with monoclonal antibodies (MAbs) to an invariable segment of CD45 (*1*), the transferrin receptor, or actin, and then analyzed by one-dimensional SDS–polyacrylamide gel electrophoresis (SDS-PAGE) (*9*) (Fig. 1A). The incorporation of the myristic acid label into CD45 was comparable to the incorporation into the transferrin receptor and actin, a representative fatty acylated protein (*10*) and an *N*-acetylated protein (*11*), respectively. The incorporation of palmitic acid into the transferrin receptor was as efficient as that of myristic acid. In contrast, palmitic acid was poorly incorporated into CD45 and actin. When a duplicate gel was treated with mild alkaline methanol (Fig. 1B), the fatty acid label incorporated into the transferrin receptor disappeared almost completely, while the treatment only slightly affected the label incorporated into CD45 and actin. Alkaline methanolysis releases fatty acyl groups from thio- as well as hydroxy-ester linkages without affecting peptide bonds (*12*); accordingly, the treatment did not alter the methionine label for any of the molecules examined (*13*).

The incorporation of the fatty acid label into actin was probably due to degradation of fatty acid into the acetyl form, which could be amide-linked to the NH_2-terminus of actin. The myristic acid label incorporated into the transferrin receptor may be in a palmitoyl form because of fatty acid chain elongation. However, in a cell-free system, either palmitate or myristate can be covalently attached to the transferrin receptor (*14*). A significant degree of fatty acid turnover into amino acids is unlikely, because the label incorporated into the transferrin receptor was labile to mild alkaline methanolysis, indicating that the label was indeed incorporated as the fatty acyl–thioester form.

In order to confirm that the [^{3}H]myristic acid incorporated into CD45 had not been converted into amino acids, we analyzed tryptic peptides of CD45 by two-dimensional (2-D) "mapping." CD45 immunoprecipitates were prepared from YAC-1 cells which were cultured in the presence of either [^{3}H]myristic acid, a ^{14}C-labeled amino acid mixture, or [^{35}S]methionine, or were surface-radioiodinated with ^{125}I (*15*). CD45 was isolated from excised gel pieces of preparative SDS-PAGE by electroelution, digested with trypsin overnight, and subjected to electrophoresis in the first dimension and chromatography in the second dimension (*3, 16*) (Fig. 2). Clearly, the fatty acid label was not incorporated in a form of amino acids, because in that event ^{3}H- and ^{14}C-labeled peptides should have shared a similar distribution pattern. Instead, virtually all ^{3}H-labeled peptides were found along the electrophoresis axis, whereas only ap-

Department of Pathology, Roger Williams General Hospital, Brown University, Providence, RI 02908.

*To whom correspondence should be addressed.

