Clearance of NH₂-Terminal Propeptides of Types I and III Procollagen Is a Physiological Function of the Scavenger Receptor in Liver Endothelial Cells

By Jukka Melkko,* Turid Hellevik,§ Leila Risteli,* Juha Risteli,‡ and Bård Smedsrød§

From the Departments of *Medical Biochemistry and ‡Clinical Chemistry, University of Oulu, SF-90220 Oulu, Finland; and the §Department of Experimental Pathology, University of Tromsø, N-9037 Tromsø, Norway

Summary

This study was undertaken to determine the fate of circulating NH₂-terminal propeptide of type I procollagen (PINP) in rats. Radiolabeled PINP showed a biphasic serum decay curve after intravenous injection. 79% of the material disappeared from the blood during the initial α-phase (t1/2α = 0.6 min), while the remaining 21% was eliminated with a t1/2β of 3.3 min. The major site of uptake was the liver, 78, 1, and 21% of its radioactivity being recovered in isolated liver endothelial cells (LEC), Kupffer cells, and parenchymal cells, respectively. In LEC, fluorescently labeled PINP accumulated in small (0.1 μm) peripheral and larger (>0.1 μm) perinuclear vesicles within 10 min at 37°C after a binding pulse at 4°C. These grew in size with increasing chasing time, reaching a maximum diameter of 1 μm or more after 30 min, and taking the shape of rings that were stained only along their periphery. At chase intervals exceeding 30 min, the size of the vesicles decreased, and after 60 min the stain appeared in smaller, densely stained perinuclearly located vesicles. Degradation of 125I-PINP to free smaller fragments and 125I⁻ was significant after 30 min. Only formaldehyde-treated albumin, acetylated LDL, polyinosinic acid and NH₂-terminal propeptide of type III procollagen (PIIINP) competed with PINP for uptake. These findings indicate that clearance of PINP and PIIINP, which are normal waste products generated in large quantities, is a physiological function of the scavenger receptor in LEC.

Type I collagen is the most abundant collagen species in soft tissues and accounts for >90% of the organic matrix of mineralized bone. It is synthesized in the form of a larger protein, type I procollagen, which contains large additional domains at both ends (1). These parts, known as the NH₂- and COOH-terminal propeptides of type I procollagen, are removed by two specific proteinases in the extracellular space. Proper cleavage of these precursor-specific parts of the molecule is a prerequisite for the appropriate assembly of type I collagen molecules into collagen fibrils and fibers (2).

Although collagen makes up about 30% of all animal protein, literature dealing with the catabolism of this protein and its propeptides is not extensive. Since the serum concentration of these substances is very low, powerful mechanisms evidently exist to eliminate these molecules. NH₂-terminal propeptide of type I procollagen (PINP) exists in two forms in serum: the native trimeric PINP (35,000 Mr), and a smaller related to its NH₂-terminal Coll domain (7,000 Mr), the former predominating. There is no solid evidence to suggest that local breakdown is responsible for the catabolism of these peptides.

We have recently found that gelatinized collagens of several types and the NH₂-terminal propeptide of type III procollagen (PIIINP), as well as the COOH-terminal propeptide of type I procollagen (PICP), are cleared from the circulation efficiently and almost exclusively by receptor-mediated endocytosis in sinusoidal liver endothelial cells (LEC) (3, 4). We show here that also the PINP is eliminated by the same cells in the rat, identify the receptor involved in this process as the scavenger receptor, and describe the specificity, kinetics, and morphology of endocytosis, and intracellular transport and degradation of PINP.

