A new sandwich enzyme immunoassay for measurement of plasma pre-β1-HDL levels

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Abstract Pre-β1-HDL, a putative discoid-shaped high density lipoprotein (HDL) of approximately 67-kDa mass that migrates with pre-β mobility in agarose gel electrophoresis, contains apolipoprotein A-I (apoA-I), phospholipids, and unesterified cholesterol. It participates in the retrieval of cholesterol from peripheral tissues. In this study we established a new sandwich enzyme immunoassay (EIA) for measuring plasma pre-β1-HDL using mouse anti-human pre-β1-HDL monoclonal antibody (MAb 55201) and goat anti-human apoA-I polyclonal antibody. MAb 55201 reacted with apoA-I in lipoprotein [A-I] with molecular mass less than 67 kDa, and with pre-β1-HDL separated by nondenaturing two-dimensional electrophoresis, whereas it did not react with apoA-I in α-HDL. Pre-β1-HDL levels measured by this method declined when incubated at 37°C for 2 h, whereas this decrease was not observed in the presence of 2 mM lecithin:cholesterol acyltransferase inhibitor 5,5′-dithiobis (2-nitrobenzoic acid). To clarify the clinical significance of measuring pre-β1-HDL by this method, 47 hyperlipidemic subjects [male/female 22/25; age 55 ± 14 years; body mass index 25 ± 4.5 kg/m^2; total cholesterol (TC) 245 ± 64 mg/dl; triglyceride (TG) 232 ± 280 mg/dl; HDL cholesterol (HDL-C) 51 ± 23 mg/dl] and 25 volunteers (male/female 15/10; age 36 ± 9.3 years; body mass index 23 ± 3.5 kg/m^2; TC 183 ± 28 mg/dl; TG 80 ± 34 mg/dl; HDL-C 62 ± 15 mg/dl) were involved. Plasma pre-β1-HDL levels were significantly higher in hyperlipidemic subjects than in volunteers (39.3 ± 10.1 vs. 22.5 ± 7.5 mg/ml, $P < 0.001$) whereas plasma apoA-I levels did not differ (144.2 ± 28.4 vs. 145.3 ± 16.3 mg/dl). These results indicate that this sandwich EIA method specifically recognizes apoA-I associated with pre-β1-HDL.—Miyazaki, O., J. Kobayashi, I. Fukamachi, T. Miida, H. Bujo, and Y. Saito. **A new sandwich enzyme immunoassay for measurement of plasma pre-β1-HDL levels. *J. Lipid Res.* 2000.** 41: **2083–2088.**

Supplementary key words BALB/c mice • hypoxanthine-aminopterin-thymidine medium • SDS-PAGE • anti-apoA-II polyclonal antibody • horseradish peroxidase

High density lipoprotein (HDL) is defined as lipoprotein in the density range of 1.063–1.21 kg/L (1). HDL particles are heterogeneous in size, apolipoprotein composition, and function (2, 3). Earlier studies by Castro and Fielding (4) showed that the preferred initial plasma acceptor of cell membrane cholesterol is a minor subpopulation of small, lipid-poor, pre-β-migrating HDL particles.

Pre-β1-HDL, a putative discoid-shaped HDL of approximately 67 kDa mass that migrates with pre-β mobility on agarose gel electrophoresis (4), contains apolipoprotein A-I (apoA-I), phospholipids, and unesterified cholesterol. It participates in a cyclic process involved in the retrieval of cholesterol from peripheral tissues.

In this cycle, unesterified cholesterol from cells is incorporated into pre-β1-HDL, providing a substrate for esterification of cholesterol by lecithin:cholesterol acyltransferase (LCAT). Pre-β1-HDL then becomes incorporated into larger HDL species of α mobility as esterification proceeds and is regenerated during transfer of cholesteryl esters from α-HDL particles to acceptor lipoproteins (4, 5). Although pre-β1-HDL has been observed both in plasma (4, 6) and peripheral lymph (7), the origin and metabolic fate remain obscure. Pre-β1-HDL has been shown to be a preferred plasma acceptor of cholesterol released from HepG2 cells (4, 8, 9). The importance of pre-β1-HDL as an acceptor of cell membrane cholesterol has been consolidated in studies in which their depletion from plasma resulted in a reduction, by more than half, of the ability of the plasma to promote cholesterol efflux from cultured fibroblast (10). This result was subsequently confirmed in studies with a monoclonal antibody (MAb) that recognizes an epitope of apoA-I that is exposed only in pre-β1-HDL (11).

Abbreviations: apoA-I, apolipoprotein A-I; BSA, bovine serum albumin; EIA, enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; FPLC, fast protein liquid chromatography; HDL, high density lipoprotein; HDL-C, HDL cholesterol; HRP, horseradish peroxidase; LCAT, lecithin:cholesterol acyltransferase; Lp[A-I], lipoprotein [A-I]; MAb, monoclonal antibody; PAb, polyclonal antibody; PBS, phosphate-buffered saline; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TC, total cholesterol; TG, triglyceride.