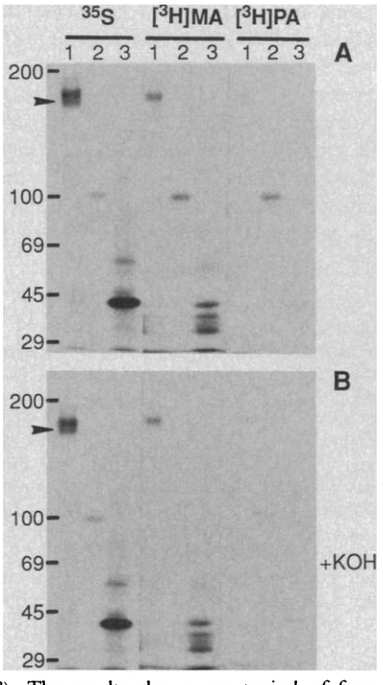

Fig. 1. Incorporation of myristic acid label into CD45. YAC-1 cells (1×10^6 cells per milliliter) were cultured either in methionine-free RPMI 1640 with supplements of 2 mM glutamine, penicillin (100 units per milliliter), streptomycin (0.1 mg/ml), 25 mM Hepes-NaOH (pH 7.4), and dialyzed fetal calf serum (10%) for [^{35}S]methionine labeling, or in Iscove's modified medium containing 5×10^{-5} M 2-mercaptoethanol, fetal calf serum (10%), and the above concentrations of glutamine, penicillin, and streptomycin for [^{3}H]myristic acid and [^{3}H]palmitic acid labeling. The cells were cultured for 1 hour in the media before the addition of the radioactive precursors. Then, 0.01 mCi of [^{35}S]methionine (Tran^{35}S-label, 1105 Ci/mmol, ICN Pharmaceuticals), 0.1 mCi of [9,10-^{3}H]myristic acid (39.3 Ci/mmol, New England Nuclear), or 0.1 mCi of [9,10-^{3}H]palmitic acid (30.0 Ci/mmol, New England Nuclear) was added per milliliter of culture followed by overnight incubation. The cells were lysed in polyoxyethylene 10 oleyl ether (BRIJ 96) (0.8%) containing 50 mM tris-HCl (pH 8.0), 150 mM NaCl, 0.1 mM EGTA (pH 8.0), 1 mM $MgCl_2$, 50 mM iodoacetamide, and 1 mM phenylmethylsulfonyl fluoride. Immunoprecipitates were prepared from the cell lysates by MAbs to an invariable segment of CD45 (lane 1), the transferrin receptor (lane 2), or actin (lane 3), and were subjected to SDS-PAGE (*9*) in a 3.5 to 8% acrylamide gradient. The gels were treated overnight at room temperature in 90% methanol without (**A**) or with (**B**) 0.1 N KOH. The position of molecular mass markers (expressed in kilodaltons) are shown on the left. The band indicated by arrowheads is discussed in (*13*). The results shown are typical of four independent trials. ([^{3}H]MA, [^{3}H]myristic acid; [^{3}H]PA, [^{3}H]palmitic acid.)

proximately one-third of ^{14}C-labeled peptides remained on the electrophoresis axis, and the rest were widely scattered. The peptides that remained on the electrophoresis axis were probably large, partially digested peptides, mostly derived from the extracellular, protease-resistant portion of CD45 (*3*), since they were prominent in ^{125}I-labeled peptides which represented the extracellular domain of CD45. In contrast, only a minor portion of ^{35}S-labeled peptides was found on the electrophoresis axis; ^{35}S-labeled peptides largely represent the protease-sensitive, intracellular portion of CD45, because 17 of the 19 methionine residues of CD45 are located intracellularly (*2*). The mapping pattern of ^{3}H-labeled peptides indicates that the fatty acid label was incorporated into the protease-resistant domain, that is, either into or in the proximity of the extracellular portion of CD45. This is in agreement with our results obtained by another comparative peptide mapping of CD45 partially digested by V8 protease (*17*).

The preceding results do not entirely eliminate the possibility that the fatty acid labeling of CD45 is a result of myristic acid turnover into the acetyl form, which may be