Materials and Methods

Ligands. Human (5), bovine (6), and porcine (7) PIIINP and human type I collagen (8) were purified as described previously. To prepare PINP, type I procollagen was first purified from the culture medium of human osteosarcoma MG-63 cell line (Amer-
ican Type Culture Collection, Rockville, MD) by precipitation with ammonium sulfate and gel filtration on Sephacryl S-500 (Pharmacia, Uppsala, Sweden). The procollagen was digested with purified bacterial collagenase (grade CLSPA; Worthington Biochem Corp., Freehold, NJ). PINP was purified from the digest by anion exchange HPLC (Protein-Pak DEAE-5PW column; Waters Associates, Milipore Corp., Milford, MA), gel filtration on Sephacryl S-300 (Pharmacia), and reverse phase HPLC (Vydac, Hesperia, CA). PINP purified in this manner was injected intradermally into rabbits to produce antiserum, which was used for assay and further purification of PINP from human ascitic fluid. SEDS-PAGE revealed a 14,000-M, major band representing the NH2-terminal segment of the procollagen chain, and a 7,000-M minor band representing the NH2-terminal segment of the proalpha2(I) chain. The molecular weights were obtained by comparison with collagenous standards. Healon (high molecular weight hyaluronan) was from Pharmacia, and heparin (free glycosaminoglycan chains) was a gift from Professor U. Lindahl (University of Uppsala, Uppsala, Sweden). Formaldehyde-treated bovine serum albumin (BSA) and acetylated light density lipoprotein (AcLDL) were prepared exactly as described (9-11). Poly(ionic acid (Poly I) was purchased from Sigma Chemical Co. (St. Louis, MO). 125I was from Amersham International, Amersham, Bucks, UK).

Labeling Techniques. Proteins were labeled with 125I (carrier-free Na125I) from Amersham International either by a direct reaction employing Iodobeads (Pierce, Rockford, IL) as described (12), or by conjugating the protein with 125I-labeled tyraminyl cellobiose (TC) exactly as described (3). LDL and AC LDL were labeled only with the latter method. Either method gave specific radioactivities of 1-3 x 105 cpm/μg protein. 125I labeling of tyrosine was done by the Iodobeads method. On the basis of hydrophobic interaction with the gel material, 125I-labeled tyrosine was purified free of unbound 125I by elution through a column of Sephadex G-25. FITC-labeled PINP was prepared by incubating PINP (1 mg/ml) with FITC (1 mg/ml) in 0.5 M sodium bicarbonate buffer (pH 9.5) for 20 h at +4°C. Unreacted dye was removed by gel filtration on a PD-10 column (Sephadex G-25; Pharmacia).

Determination of Anatomical Distribution and Serum 1/2. Serum half-life, and organ and hepatocellular distribution of intravenously administered labeled PINP were determined in rats as described (13). 125I- or 125I-TC-labeled PINP (5 μg) was injected into a lateral tail vein. Blood sampling was started after 1 min by collecting 25-μl volumes from the tip of the tail into calibrated capillary tubes. To determine the α- and β-phases of the elimination from blood, about six samples were taken per minute during the first 5 min, and two samples per minute thereafter.

Isolation and Culture of Liver Cells. Preparation of pure cultures of functionally intact Kupffer cells (KC), LEC, and parenchymal cells (PC) from a single rat liver has been detailed elsewhere (14). After collagenase perfusion of the liver, and isopycnic centrifugation of the resulting dispersed cells through Percoll (Pharmacia), pure monolayer cultures of KC and LEC were established by selective attachment on substrates of fibronectin and glutaraldehyde-treated serum albumin, respectively (15). PC were made free from sinusoidal cells by sedimentation through 50% Percoll.

Endocytosis Studies In Vitro. Monolayer cultures established in 28 cm2 wells and maintained in serum-free RPMI 1640 medium, were washed and supplied with fresh medium containing 1% serum albumin and labeled proteins. Incubations carried out for various lengths of time at +37°C to measure endocytosis or at +4°C to measure binding, were terminated by transferring the media, along with one wash, to tubes containing 20% TCA. This precipitates only intact, undegraded protein or intermediate degradation products of high molecular weight. The extent of degradation was determined by measuring the radioactivities in pellet and supernatant after centrifugation. Cell-associated ligand was quantified by measuring the amount of label solubilized in PBS containing 1% SDS.

Pulse-chase Studies on Kinetics of Uptake and Degradation In Vitro. Monolayer cultures of LEC established in 28 cm2 dishes were allowed to bind 125I-PINP or 125I-TC-PINP at +4°C for 2 h. After washing the cultures to remove unbound ligand, the incubation temperature was raised to +37°C, and after various periods of incubation, cultures were solubilized in 1% SDS in PBS containing 1 mM PMSF, 2 mM EDTA, 2 mM N-ethylmaleimide, and 1 μg/ml pepstatin A. The solubilates were analyzed by gel filtration on Sephacryl S-300 eluted with PBS containing 0.1% SDS.