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Plasma pre-β1-HDL is known to decrease during incubation of plasma in the presence of LCAT (12). Although several methods have been developed and used to determine plasma and serum levels of pre-β1-HDL (13–17), no methods using antibody specifically recognizing this particle are available. In this study we describe the development of a novel method of quantitation of plasma pre-β1-HDL levels by a sandwich enzyme immunoassay (EIA) using a specific MAb raised against apoA-I associated with pre-β1-HDL.

MATERIALS AND METHODS

Subjects

Forty-seven hyperlipidemic subjects [male/female 22/25; average age 55 ± 14 years; body mass index 25 ± 4.5 kg/m^2; total cholesterol (TC) 245 ± 64 mg/dl; triglyceride (TG) 232 ± 280 mg/dl; HDL cholesterol (HDL-C) 51 ± 23 mg/dl] and 25 normal volunteers (male/female 15/10, average age 36 ± 9 years; body mass index, 23 ± 3.5 kg/m^2; TC 183 ± 28 mg/dl; TG 80 ± 34 mg/dl; HDL-C 62 ± 15 mg/dl) were recruited for the present study.

The study protocol was approved by the institutional review board of Chiba University Medical School (Chiba, Japan). All participants gave informed consent. The participants were studied in the Lipid Research Clinic in Chiba University Hospital (Chiba, Japan).

Preparation of antigen

The HDL fraction (1.063 g/ml < d < 1.21 g/ml) was obtained by ultracentrifugation (1) from the plasma of a normal volunteer.

The obtained HDL were delipidated with diethyl ether–ethanol 3:2 (v/v) at 4°C (18).This mixture was subjected to centrifugation at 5,000 rpm for 10 min and the obtained pellet was washed with ethyl ether. The pellet, resolved with 8 M urea, was applied to Sephacryl S-200 (Amersham Pharmacia, Piscataway, NJ) for gel filtration (19) followed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The obtained protein, corresponding to a molecular mass of 28,000 Da, was pooled and then condensed with a Vivapore concentrator (Vivascience, Westford, MA). This pooled fraction was stocked at −80°C after dialysis against phosphate-buffered saline (PBS) until used for immunization of mice.

Preparation of MAb

Purified human apoA-I emulsified with complete Freund's adjuvant (GIBCO, Grand Island, NY) (volume ratio of 1:1, 0.1 mg/0.1 ml) was subcutaneously injected into BALB/c mice (6-week-old females) five times with a 1-week interval between injections. Two days after the final injection of the antigen, spleen cells were extracted and fused with murine myeloma cells (SP2/0-Ag14) at the ratio of 6:1 in the presence of 50% polyethylene glycol 1540 (Wako Chemical, Kyoto, Japan). The fused cells (2.5 × 10^6/ml) were suspended in hypoxanthine-aminopterin-thymidine medium and seeded into 96-well plates (Corning, Acton, MA), followed by incubation in 5% CO_2 at 37°C for 2 weeks.

Screening of monoclonal cells

To select monoclonal cells producing anti-pre-β1-HDL MAb, the supernatant of the hybrid cells was added to a microplate (Nunc, Roskilde, Denmark) coated with goat anti-mouse IgG (FMC, Rockland, ME) antibody (Jackson ImmunoResearch, West Grove, PA) and incubated. After washing the plates, apoA-I-containing lipoproteins (mainly HDL) were added to the plates and incubated. After washing the plates, biotin-conjugated goat anti-apoA-I polyclonal antibody (PAb) (or anti-apoA-II antibody) prepared by immunizing goats with purified human apoA-I (or apoA-II) was added and incubation was carried out. Conjugation of goat anti-apoA-I (or apoA-II) PAb with biotin was conducted using biotin-*N*-hydroxyl succimide. The plates were then washed and then streptavidin conjugated with horseradish peroxidase (HRP) was added. After washing the plates, the peroxidase activity was measured by absorbance at 492 nm, using *o*-phenylenediamine in citrate buffer (pH 5.2) containing H_2O_2 as substrate. We then selected hybridomas that showed strong reactivity against biotin-conjugated anti-apoA-I antibody but did not show reactivity against biotin-conjugated anti-apoA-II antibody. The selected hybridomas were cloned by limited dilution and then injected into the peritoneal region of mice, using cell counts of 0.5 × 10^6/ml. Fourteen days later, ascites were harvested from the mice and IgG was purified with protein A-Sepharose CL-4B from ascites fluid. This purified MAb was named MAb 55201.