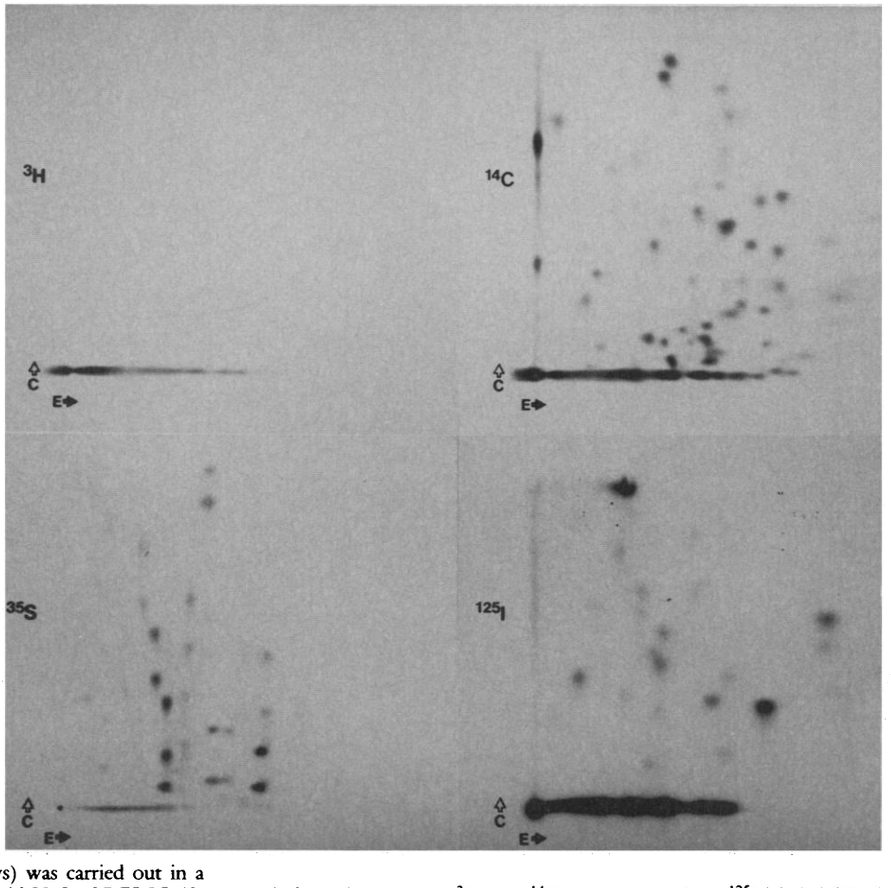

Fig. 2. Two-dimensional–tryptic peptide mapping of CD45. YAC-1 cells were labeled with [^{3}H]myristic acid overnight or with [^{35}S]methionine for 5 hours as described in Fig. 1, or overnight with an L-^{14}C(U)-labeled amino acid mixture [a mixture of A, R, D, E, G, H, I, L, K, F, P, S, T, Y, and V (*30*), 55.5 mCi/mmol of carbon, ICN Pharmaceuticals] added (2 μCi per milliliter of culture media; RPMI 1640 free of the 15 amino acids described above but containing the supplements described in Fig. 1), or ^{125}I-labeled with lactoperoxidase (*15*). Immunoprecipitates with MAb to CD45 were prepared from each cell lysate and were subjected to preparative SDS-PAGE. CD45 bands in the gel were excised by referring to locations of molecular mass standards, and CD45 was electrophoretically eluted from the gel pieces. The protein in the eluates was precipitated in 15% TCA with bovine γ-globulin as a carrier, washed in acetone, dried, dissolved in 6 M guanidine-HCl and 0.5 M tris-HCl (pH 8.0), and treated with dithiothreitol and iodoacetamide successively (*3*). The protein was reprecipitated in TCA, washed in acetone, dried, and redissolved in 0.05 M NH_4HCO_3. Each sample was digested with tosylamido phenylethyl chloromethyl ketone (TPCK)–trypsin (specific activity 12,200 units per milligram of protein, 0.17 mg/ml added for the first 17 hours and then 0.25 mg/ml for the following 6 hours) at room temperature. The dried samples were dissolved in the electrophoresis buffer and spotted on thin-layer cellulose plates (20 by 20 cm, 0.1 mm layer thickness). Electrophoresis in the first dimension (E; the direction toward the cathode is shown by arrows) was carried out in *n*-butanol:pyridine:acetic acid:H_2O (2:1:1:18, v/v/v/v) at 1000 V for 1 hour. Ascending chromatography in the second dimension (C; the direction as indicated by arrows) was carried out in a chamber saturated with *n*-butanol:pyridine:acetic acid:H_2O (97:75:15:60, v/v/v/v) for 4 hours. The peptide mapping patterns were reproducible when two independent trials on ^{3}H- and ^{14}C- and three trials on ^{125}I-labeled CD45 were carried out.

incorporated as a part of acetylated saccharides. CD45 immunoprecipitates prepared from YAC-1 cells that were labeled with [^{35}S]methionine or [^{3}H]myristic acid overnight were subjected to glycosidase treatments (Fig. 3). Without any enzyme, incubating samples at 37°C, in contrast with incubating them at −20°C, resulted in the loss of noticeable amounts of either label. Compared to those 37°C controls, endoglycosidase F as well as N-glycanase (*18*) treatments of CD45 reduced the size of the molecule by a few thousand daltons but did not cause any significant loss in the intensity of either label. Treatment with neuraminidase and then O-glycanase (*19*) did not affect CD45 at all, whereas the same treatment caused a decrease in size of CD45 derived from WEHI 279 cells (*20*), which expresses the B220 isotype of CD45 that contains O-linked saccharides (*2*). These results demonstrate that most of the fatty acid label incorporated into CD45 is part of a structure apart from saccharide moieties.