Pulse-chase Studies on the Intracellular Transport of Endocytosed Collagen. For morphological pulse-chase studies, monolayer cultures of LEC established on 13-cm diameter round coverslips were incubated with FITC-PINP (100 μg/ml) at +4°C for 2 h, washed, and chased for various periods of time at +37°C. The experiments were terminated by fixing the cultures with 25% glutaraldehyde in PBS. After mounting of the cultures on glass slides, the cells were examined in a Zeiss Axioshot microscope equipped with fluorescence optics. Pictures were taken on a Kodak Ektachrome film.

Results

Elimination of Circulating PINP: Serum 1/2, Distribution and Specificity of Uptake. The circulatory survival of PINP was determined by measuring the decay of blood radioactivity after intravenous injection of trace amounts of directly labeled 125I-PINP or adduct-labeled 125I-pTC-PINP. Linear decay plots indicated a very efficient clearance of both 125I-PINP and 125I-TC-PINP (Figs. 1 and 2). Acid-soluble degradation products started to appear in serum about 10 min after injection of 125I-PINP (Fig. 1). In contrast, 125I-TC-PINP yielded no degradation products in serum. Semilogarithmic decay plots revealed a biphasic pattern of elimination ( Insets, Fig. 2). 70-80% of the injected 125I-TC-PINP was eliminated during an initial rapid α-phase with a serum 1/2 of 0.6-0.8 min. The remaining 20-30% was eliminated with
Figure 2. Effect of excess PINP or FSA on elimination of 125I-TC-PINP from the circulation. 125I-TC-PINP (5 μg) was injected either alone (a, n = 3) or together with 2 mg PINP (b, n = 1) or 5 mg FSA (c, n = 4) through the tail vein of rats, and radioactivity in blood samples was plotted against time after injection. See legend to Fig. 1 for explanation. Radioactivity at 1 min after injection was taken as 100%. 125I-labeled degradation products could not be detected. Semilogarithmic plots (insets) were used to determine the slope, β, of the terminal phase ("β-phase"), taken from about 3–10 min after injection. The extrapolated values of the curve with slope β were subtracted from the experimental values obtained during the initial phase (1–3 min after injection) to give the curve with slope α (insets) describing the kinetics of the initial clearance phase ("α-phase"). See Table 1 for pharmacokinetic data calculated on the basis of the α- and β-slopes.

Figure 3. Anatomical distribution of intravenously injected 125I-TC-PINP (a) and 125I-PINP (b). The animals used in the serum half-life studies (Figs. 1 and 2) were analyzed for anatomical distribution of radioactivity 50 min after injection. About 80 and 50% of injected dose were recovered in the tissues listed in a and b, respectively. Results are expressed as percent total radioactivity recovered. In a, 125I-TC-PINP was injected either alone (black bars, n = 3), or together with 2 mg PINP (shaded bars, n = 1) or 5 mg FSA (open bars, n = 4). Variation was <10%.

Table 1. Elimination of 125I-TC-PINP from the Circulation of Rat: Pharmacokinetic Parameters

| Injected material | t_{1/2}α (min ± SD) | t_{1/2}β (min ± SD) | Proportion eliminated with t_{1/2}α (percent ± SD) | Proportion eliminated with t_{1/2}β (percent ± SD) |
|-------------------|---------------------|---------------------|--------------------------------------------------|--------------------------------------------------|
| 125I-TC-PINP (10 μg) (n = 3)* | 0.59 (± 0.05) | 3.33 (± 0.45) | 78.5 (± 6.25) | 21.5 (± 6.25) |
| 125I-TC-PINP (2 mg) (n = 1) | 0 | 4.3 | 0 | 100 |
| 125I-TC-PINP (10 μg) + FSA (5 mg) (n = 4) | 1.08 (± 0.32) | 5.08 (± 0.78) | 23.9 (± 6.97) | 76.13 (± 6.98) |

* Number of experiments.
† Initial rapid α-phase of serum half-life.
§ Terminal, slower β-phase of serum half-life.
Figure 4. Hepatocellular distribution of intravenously injected $^{125}$I-PINP. 60 min after intravenous administration of $^{125}$I-TC-PINP (5 μg) LEC, KC, and PC were established in pure cultures. Content of $^{125}$I is given either as cpm per 10$^6$ cells, expressed in relative units setting cpm per 10$^6$ KC equal to 1 (a), or as cpm per total cell population in liver (b), making use of the fact that the total numbers of KC, LEC, and PC in rat liver relate to each other as 1:2.5:7.7 (14). Means from two experiments are given, variation being <10%.

