METHODS  (full-length version for the online supplement)

**Animals**: Female NMRI mice were purchased from the Viikki Laboratory Animal Center, University of Helsinki.

**Preparation of human tryptase**: β-tryptase was purified from human lung tissue to apparent homogeneity, using a modification of the methods described,\(^1\)-\(^3\) and was also produced as a recombinant protein. Determination of the specific activities of both the lung-derived and the recombinant tryptase preparations by fluorometric active site titration yielded values that were > 85% of the theoretical value.

**Determination of tryptase activity**: Tryptase activity was determined spectrophotometrically, using n-tosyl L-arginine methyl ester (TAME) as described with minor modifications.\(^3\) The assay was performed in 3 mL of 50 mM Tris, pH 7.4, containing 1 mM TAME and the samples. A tryptase activity of 1 Unit was defined as the activity of the enzyme which, at ambient temperature, cleaves 1 \(\mu\)mol of the TAME substrate in 1 min.

**Preparation of aortic proteoglycans**: Proteoglycans were prepared from the intima and media of human aortas that were obtained at autopsy within 48 h after death,\(^4\) and were quantified by the Alcian Blue method\(^5\) with commercial heparin as standard. After treatment of the proteoglycans with chondroitinases ABC and AC, the disaccharide composition of the proteoglycans was analyzed by HPLC, using a 5-\(\mu\)m NH\(_2\) column, as described.\(^6\) This analysis indicated that the isolated human aortic proteoglycans contained, on average, 50% condroitin-6-sulfate, 35% condroitin-4-sulfate, and 20% dermatan sulfate. The isolated proteoglycans, like the commercial
glycosaminoglycan used in these studies (Heparin Leo, MW$_{av}$ 15 000; Leo, Denmark), were all devoid of any endogenous proteolytic activity against human HDL$_3$.

**Isolation, chemical modification, and radioactive labeling of plasma lipoproteins:**

LDL (1.019 – 1.050 g/mL) and HDL$_3$ (1.125 – 1.210 g/mL) were prepared from freshly isolated normolipidemic human plasma by sequential ultracentrifugation, using KBr. The quantities of the lipoproteins are expressed in terms of their protein content. We have observed that the ultracentrifugally isolated HDL$_3$ fraction contains both α- and preβ-migrating HDL. LDL was acetylated (acetyl-LDL) by repeated additions of acetic anhydride. Acetyl-LDL was radiolabeled by treatment with $^3$H-cholesteryl linoleate (1,2(n)$^3$H-cholesteryl linoleate, Amersham International) dissolved in 10 % dimethylsulfoxide, yielding preparations of $^3$H-cholesteryl linoleate incorporated into acetyl-LDL ($^3$H-CL-acetyl-LDL) with specific activities ranging from 30 to 100 dpm/ng protein. $^3$H-HDL was prepared by labeling the protein component of the lipoprotein by the Bolton-Hunter procedure using N-Succinimidyl [2,3-$^3$H] propionate to label the apolipoproteins with tritium (Amersham Pharmacia Biotech, code TRK556). The radioactive labeling did not cause any changes in the electrophoretic mobility of the lipoproteins, as examined on agarose gels.

**Isolation of apoA-I from human plasma:** HDL (1.063 – 1.210 g/mL) was delipidated with ethanol:ether as previously described, followed by apoA-I isolation from other HDL apolipoproteins by anion exchange chromatography on a HiTrap Q column (Pharmacia LKB Biotechnology). The purity of the apolipoprotein was ensured by electrophoretic analysis in a 15% SDS-PAGE gel under nonreducing conditions.
Proteolysis of HDL₃ by tryptase: One mg of ³H-labeled HDL₃ and 10 or 30 µg of tryptase (specific activity 0.82 TAME Units/µg) were incubated at 37°C in 1 mL of 5 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, pH 7.4, in the absence or presence of heparin glycosaminoglycans for the indicated time periods. The incubations were fully stopped by addition of leupeptin (Sigma; 0.2 mg/mL, final concentration). The degree of proteolysis was estimated by measuring the generation of ³H-labeled TCA-soluble peptides from the ³H-HDL₃, and was expressed as the fraction of the radioactivity that was soluble in 10% TCA relative to the total radioactivity present at the start of the incubation. Degradation products from parallel incubations using nonlabeled HDL₃ were analyzed on SDS-PAGE (15% gel) and the protein bands were stained with Coomassie Brilliant Blue. The gels were then quantitated with a Gel Doc 2000 gel documentation system (Bio-Rad).