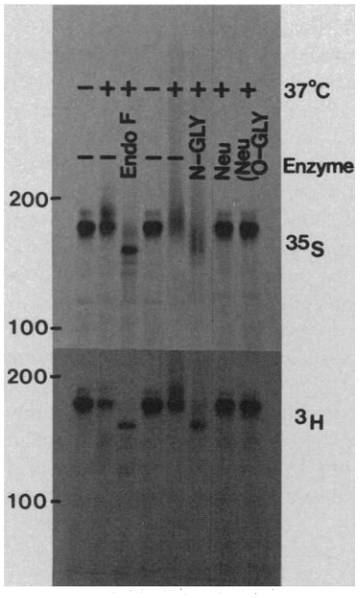

Fig. 3. Glycosidase treatment of CD45. YAC-1 cells were cultured overnight as described in Fig. 1 in the presence of either [^{35}S]methionine or [^{3}H]myristic acid. Immunoprecipitate prepared with MAb to CD45 was separated into equal aliquots. Each aliquot was either kept at −20°C (−) or incubated at 37°C overnight without enzyme (−) or with endoglycosidase F (Endo F) (*18*); N-glycanase (N-GLY) (6.25 units per milliliter; Genzyme); neuraminidase (Neu) (0.9 unit per milliliter; from *Arthrobacter ureafaciens*, Boehringer Mannheim); or O-glycanase (O-GLY) (0.032 unit per milliliter; Genzyme) (*19*), then analyzed on SDS-PAGE in a 3.5 to 8% acrylamide gradient. The samples in lanes 1 to 3 were in the solution consisting of 145 mM sodium phosphate (pH 8.6), SDS (0.37%), 2-mercaptoethanol (0.37%), 7.4 mM EDTA, NP-40 (0.74%), and glycerol (10%). The samples in lanes 4 to 6 were in 145 mM sodium phosphate (pH 8.6), SDS (0.37%), 2-mercaptoethanol (0.37%), and NP-40 (2.2%). The samples in lanes 7 and 8 were in 15 mM sodium phosphate (pH 6.0), SDS (0.3%), NP-40 (1.8%), and 10 mM calcium acetate. The position of molecular mass markers (expressed in kilodaltons) are shown on the left. The results are typical of three independent trials.