Figure 5. Kinetics of endocytosis of $^{125}$I-PINP in cultured LEC. $^{125}$I-PINP (10 ng) was incubated with cultures of LEC in 2-cm$^2$ diameter dishes, and cell-associated (□) and degraded (●) ligand were determined after various periods as described in the Materials and Methods section. Extent of endocytosis (○) is the sum of cell-associated and degraded ligand. Results are means of triplicates, variation being <10%.

4.3 min (Table 1). Coinjection with excess amounts of FSA (5 mg) reduced the proportion eliminated with α kinetics to 24% ($t_{1/2,α} = 1.1$ min), leaving 76% to be eliminated with a $t_{1/2,β}$ of 5.1 min.

Figure 6. Morphological pulse-chase study on intracellular transport of endocytosed PINP. Cultures of LEC were pulsed with FITC-PINP (100 μg/ml) for 2 h at 4°C. Chasing was performed after removal of unbound ligand by washing, and transferring of the cultures to 37°C. The cultures were fixed after chase periods of 0 min (a), 10 min (b), 30 min (c), and 60 min (d) and examined in a fluorescence microscope. At chase start (a) the probe is diffusely distributed over the cell surface. After 10 min (b) the probe is concentrated in vesicles of varying size and location. The endocytic vesicles containing the probe grow in size until 30 min, at which time they reach their maximal diameter of 1 μm or more (c). Note that the larger vesicles appear stained only in their peripheral aspects (arrow). After 60 min the probe is concentrated in smaller, densely stained perinuclear vesicles (d). Bar, 10 μm.
Radioactivity was measured in various organs 50 min after injection. The liver was the main site of uptake (Fig. 3). When 125I-PINP was injected, uptake in liver and kidneys amounted to 60 and 30% of the total, respectively (Fig. 3 b). In contrast, when 125I-TC-PINP was administered, nearly 90% of recovered radioactivity resided in the liver, and <10% in the kidneys (Fig. 3 a). Injection of 125I-TC-PINP together with excess amounts of unlabeled PINP (2 mg) or FSA (5 mg) led to a reduced sequestration in liver corresponding to 5 and 10%, respectively, the kidneys taking up the spillover from liver. Only insignificant amounts were recovered in the spleen.

Pure cultures of various types of liver cells were prepared after injection of 125I-TC-PINP. In LEC, the uptake was 23- and 10-fold more active per cell than in KC and PC, respectively (Fig. 4). We calculated that in the whole liver, the population of LEC was responsible for 78% of the uptake, whereas KC and PC took up 1 and 21%, respectively.

In Vitro Studies: Kinetics of Endocytosis, Degradation, Intracellular Transport, and Specificity of Uptake. To study the kinetics of endocytosis, trace amounts of labeled PINP were incubated with cultures of LEC, and cells and medium was analyzed for radioactivity at various time points (Fig. 5). Endocytosis proceeded at a rapid rate for the first 4 h of incubation, reaching 50% of added amount. At this time point, as much as 40% of added label was recovered in the medium as acid-soluble, degraded material.

Intracellular transport of endocytosed PINP was studied with FITC-labeled PINP in cultured LEC. After binding at 4°C, only a diffuse staining was observed on the cells. After a chase period of 10 min, the probe was observed both in peripherally located small vesicles (diameter ~0.1 μm) and perinuclearly located larger vesicles (diameter 0.1-1.0 μm) (Fig. 6 a). At chase intervals exceeding 10 min (Fig. 6 b) the probe disappeared from the peripheral area, and appeared along the rim of vesicles that grew in size until about 30 min, containing diameters of 1 μm or more (Fig. 6 c). These large, characteristically looking ring structures decreased in size after 30 min, and by 60 min appeared exclusively as smaller (diameter ≈ 1 μm) densely, intensely stained perinuclear vesicles (Fig. 6 d).