Reactivity of MAb 55201 against apoA-I by Western blotting

After separation from human plasma by SDS-PAGE, the gels were electrophoretically transferred onto a polyvinylidene difluoride membrane (Millipore, Danvers, MA). The membrane was blocked with PBS containing 0.05% Tween 20 (pH 7.2) and 3% skim milk. The membrane was first reacted with MAb 55201 and was then reacted with HRP-conjugated goat anti-mouse IgG antibody. The membrane was then stained with diaminobenzidine.

Reactivity of MAb 55201 against Lp[A-I] and Lp[A-I:A-II] by enzyme-linked immunosorbent assay

Reactivity of MAb 55201 against lipoprotein [A-I] (Lp[A-I]) and Lp[A-I:A-II] was investigated by a sandwich EIA. In the immunosorbent procedure, the plates were coated with MAb 55201 (5 μg/ml in PBS, pH 7.2) at 4°C overnight. After rinsing the plates with PBS three times and blocking the plates with PBS containing 1% bovine serum albumin (BSA), diluted human plasma was added and incubated at room temperature for 1 h. After the plates were rinsed, goat anti-apoA-I PAb (or anti-apoA-II PAb), 5 μg/ml, conjugated with HRP was added and incubated at room temperature for 1 h. The plates were then rinsed and peroxidase activity was measured by absorbance at 492 nm, using *o*-phenylenediamine in citrate buffer (pH 5.3) containing H_2O_2 as substrate.

Reactivity of MAb 55201 against each lipoprotein fraction by gel filtration

To determine which lipoprotein fraction MAb 55201 showed reactivity, gel filtration with a Pharmacia fast protein liquid chromatography (FPLC) system composed of two TSK gel G3000SW columns (7.5-mm i.d. × 60 cm), a TSK gel G3000SW column (7.5-mm i.d. × 30 cm), and a Superdex 200 HR 10/30 column (10-mm i.d. × 30 cm) was carried out. Two hundred microliters of normal human plasma was applied to the gel and filtrated with 0.9% NaCl solution at a flow rate of 0.15 ml/min. Reactivity of MAb 55201 against each fraction was determined with a sandwich EIA using MAb 55201 and HRP-labeled goat anti-human apoA-I PAb. ApoA-I, apoA-II, and apoE concentrations were determined with a sandwich EIA, using PAb.

Reactivity of MAb 55201 against pre-β1-HDL by two-dimensional electrophoresis

The distribution of lipoprotein fractions, particularly those of HDL, was determined by two-dimensional agarose-polyacry-

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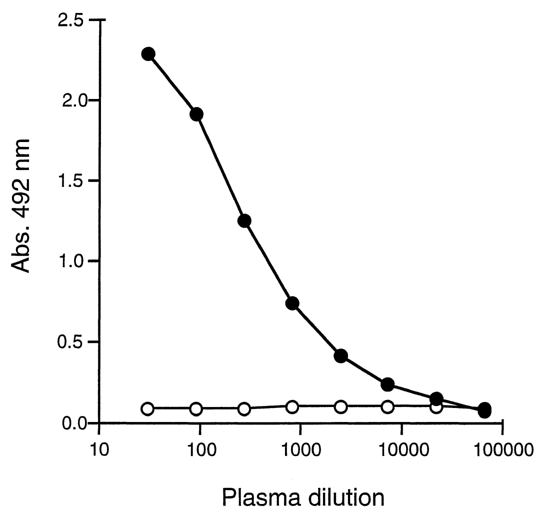

Fig. 1. Reactivity of MAb 55201 against Lp[A-I] and Lp[A-I:A-II] by ELISA. The supernatant of the hybrid cells was added to a microplate (Nunc) coated with goat anti-mouse IgG (Fc) antibody (Jackson ImmunoResearch) and incubated. After washing the plates, apoA-I-containing lipoproteins (mainly HDL) were added to the plates and incubated. After washing the plates, biotin-conjugated goat anti-apoA-I PAb (solid circles) or anti-apoA-II PAb (open circles) prepared by goat immunization with purified human apoA-I (or apoA-II) was added and the incubation was carried out. The plates were then washed and streptavidin conjugated with horseradish peroxidase was added. After washing the plates, the peroxidase activity was measured by absorbance at 492 nm, using *o*-phenylenediamine in citrate buffer (pH 5.2) containing H_2O_2 as substrate.

lamide gel electrophoresis (2, 4, 6, 10–12). In the present study, fasting plasma from a normal volunteer was preincubated for 5 min on ice-water with MAb 55201 or nonimmune mouse IgG before conducting agarose gel electrophoresis.

A sandwich EIA to measure plasma pre-β1-HDL levels

The obtained MAb 55201 (5 μg/ml in 50 ml of PBS, pH 7.2) was incubated in a 96-well enzyme-linked immunosorbent assay (ELISA) plate (Nunc) at 4°C overnight. The plates were rinsed three times with PBS, and 100 μl of BSA (1% in PBS) was added and then incubated at room temperature for 1 h. The purified human apoA-I as standard and the sample plasma (50 μl/well) diluted with PBS containing 1% BSA were placed into wells and incubated for 1 h at room temperature. The plates were then rinsed three times with PBS containing 1% BSA and goat anti-human apoA-I PAb (5 μg/ml) conjugated with HRP was reacted at room temperature for 1 h. The plates were then rinsed three times and the peroxidase activity was measured by absorbance at 492 nm, using *o*-phenylenediamine in citrate buffer (pH 5.3) containing H_2O_2 as substrate.