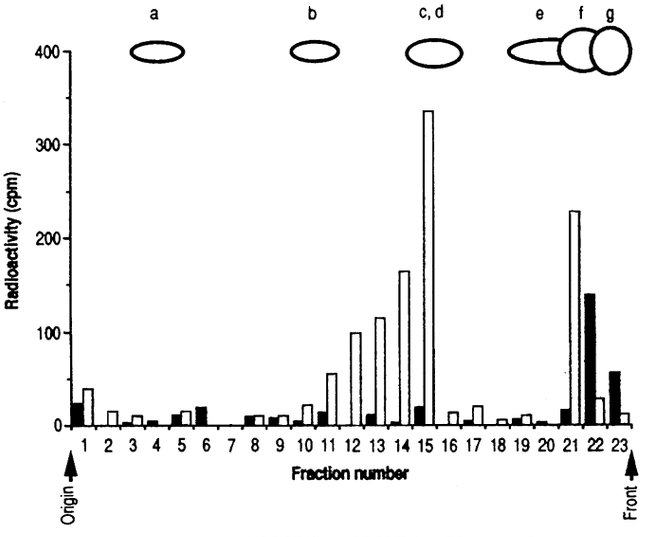

Fig. 4. TLC analysis of acid hydrolysis product derived from CD45 labeled with [^{3}H]myristic acid. YAC-1 cells were cultured overnight with [^{3}H]myristic acid as described in Fig. 1, and cell lysates were immunoprecipitated with MAb to CD45. The immunoprecipitate was subjected to preparative gel electrophoresis, and the CD45 band was excised from the gel for electroelution as in Fig. 2. The protein in the eluate was allowed to precipitate in 15% TCA on ice for 1 hour. After successive washing in cold ethanol and in a mixture of cold ethanol and ether (1:1, v/v), the precipitate was hydrolyzed in 6 N HCl at 110°C for 22 hours in a vacuum-sealed tube. The hydrolysate (6000 cpm) was extracted by adding chloroform, methanol, and H_2O (final ratio, 8:4:3, v/v/v). Both the upper and the lower phase of the mixture and various lipid standards were applied to a silica gel G plate (20 by 20 cm, 0.25 mm layer thickness). The plate was developed first in acetone:petroleum ether (1:3, v/v), and then in chloroform:methanol:acetic acid:H_2O (25:15:4:2, v/v/v/v). The silica gel was scraped from the sample lanes into each fraction of 6.4-mm length for the determination of radioactivity, and the rest of the plate was sprayed with Rhodamine B in order to locate the standards (Sigma): sphingomyelin (a, from bovine brain), psychosine (b, 1-β-D-galactosyl-sphingosine), sphingosine (c, derived from bovine brain sphingomyelin), dihydrosphingosine (d, DL-1,3-dihydroxy-2-amino octadecane), cerebroside (e, from bovine brain), myristic acid (f, tetradecanoic acid), methyl esters of fatty acids (g, methyl esters of palmitic acid, stearic acid, oleic acid, linolenic acid, and arachidic acid). The results are typical of six independent trials. (■, Lower phase; □, upper phase.)

We next sought to identify the lipid moiety attached to CD45. CD45 labeled with [^{3}H]myristic acid was isolated by electroelution from a preparative SDS-PAGE gel and was subjected to acid hydrolysis with 6 N HCl at 110°C for 22 hours. Under these hydrolysis conditions, all lipids, saccharides, and peptides will be broken down to their simplest structural components (*12*). The hydrolysate was extracted with a chloroform:methanol:H_2O(HCl) mixture (*21*), and both the upper and the lower phases were analyzed by thin-layer chromatography (TLC) (*22*) (Fig. 4). The TLC condition used here is appropriate for resolving phospholipids and lipids of high polarity, and, as a result, most neutral lipids migrate near or with the solvent front. The TLC of the upper phase revealed two main radioactive peaks, one of which comigrated with myristic acid, whereas the other comigrated with sphingosine-dihydrosphingosine standards with a considerable amount of "tailing" material. On the other hand, analysis of the lower phase resulted in only one peak which comigrated with myristic acid and the solvent front. Sphingosine is difficult to extract into a chloroform phase by the typical chloroform:methanol:H_2O mixture used for lipid extraction, especially in highly acidic conditions, because of the compound's high polarity and amino group (*12*). The material "tailing" behind the sphingosine-dihydrosphingosine standards may represent a group of related compounds with hydrocarbon chains shorter than those of the standards, which are either derived from brain sphingomyelin or chemically synthesized octadecane (C_{18}). In addition, the presence of methanol during extraction and storage of lipid preparations is expected to cause the partial conversion of free fatty acids into their methyl ester forms, which migrate with the solvent front (*12*). In order to confirm the identity of the lipid components, we subjected similar hydrolysis products to another TLC condition intended for the separation of neutral lipids (*22*). The radioactivity was again found in three locations where sphingosine-dihydrosphingosine, fatty acid, and methyl ester of fatty acid standards were detected (*23*). Without the acid hydrolysis, no radioactive material could be extracted into the chloroform phase, and all the radioactivity remained with aggregated material in the upper phase.

Our data suggest that CD45 of YAC-1 exists in a lipid-linked form. It is unlikely

that the lipid is noncovalently associated with CD45, since the lipid attachment survives boiling in the presence of SDS followed by separation by electrophoresis, precipitation in trichloroacetic acid (TCA) and washing in ethanol:ether or acetone, and extraction with chloroform:methanol. At present, there are four forms of lipid linkages known to occur among proteins of eukaryotic origin: (i) fatty acylation through cysteine residues forming thioester linkage, as in the case of the transferrin receptor (*10*); (ii) polyisoprenoid attached to COOH-terminal cysteine forming thioether linkage as reported for p21Ras (*24*); (iii) phosphatidyl inositol (PI) as a part of PI-glycan membrane anchor attached to the COOH-terminus of numerous membrane molecules such as Thy-1 (*25*); and (iv) myristoylation on NH_2-terminal glycine forming amide linkage in such proteins as $p60^{src}$ (*26*). Earlier studies of CD45 detected no incorporated radioactive palmitic acid (*10*) and no sensitivity to PI-specific phospholipase C treatment (*27*). Thus, the first and the third types of the possible lipid linkages seem unlikely. This is confirmed by our data because the lipid attached to CD45 is resistant to mild alkaline treatment, to which thioester and the PI-glycan types of fatty acyl linkages should be labile. Moreover, none of the HCl hydrolysis products migrates as long-chain hydrocarbons such as the polyisoprenoids that form thioether linkages with certain proteins. The presence of a fatty acyl and a sphingosine-type material in a comparable ratio in the acid hydrolysates renders it likely that they are derived from a form of sphingolipids. It is possible that such lipids are covalently linked to proteins.