The extent of degradation of the ligand in the various endocytic compartments was studied in another set of pulse-chase experiments using PINP labeled with either 125I or 125I-TC. SDS-solubilized cultures were analyzed by gel filtration on Sephacryl S-300 after various chase periods. After 20, 30, and 60 min about 30, 50, and 70% of the endocytosed propeptide had been transferred to degradation products small enough to elute after relative elution position (Kav) 0.75. A substantial proportion of the ligand had been degraded to free 125I (Kav ≈ 1.4), whereas only a minor fraction eluted at Kav = 1.

Neither LDL, gelatinized human type I collagen, heparin, nor hyaluronan affected the uptake of 125I-TC-PINP. Endocytosis of 125I-TC-PIIINP (Fig. 8 a), 125I-FSA (Fig. 9 a), or 125I-TC-AcLDL (Fig. 9 b) was also inhibited by 74%. Neither LDL, gelatinized human type I collagen, heparin, nor hyaluronan affected the uptake of 125I-TC-PINP. Endocytosis of 125I-TC-PIIINP (Fig. 8 a), 125I-FSA (Fig. 9 a), or 125I-TC-AcLDL (Fig. 9 b) was also inhibited...
Discussion

A number of physiological macromolecular waste products have been shown to be efficiently eliminated from the circulation by receptor-mediated endocytosis in LEC (for a review see reference 4). These cells are more important than the KC in the elimination of connective tissue macromolecules, such as α chains of most collagen types, hyaluronan, chondroitin sulfate, laminin, nidogen, PICP, and PIIINP. A share of work between the two cells of the hepatic reticuloendothelial system seems probable, KC clearing particulate matter and LEC taking up soluble macromolecules. We show here that PINP is another major connective tissue–related protein that is cleared by LEC.

80% of the PINP is removed from the bloodstream with a t1/2α as short as 0.6 min, and the remaining 20% with a t1/2β of >3 min. The capacity of the rapid removal seems limited, as it could be suppressed with large amounts of either PINP itself or FSA, whereas the reserve capacity for the slower β-phase was unsaturable at the amounts of ligand tested here. Thus, the total capacity for hepatic uptake of circulating PINP is much greater than what is needed under normal physiological conditions. The ability of FSA to inhibit the uptake in vivo suggests that the elimination is mediated by the scavenger receptor (16).

Figure 10. Specificity of endocytosis of PIIINP from different species. 125I-PIIINP (10 ng) of porcine (a) or bovine (b) origin were incubated with cultured LEC in 2 cm² dishes for 2 h at 37°C with or without (Control) the presence of 100 μg/ml unlabeled human PIIINP (hPIIINP). (Shaded areas) Cell-associated radioactivity; (open areas) acid-soluble radioactivity (degraded ligand). Results are means of triplicate measurements, the variation being <10%.

Fluorescently labeled PINP visualized two distinct intracellular endocytic structures in LEC, the stainings of which were separated by time. Circular doughnut-shaped structures of >0.1-μm diameter started to appear after 10 min, reaching their maximum diameter at about 30 min. From these, the protein was apparently transferred to smaller, densely stained perinuclear vesicles, where virtually all probe was observed after 60 min. These compartments resemble the large acid phosphatase-negative vesicles and the smaller acid phosphatase-containing vesicles, obviously lysosomes, in which colloidal gold-labeled chondroitin sulfate proteoglycan accumulates during the initial 30–40 min and later, respectively, as revealed by electron microscopy (17). Three different receptors, i.e., the hyaluronan/chondroitin sulfate receptor (17), the mannos receptor (18), and the PINP/PIIINP or scavenger receptor described here thus seem to mediate similar morphological patterns of intracellular transport of the endocytosed ligand in LEC.

Although PINP seems to be partially degraded in the prelysosomal compartment, complete breakdown only takes place after its transfer to the perinuclear vesicles, believed to be lysosomes. The aberrant behavior of the final breakdown products of 125I-PINP is explained by interactions of 125I with the gel material.