RESULTS

Reactivity of MAb 55201

We confirmed that MAb 55201 reacted with bands corresponding to a molecular mass of 28 kDa (data not shown) by Western blot analysis. Reactivity of MAb 55201 against Lp[A-I] and Lp[A-I:A-II] by ELISA was also investigated (**Fig. 1**). The obtained MAb 55201 showed strong reactivity against the system of HRP-labeled goat anti-apoA-I PAb whereas it did not show any reactivity against the system of HRP-labeled goat anti-apoA-II PAb. These results indicate that MAb 55201 reacts specifically with apoA-I in Lp[A-I]. Reactivity of MAb 55201 against each lipoprotein fraction by gel filtration was also confirmed (**Fig. 2**). The antibody 55201 showed a strong reactivity against the lipoprotein fraction with a molecular mass of less than 67 kDa, which contained a considerable amount of apoA-I but not apoE or apoA-II. To determine whether lipoprotein fractions recognized by MAb 55201 corresponded to the pre-β1-HDL reported by Fielding et al. (11), two-dimensional electrophoretic analysis of HDL fraction preincubated with MAb 55201 (or control mouse IgG) was carried out (**Fig. 3**). When human plasma was preincubated with MAb 55201 followed by

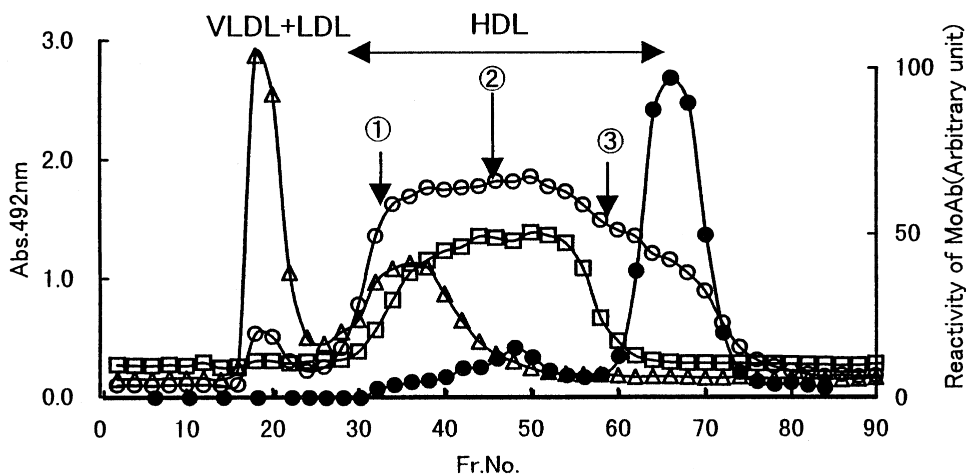

Fig. 2. Reactivity of anti-human pre-β1-HDL MAb 55201 against each lipoprotein fraction separated by gel filtration, using an FPLC system. Numbers 1, 2, and 3 (circled) correspond to MW 440,000, MW 150,000, and MW 67,000, respectively. Open circles, open squares, and open trianges represent apoA-I, apoA-II, and apoE levels, respectively, measured by ELISA with PAbs. Solid circles indicate the apoA-I level, measured by ELISA with MAb 55201.

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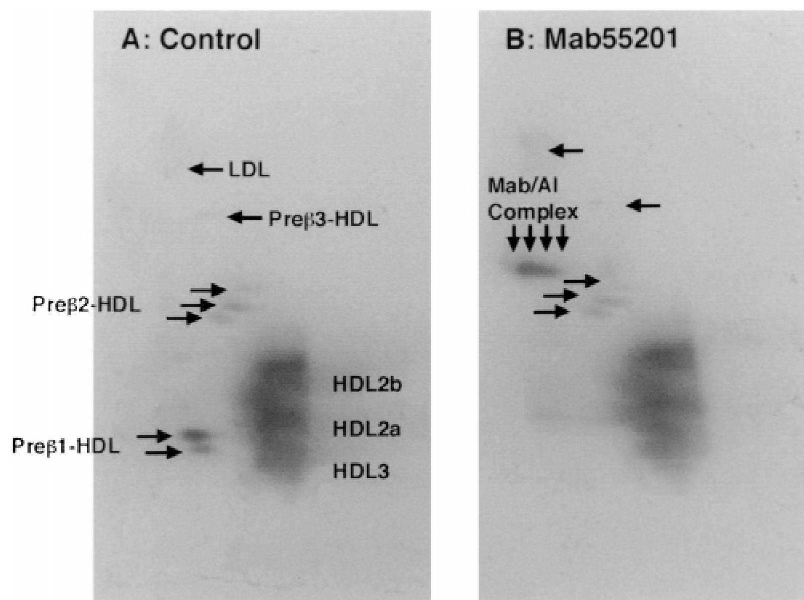