Sphingolipids and their breakdown products, sphingosine and lysosphingosine, have numerous biological effects, including modulation of cell proliferation and junction formation, and cell contact inhibition (*28*). Furthermore, sphingolipid breakdown products inhibit protein kinase C and signal transduction, implicating a role for them as "negative" messengers (*28, 29*). Because CD45 is already known to affect at least one kinase, $p56^{lck}$ (*5*), it is tempting to speculate a role for CD45 and its sphingolipid in regulating protein kinases. CD45 may partially exert its effect by adjusting the amount of free, kinase-inhibiting sphingosine through formation and breakdown of sphingolipid-peptide linkage.

REFERENCES AND NOTES

1. T. Springer, G. Galfre, D. S. Secher, C. Milstein, *Eur. J. Immunol.* **8**, 539 (1978).
2. Y. Saga, J.-S. Tung, F.-W. Shen, E. A. Boyse, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 6940 (1986); M. L. Thomas, P. J. Reynolds, A. Chain, Y. Ben-Neriah, I. S. Trowbridge, *ibid.* **84**, 5360 (1987).
3. M. B. Omary and I. S. Trowbridge, *J. Biol. Chem.* **255**, 1662 (1980).
4. N. K. Tonks, H. Charbonneau, C. D. Diltz, E. H. Fischer, K. A. Walsh, *Biochemistry* **27**, 8695 (1988).
5. T. Mustelin, K. M. Coggeshall, A. Altman, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 6302 (1989); H. L. Ostergaard *et al.*, *ibid.*, p. 8959.
6. J. A. Ledbetter, N. K. Tonks, E. H. Fischer, E. A. Clark, *ibid.* **85**, 8628 (1988); P. A. Kiener and R. S. Mittler, *J. Immunol.* **143**, 23 (1989); J. P. Deans, J. Shaw, M. J. Pearse, L. M. Pilarski, *ibid.*, p. 2425.
7. D. A. Towler, J. I. Gordon, S. P. Adams, L. Glaser, *Annu. Rev. Biochem.* **57**, 69 (1988).
8. M. Cikes, S. Friberg, Jr., G. Klein, *J. Natl. Cancer Inst.* **50**, 347 (1973); R. Le Corre *et al.*, *Eur. J. Immunol.* **17**, 327 (1987).
9. U. K. Laemmli, *Nature* **227**, 680 (1970).
10. M. B. Omary and I. S. Trowbridge, *J. Biol. Chem.* **256**, 4715 (1981).
11. S. Tsunasawa and F. Sakiyama, *Methods Enzymol.* **106**, 165 (1984).
12. J. C. Dittmer and M. A. Wells, *ibid.* **14**, 482 (1969).
13. The lowest molecular weight moiety of CD45 seen with the methionine label (the band indicated by arrows in Fig. 1) was absent from those preparations labeled with the fatty acid. Hence, it provides evidence that peptide synthesis and incorporation of the fatty acid label did not always coincide. This fast-migrating band of CD45 tended to appear only after long-term methionine labeling. Furthermore, surface-radioiodinated preparations of CD45 also did not reveal the fast-migrating band (A. Takeda, unpublished data). These observations suggest that the fast-migrating form of CD45 may be a modified or degraded intracellular form or may represent a minor isotype.
14. M. Adam *et al.*, *J. Biol. Chem.* **259**, 15460 (1984).
15. R. E. Cone and W. C. Brown, *Immunochemistry* **13**, 571 (1976).
16. W. Gibson, *Virology* **62**, 319 (1974).
17. YAC-1 cells were labeled by [^{3}H]myristic acid or [^{35}S]methionine incorporation, or by surface-^{125}I labeling, and CD45 electroeluates were prepared as described in Fig. 2. The eluates were digested with V8 protease for partial proteolysis; then the digests were analyzed by SDS-PAGE. The fatty acid and the surface-iodine–labeled samples showed a remarkable resemblance, producing only a few dominant peptides among which the largest two bands comigrated. The methionine-labeled sample, on the other hand, exhibited a pattern quite different from the other two, producing many peptides of various sizes. The resemblance between the myristic acid and the surface-iodine patterns implies that the myristic acid label was incorporated either into or in the proximity of the extracellular portion of CD45.
18. J. H. Elder and S. Alexander, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4540 (1982).
19. J. Umemoto, V. P. Bhavanandan, E. A. Davidson, *J. Biol. Chem.* **252**, 8609 (1977).
20. A. Takeda, unpublished data.
21. J. Folch *et al.*, *J. Biol. Chem.* **226**, 497 (1957).
22. V. P. Skipski and M. Barclay, *Methods Enzymol.* **14**, 530 (1969).
23. A. Takeda, unpublished data.
24. J. F. Hancock, A. I. Magee, J. E. Childs, C. J. Marshall, *Cell* **57**, 1167 (1989).
25. A. G. D. Tse, A. N. Barclay, A. Watts, A. F. Williams, *Science* **230**, 1003 (1985).
26. B. M. Sefton, I. S. Trowbridge, J. A. Cooper, E. M. Scolnick, *Cell* **31**, 465 (1982).
27. J. Stiernberg, M. G. Low, L. Flaherty, P. W. Kincade, *J. Immunol.* **138**, 3877 (1987).
28. Y. A. Hannun and R. M. Bell, *Science* **243**, 500 (1989).
29. Y. A. Hannun *et al.*, *J. Biol. Chem.* **261**, 12604 (1986); Y. A. Hannun, C. S. Greenberg, R. M. Bell, *ibid.* **262**, 13620 (1987).
30. Single-letter abbreviations for the amino acid residues are A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.