The observation that FSA inhibited uptake of PINP in vivo, and of both PINP and PIIINP in vitro, along with the finding that AcLDL and Poly I competed with PINP for uptake in cultured LEC, strongly suggest that the propeptides are endocytosed in LEC via the scavenger receptor. The term scavenger receptor was originally coined to denote a receptor species in macrophages that recognizes and endocytoses modified...
proteins, e.g., AcLDL and malondialdehyde-treated serum albumin (for a review see reference 19). However, circulating AcLDL was later found to be cleared almost exclusively by a similar receptor in LEC (20, 21), and FSA (16) and Poly I have also been found to be ligands for this scavenger receptor (16). Oxidized LDL, which is likely to be a more physiological modification product than AcLDL, is cleared by both KC and LEC (22). FSA is a purely experimental ligand and it has not been possible to find AcLDL in the body. Two macrophage scavenger receptor proteins have been cloned and sequenced (23), but it still remains to be determined whether the receptor on LEC is identical with these. Nevertheless, our observation that both PINP and PIIINP are cleared by the scavenger receptor in LEC, gives the first clue to the physiological function of this receptor.

The procollagen propeptides are produced in large amounts when collagen is secreted from connective tissue cells, i.e., fibroblasts and osteoblasts. Since they fulfill the function of preventing premature association of the procollagen molecules to fibrils while still being part of the procollagen molecule, they can be considered as waste products once they have been cleaved off. The structure common to these propeptides that is recognized by the scavenger receptor remains to be determined. The primary structures of PINP and PIIINP are clearly different, though homologous, and there is no immunological cross-reactivity. On the other hand, their three-dimensional structures are similar and both have an isoelectric point of about 3 (24-27). AcLDL, FSA, and Poly I are also negatively charged. However, our finding that neither hyaluronan nor heparin, two highly negatively charged glycosaminoglycans, could compete with PINP for uptake, suggests that a negative charge per se is not sufficient as a signal for uptake.

The authors acknowledge the expert technical help of Mr. Trond Jakobsen (University of Tromsø).

This work was supported by grants from the Norwegian Council for Medical Research (312.92/017), the Norwegian Cancer Foundation, the Aakre Foundation Against Cancer (Tromsø, Norway), the Finnish Medical Foundation, and the Finnish Cancer Foundation.

Address correspondence to Dr. Bård Smedsrød, Department of Experimental Pathology and Anatomy, Institute of Medical Biology, University of Tromsø, N-9037 Tromsø, Norway.

Received for publication 16 August 1993 and in revised form 27 October 1993.

References

1. Prockop, D.J., K.I. Kivirikko, L. Tuderman, and N.A. Guzman. 1979. The biosynthesis of collagen and its disorders. N. Engl. J. Med. 301:13.
2. Miyahara, M., F.K. Njieha, and D.J. Prockop. 1982. Formation of collagen fibrils in vitro by cleavage of procollagen with procollagen proteinases. J. Biol. Chem. 257:8442.
3. Smedsred, B., J. Melklo, L. Risteli, and J. Risteli. 1990. Circulating C-terminal propeptide of type I procollagen is cleared mainly via the mannose receptor in liver endothelial cells. Biochem. J. 271:345.
4. Smedsred, B., H. Pertoft, S. Gustafson, and T.C. Laurent. 1990. Scavenger functions of the liver endothelial cell. Biochem. J. 266:313.
5. Niemelä, O., J. Risteli, J. Parkkinen, and J. Risteli. 1985. Purification and characterization of the N-terminal propeptide of human type I procollagen. Biochem. J. 232:145.
6. Bruckner, P., H.P. Bächinger, R. Timpl, and J. Engel. 1978. Three conformational distinct domains in the amino-terminal segment of type III procollagen and its rapid triple helix ⇐⇒ coil transition. Eur. J. Biochem. 90:595.
7. Jensen, L.T., H.P. Ølesen, J. Risteli, and I. Lorenzen. 1990. External thoracic duct-venous shunt in conscious pigs for long term studies of connective tissue metabolites in lymph. Lab Anim. Sci. 40:620.
8. Miller, E.J., and R.K. Rohdes. 1982. Preparation and characterization of the different types of collagen. Methods Enzymol. 82:33.
9. Mego, J.L., F. Bertini, and D.J. McQueen. 1967. The use of formaldehyde-treated 131I-albumin in the study of digestive vacuoles and some properties of these particles from mouse liver. J. Cell Biol. 32:699.
10. Regrave, T.G., D.C.K. Roberts, and C.E. West. 1975. Separation of plasma lipoproteins by density-gradient ultracentrifugation. Anal. Biochem. 65:42.
11. Basu, S.K., J.L. Goldstein, R.G.W. Anderson, and M.S. Brown. 1976. Degradation of carboxylated low density lipoprotein and regulation of cholesterol metabolism in homozygous familial hypercholesteremia fibroblasts. Proc. Natl. Acad. Sci. USA. 73:3178.
12. Markwell, M.A.K. 1982. A new solid-state reagent to iodinate proteins: conditions for the efficient labeling of antisera. Anal. Biochem. 125:427.
13. Smedsred, B., and M. Einarrson. 1990. Clearance of tissue plasminogen activator by mannose and galactose receptors in liver. Thromb Haemostasis. 63:60.
14. Pertoft, H., and B. Smedsred. 1987. Separation and characterization of liver cells. In Cell Separation. Methods and Selected Applications. Vol. 4. T.G. Pretlow II and T.P. Pretlow, editors. Academic Press, New York. 1–24.
15. Laakso, T., and B. Smedsred. 1987. Cellular distribution in rat liver of intravenously administered polyacryl starch and chondroitin sulfate microparticles. Int. J. Pharmacol. 36:253.
16. Eskild, W., and T. Berg. 1982. Scavenger receptors in rat non-parenchymal cells. In Sinusoidal Liver Cells. D.L. Knook and
17. Smedsrod, B., M. Malmgren, J. Ericsson, and T.C. Laurent. 1988. Morphological studies on endocytosis of chondroitin sulphate proteoglycan by rat liver endothelial cells. *Cell Tissue Res.* 253:39.