Fig. 3. Two-dimensional nondenaturing electrophoresis of human plasma preincubated with the anti-human pre-β1-HDL MAb 55201 (right) or nonimmune mouse IgG (left). First-dimension separation (horizontal axis) was in 0.75% agarose; second-dimension separation (vertical axis) was in a nondenaturing gradient (2–15%, w/v) polyacrylamide gel. The separated proteins were transferred to a nitrocellulose membrane and apoA-I-containing complexes were identified by reaction first with polyclonal rabbit anti-human apo-AI antibody and then with ^{125}I-labeled goat anti-rabbit IgG. The distribution of reactivity was visualized after autoradiography. The concentration of MAb 55201 (or nonimmune IgG) added to plasma prior to 2D electrophoresis was 3.1 mg/ml in a volume ratio of 1:1. The concentration of pre-β1-HDL used in this experiment was 25 μg/ml. We repeated this experiment five times, using plasma from control and hyperalphalipoproteinemic subjects and obtained identical electrophoretic patterns. The spot labeled as "Mab/AI Complex" showed reactivity with both anti-apoA-I Ab and anti-mouse IgG.

two-dimensional electrophoresis, the spot of pre-β1-HDL visualized by autoradiography was found to have almost completely disappeared, which was not the case when preincubated with nonimmune mouse IgG. This result indicates that MAb 55201 reacted specifically with apoA-I in pre-β1-HDL.

Standard curve and precision of EIA using MAb 55201

Plasma specimens with 3 different concentrations of pre-β1-HDL were measured 12 consecutive times. The assay coefficient of variations was 5.2%, 5.0%, and 3.1%, at concentration of 64.7, 39.6, and 17.5 ng/ml, respectively. The standard curve showed that the absorbance was proportional to the added purified human apoA-I (**Fig. 4A**). A dilution test showed that the curve produced by serially diluted plasma samples was parallel to the original standard curve, indicating that this assay system specifically determines concentration of pre-β1-HDL (Fig. 4B).

Effect of incubation at 37°C on plasma levels of pre-β1-HDL in normal volunteers

When plasma from normal volunteers (n = 3) was incubated at 37°C, the pre-β1-HDL levels declined in a time-dependent manner, while this decrease was not observed when the sample was incubated in the presence of 2 mM LCAT inhibitor 5,5′-dithiobis (2-nitrobenzoic acid) (DTNB) (**Fig. 5**). This result suggests that pre-β1-HDL measured by the present method disappeared from the plasma due to the function of plasma LCAT, which is well known to

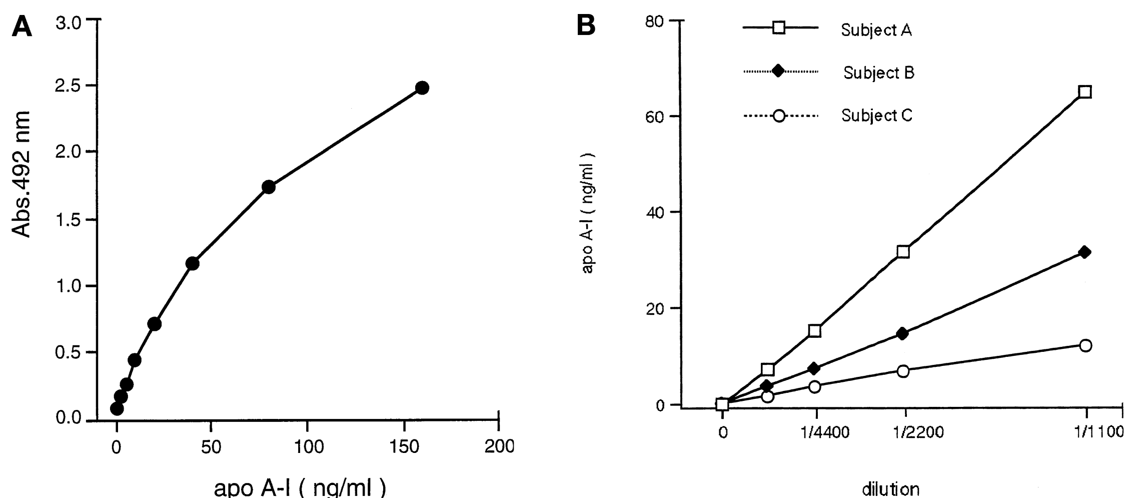

Fig. 4. 22. Linearity of human pre-β1-HDL in the developed immunoassay system. (A) Standard curve of plasma pre-β1-HDL measured by the sandwich EIA. (B) Serial dilutions of human plasma from volunteers were incubated as standard. Each point represents the average of duplicate values, and the duplicate values differed by less than 5%.