31. We thank R. Frackelton, P. Knopf, S. Mehta, and P. Wiest for useful comments on the paper; J. Kaufman for providing endoglycosidase F; J. Wu for technical assistance; R. Frackelton for help in peptide mapping; and J. Morgan for help in preparation of the manuscript. Supported by American Cancer Society grant ACS-IN 45-30 (to A.T.) and National Cancer Institute grant CA 45148 (to A.L.M.).

27 March 1990; accepted 16 August 1990

Expression of Murine CD1 on Gastrointestinal Epithelium

PAUL A. BLEICHER,* STEVEN P. BALK, SUSAN J. HAGEN, RICHARD S. BLUMBERG, THOMAS J. FLOTTE, COX TERHORST

Cluster of differentiation 1 (CD1) in humans is a family of major histocompatibility complex (MHC) class I–like molecules expressed on the surface of immature thymocytes, Langerhans cells, and a subpopulation of B cells. The only function identified for human CD1 is as a ligand recognized by a subpopulation of T lymphocytes. In order to study the distribution and function of these molecules in the mouse, a murine CD1 complementary DNA was expressed in mouse fibroblasts and used to produce monoclonal antibodies. These antibodies revealed prominent expression of murine CD1 only on gastrointestinal tract epithelium and in the cytoplasm of hepatocytes. Low levels of expression were also detected on thymocytes and peripheral lymphocytes. The gastrointestinal distribution of murine CD1 suggests that this molecule may be important in epithelial immunity.

THE INTRAEPITHELIAL COMPARTMENT of mouse skin, lung, vagina, uterus, and tongue each contain T cells bearing γδ receptors of limited diversity (*1–3*). Similarly, the intestinal epithelium is populated by γδ-bearing T cells, which use a limited number of V_γ genes, but do have extensive junctional diversity (*4*). These observations suggest that antigen recognition in epithelial immunity may differ from antigen recognition as it is understood in systemic immunity. The limited V_γ gene usage and restricted heterogeneity of the γδ receptors on intraepithelial T cells suggest that