Fractionation of HDL₃ by size exclusion chromatography: Samples of HDL₃ which had been incubated at 37°C overnight in the absence or presence of tryptase were passed through a Superose 12 HR 10/30 column (Amersham Pharmacia Biotech) at room temperature at a flow rate of 0.4 mL/min, using a solution of 5 mM Tris-HCl, 150 mM NaCl, and 1 mM EDTA, pH 7.4, as eluant. Fractions were collected and monitored continuously at 280 nm (SMART, Pharmacia, LKB). The column was calibrated with thyroglobulin (670 kDa), gamma globulin (158 kDa), ovalbumin (44 kDa), myoglobin (17 kDa), and vitamin B-12 (1.3 kDa) used as standards (all from BioRad). Fractions containing the main peak of HDL₃ and the two other minor fractions were collected and further analyzed for their contents of protein and phospholipids.
Demonstration of HDL subclasses by agarose gel electrophoresis or by nondenaturing two-dimensional electrophoresis and immunoblotting: Agarose gel electrophoresis was performed, using the Beckman Paragon system according to the instructions of the manufacturer. Proteins were transferred from the agarose gel to a PDVF membrane (Bio-Rad) by pressure blotting. ApoA-I was visualized using a polyclonal anti-human apoA-I antibody and AP-conjugated anti-mouse IgG as the second antibody (Dako, Denmark). Nondenaturing two-dimensional polyacrylamide gradient gel electrophoresis (2D-PAGGE) was performed in the sequence agarose gel electrophoresis, polyacrylamide gradient gel electrophoresis, as described previously. After electroblotting onto a nitrocellulose membrane, apoA-I- or apoA-IV-containing lipoproteins were immunocomplexed with goat antibodies against human apoA-I (Roche, Mannheim) or with rabbit antibodies against human apoA-IV (a kind gift from Dr. A. Steinmetz, Marburg, Germany), respectively. Antigen-antibody complexes were visualized with peroxidase-conjugated anti-goat antibodies from rabbit (DAKO, Denmark) or anti-rabbit antibodies from donkey, respectively, and chloronaphthol and hydrogen superoxide as substrates.

Cell cultures and loading of macrophages with cholesteryl esters: Peritoneal cells were harvested from unstimulated mice into PBS containing 1 mg/mL BSA. The cells were recovered after centrifugation, resuspended in DMEM (GIBCO) containing 100 U/mL penicillin, 100 µg/mL streptomycin, and 20% fetal calf serum, and plated onto 24-well plates (Becton Dickinson Labware, Lincoln Park, NY). After incubation at 37°C for 2 h in a humidified CO₂ incubator, nonadherent cells were removed by washing with PBS. The adherent cells (i.e. macrophages) were loaded and
radiolabeled by incubation for 18 h in the presence of 20 µg/mL of \(^{3}\)H-cholesteryl linoleate-acetyl-LDL in DMEM supplemented with 20% fetal calf serum.

**Cholesterol efflux assay:** \(^{3}\)H-Cholesterol-loaded macrophages were washed with PBS and incubated with DMEM supplemented with leupeptin (0.2 mg/mL) and the indicated concentrations of HDL\(_{3}\). After 6 h, the media were collected and centrifuged at 200 g for 5 min, and the radioactivity in each supernatant was determined by liquid scintillation counting. Under the conditions used, the \(^{3}\)H-cholesterol efflux from the macrophage foam cells is linear for up to 6 h of incubation and reflects the net flux of cholesterol from the macrophages into the medium."\(^{7,14}\) The data presented are means of triplicate incubations.

**Other methods:** The protein concentrations of LDL, HDL\(_{3}\), and apoA-I were measured by the method of Lowry, with BSA as standard. Phospholipids of HDL\(_{3}\) were determined by a commercial kit (BioMerieux S.A., France).

**REFERENCES**