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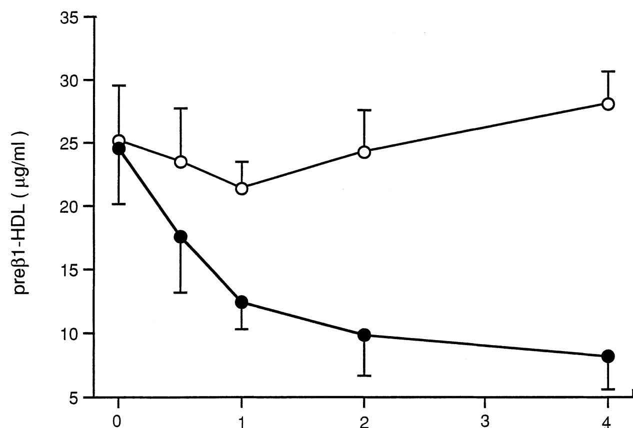

Fig. 5. Effects of incubation at 37°C for the indicated times on the plasma level of pre-β1-HDL from three volunteers (male/female 1/2; average age 32 ± 4.2 years; body mass index 21 ± 1.8 kg/m^2; TC 178 ± 24 mg/dl; TG 72 ± 13 mg/dl; HDL-C 70 ± 8.7 mg/dl) in the presence or absence of 2 mM LCAT inhibitor DTNB. Open circles show time course for plasma pre-β1-HDL level with incubation at 37°C with DTNB. Closed circles show time course for plasma pre-β1-HDL level with incubation at 37°C without DTNB. Values represent means ± SD.

esterify the free cholesterol in HDL particles during the process of maturation.

Comparison of plasma pre-β1-HDL level between hyperlipidemic and control subjects

Plasma pre-β1-HDL levels were significantly higher in subjects with hyperlipidemia than in normal control subjects, whereas plasma apoA-I levels did not differ between these two groups (**Fig. 6**).

DISCUSSION

In this study we have reported the specificity of MAb 55201 and a novel method for measurement of plasma pre-β1-HDL levels, using a sandwich EIA and MAb 55201. One of the notable aspects of this method for measurement of pre-β1-HDL is that we have used a specific MAb that recognizes apoA-I associated with pre-β1-HDL particles. We have proved that MAb 55201 specifically reacts with apoA-I associated with pre-β1-HDL, because MAb 55201 reacts with Lp[A-I] in the gel-filtration fraction corresponding to a molecular weight of less than 6.7 × 10^4 and with the pre-β1-HDL fraction separated by nondenaturing two-dimensional electrophoresis.

The fact that the pre-β1-HDL levels measured by the sandwich EIA method declined drastically when plasma was incubated at 37°C, while this decrease was not observed in the presence of 2 mM LCAT inhibitor DTNB, was compatible with the observation by Miida et al. (12). This result suggests that pre-β1-HDL measured by the present method disappears from the plasma during the maturation of HDL particles, mainly because of the function of plasma LCAT, which is well known to cause esterification of free cholesterol in the immature HDL particles. Several methods were developed and used to measure plasma pre-β1-HDL levels, among which was secondary electrophoresis (4, 10). In 1997, O'Connor et al. (14) reported an ultrafiltration-isotope dilution technique to quantitate pre-β1-HDL levels in human plasma, in which they used a special membrane with a permeability limit of 100 kDa discriminating categorically between pre-β1-HDL and larger HDL particle species.

One great advantage of measurement of plasma pre-β1-HDL levels by a sandwich EIA over other methods is its ease of use and thus suitability for assaying large numbers of samples. In addition, pre-β1-HDL levels are directly measured by this method without the need for separation by electrophoresis or ultrafiltration. Our results showing that the pre-β1-HDL level was higher in hyperlipidemic subjects than in control subjects are compatible with the previous finding by Miida et al. (17, 20), in which significanly higher pre-β1-HDL levels, measured by native two-dimensional gel electrophoresis followed by immunoblotting against apoA-I, were found in hypercholesterolemia and hypertriglyceridemia.