18. Stang, E., G.M. Kindberg, T. Berg, and N. Roos. 1990. Endocytosis mediated by the mannose receptor in liver endothelial cells. An immunocytochemical study. *Eur. J. Cell Biol.* 52:67.

19. Brown, M.S., and J.L. Goldstein. 1983. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu. Rev. Biochem.* 52:223.

20. Nagelkerke, J.F., K.P. Barto, and T.J.C. van Berkel. 1983. In vivo and in vitro uptake and degradation of acetylated low density lipoprotein by rat liver endothelial, Kupffer, and parenchymal cells. *J. Biol. Chem.* 258:12221.

21. Blomhoff, R., C.A. Drevon, W. Eskild, P. Helgerud, K.R. Norum, and T. Berg. 1984. Clearance of acetyl low density lipoprotein by rat liver endothelial cells. Implications for hepatic cholesterol metabolism. *J. Biol. Chem.* 259:8898.

22. Van Berkel, T.J.C., de Rijke, Y.B., and J.K. Krujit. 1991. Different fate in vivo of oxidatively modified-LDL and acetylated-LDL in rats—recognition by different scavenger receptors on Kupffer and endothelial cells. *J. Biol. Chem.* 266:2282.

23. Kodama, T., M. Freeman, L. Rohrer, J. Zabrecky, P. Matsuda, and M. Krieger. 1990. Type I macrophage scavenger receptor contains α-helical and collagen-like coiled coils. *Nature (Lond.)* 343:531.

24. Fisher, L.W., P.G. Robey, N. Tuross, A.S. Otsuka, D.A. Tepen, P.S. Esch, S. Shimasaki, and J.D. Termine. 1987. The M, 24,000 phosphoprotein from developing bone is the NH₂-terminal propeptide of the α1-chain of type I collagen. *J. Biol. Chem.* 37:13457.

25. Kuivaniemi, H., G. Tromp, M.L. Chu, and D.J. Prockop. 1988. Structure of the full-length cDNA clone for the prepro α2(I) chain of human type I procollagen. Comparison with the chicken gene confirms unusual patterns of gene conservation. *Biochem. J.* 252:633.

26. Tromp, G., H. Kuivaniemi, A. Stacey, H. Shikata, C.T. Baldwin, R. Jaenisch, and D.J. Prockop. 1988. Structure of a full-length cDNA clone for the prepro α1(I) chain of human type I procollagen. *Biochem. J.* 253:919.

27. Ala-Kokko, L., S. Kontusaari, C.T. Baldwin, H. Kuivaniemi, and D.J. Prockop. 1989. Structure of cDNA clones coding for the entire preproα1(III) chain of human type III procollagen. *Biochem. J.* 260:509.