It remains to be clarified why hyperlipidemic subjects had higher plasma pre-β1-HDL levels than control subjects. We hypothesize that hyperlipidemic subjects might have higher levels of lipids localized in tissues or cells such as fibroblasts and macrophages, as well as in the plasma, than control subjects did. It has been suggested that one of the most important factors affecting the plasma pre-β1-

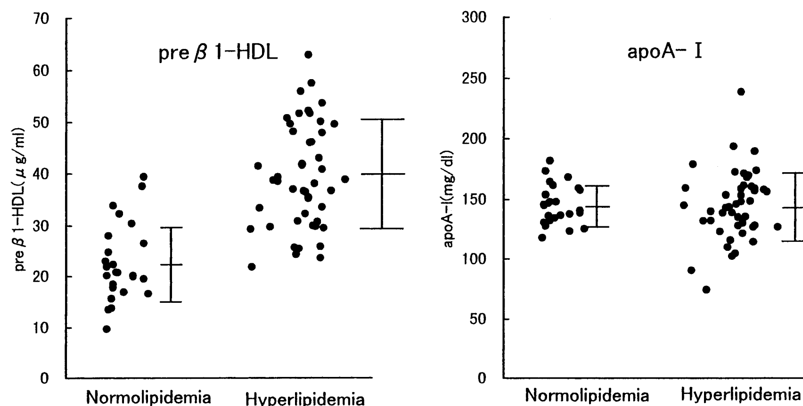

Fig. 6. Plasma pre-β1-HDL (left) and apoA-I (right) levels in control (n = 25; male/female 15/10; age 36 ± 9 years; body mass index 23 ± 3.5 kg/m^2; TC 183 ± 28 mg/dl; TG 80 ± 34 mg/dl; HDL-C 62 ± 15 mg/dl) and hyperlipidemic subjects (n = 47; male/female 22/25; age 55 ± 14 years; body mass index 25 ± 4.5 kg/m^2; TC 245 ± 64 mg/dl; TG 232 ± 280 mg/dl; HDL-C 51 ± 23 mg/dl) are plotted and mean values ± SD are shown.

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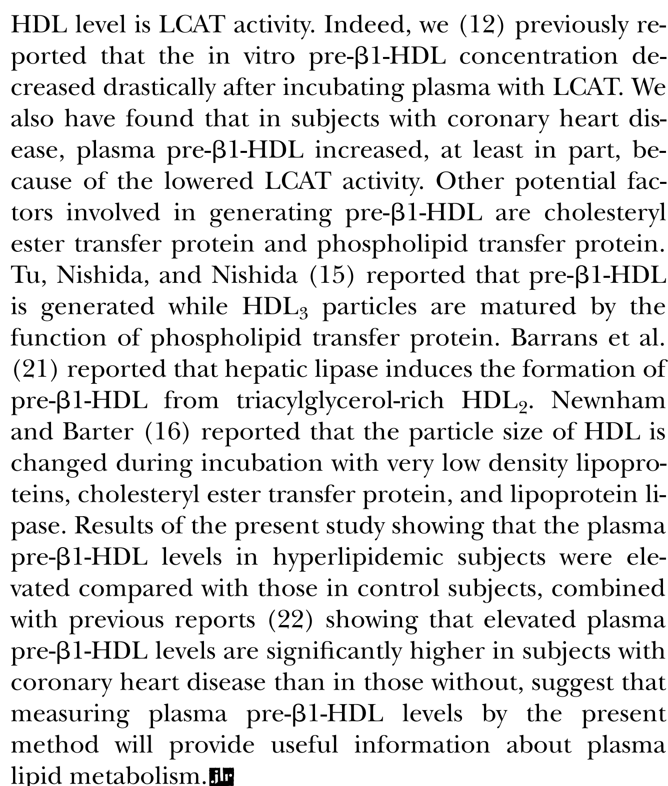

HDL level is LCAT activity. Indeed, we (12) previously reported that the in vitro pre-β1-HDL concentration decreased drastically after incubating plasma with LCAT. We also have found that in subjects with coronary heart disease, plasma pre-β1-HDL increased, at least in part, because of the lowered LCAT activity. Other potential factors involved in generating pre-β1-HDL are cholesteryl ester transfer protein and phospholipid transfer protein. Tu, Nishida, and Nishida (15) reported that pre-β1-HDL is generated while HDL_3 particles are matured by the function of phospholipid transfer protein. Barrans et al. (21) reported that hepatic lipase induces the formation of pre-β1-HDL from triacylglycerol-rich HDL_2. Newnham and Barter (16) reported that the particle size of HDL is changed during incubation with very low density lipoproteins, cholesteryl ester transfer protein, and lipoprotein lipase. Results of the present study showing that the plasma pre-β1-HDL levels in hyperlipidemic subjects were elevated compared with those in control subjects, combined with previous reports (22) showing that elevated plasma pre-β1-HDL levels are significantly higher in subjects with coronary heart disease than in those without, suggest that measuring plasma pre-β1-HDL levels by the present method will provide useful information about plasma lipid metabolism.

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REFERENCES

1. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.
2. Skinner, E. R. 1994. High density lipoprotein subclasses. *Curr. Opin. Lipidol.* **5:** 241–247.
3. Fielding, C. J., and P. E. Fielding. 1995. Molecular physiology of reverse cholesterol transport. *J. Lipid Res.* **36:** 211–228.
4. Castro, G. R., and C. J. Fielding. 1988. Early incorporation of cell-derived cholesterol into pre-beta-migrating high density lipoprotein. *Biochemistry.* **27:** 25–29.
5. Rye, K-A., M. A. Clay, and P. J. Barter. 1999. Remodelling of high density lipoproteins by plasma factors. *Atherosclreosis.* **145:** 227–238.
6. Miida, T., T. Yamaguchi, T. Tsuda, and M. Okada. 1998. High pre β1-HDL levels in hypercholesterolemia are maintained by probucol but reduced by a low-cholesterol diet. *Atherosclerosis.* **138:** 129–134.
7. Asztalos, B. F., C. H. Sloop, L. Wong, and P. S. Roheim. 1993. Comparison of apoA-I-containing subpopulations of dog plasma and prenodal peripheral lymph: evidence for alteration in subpopulations in the interstitial space. *Biochim. Biophys. Acta.* **1169:** 301–304.
8. Sviridov, D., and N. Fidge. 1995. Pathway of cholesterol efflux from human hepatoma cells. *Biochim. Biophys. Acta.***1256:** 210–220.
9. Huan, Y., A. von Eckardstein, and G. Assmann. 1993. Cell-derived unesterified cholesterol cycles between different HDLs and LDL for its effective esterification in plasma. *Arterioscler. Thromb.* **13:** 445–458.
10. Kawano, M., T. Miida, C. J. Fielding, and P. E. Fielding. 1993. Quantitation of pre β-HDL-dependent and nonspecific components of the total efflux of cellular cholesterol and phospholipids. *Biochemistry.* **32:** 5025–5028.
11. Fielding, P. E., M. Kawano, A. L. Catapano, A. Zoppo, S. Marcovina, and C. J. Fielding. 1994. Unique epitope of apolipoprotein A-I expressed in preβ1 high density lipoprotein and its role in the catalyzed efflux of cellular cholesterol. *Biochemistry.* **33:** 6981–6985.
12. Miida, T., M. Kawano, C. J. Fielding, and P. E. Fielding. 1992. Regulation of the concentration of preβ high-density lipoprotein in normal plasma by cell membranes and lecithin–cholesterol acyltransferase activity. *Biochemistry.* **31:** 11112–11117.
13. Miida, T., C. J. Fielding, and P. E. Fielding. 1990. Machanism of transfer of LDL-derived free cholesterol to HDL subfraction in human plasma. *Biochemistry.* **29:** 10469–10474.
14. O'Connnor, P. J., P. Naya-Vigne, B. Duchateau, M. Ishida, B. Mazur, M. Zysow, S. Malloy, S. Kunitake, and J. Kane. 1997. Measurement of pre beta-1 HDL in human plasma by ultrafiltration-isotope dilution technique. *Anal. Biochem.* **251:** 234–240.
15. Tu, A. Y., H. Nishida, and T. Nishida. 1993. High density lipoprotein conversion mediated by human plasma phospholipid transfer protein. *J. Biol. Chem.* **268:** 23098–23105.
16. Newnham, H. H., and P. J. Barter. 1992. Changes in particle size of high density lipoproteins during incubation with very low density lipoproteins, cholesteryl ester transfer protein and lipoprotein lipase. *Biochim. Biophy. Acta.* **1125:** 297–304.
17. Miida, T., K. Inano, T. Yamaguchi, T. Tsuda, and M. Okada. 1997. Lp A-I levels do not reflect preβ1 HDL levels in human plasma. *Atherosclerosis.* **133:** 221–226.
18. Edelstein, C., C. T. Lim, and A. M. Scanu. 1972. On the subunit structure of the protein of human serum high density lipoprotein. *J. Biol. Chem.* **247:** 5842–5849.
19. Brewer, H. B., R. Ronan, M. Meng, and C. Bishop. 1986. Isolation and characterization of apolipoprotein A-I, A-II and A-IV. *Methods Enzymol.* **128:** 223–246.
20. Miida, T., T. Yamahishi, T. Tsuda, and M. Okada. 1998. High preβ1-HDL levels in hypercholesterolemia are maintained by probucol but reduced by a low-cholesterol diet. *Atherosclerosis.* **138:** 129–134.
21. Barrans, A., X. Collet, R. Barbaras, B. Jaspard, J. Manent, C. Vieu, H. Chap, and B. Perret. 1994. Hepatic lipase induces the formation of pre-beta 1 high density lipoprotein (HDL) from triacylglycerol-rich HDL_2. A study comparing liver perfusion to in vitro incubating with lipase. *J. Biol. Chem.* **269:** 11572–11577.
22. Miida, T., Y. Nakamura, K. Inano, T. Matsuto, T. Yamaguchi, T. Tsuda, and M. Okada. 1996. Preβ1-high-density lipoprotein increase in coronary artery disease. *Clin. Chem.* **42:** 1992–1995.

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