Accelerated lipoprotein uptake by transplantable hepatomas that express hepatic lipase

Charles Donner,[1,*,†] Sungshin Choi,[*] Michael Komaromy,[2,*] and Allen D. Cooper[3,*,†]

Research Institute,* Palo Alto Medical Foundation, 860 Bryant St., Palo Alto, CA 94301, and Department of Medicine,† Stanford University School of Medicine, Stanford, CA 94305

Abstract **To test the hypothesis that hepatic lipase plays a key role in lipoprotein removal in vivo, a novel system was used. Hepatoma cells (HTC 7288c) were transfected with a cDNA encoding hepatic lipase in culture and grown as solid tumors in vivo. In culture, transfected cells degraded chylomicron remnants and low density lipoprotein (LDL) somewhat more efficiently than untransfected cells. Tumors from the transplanted cells produced hepatic lipase localized to the surface of tumors from transfected cells but not tumors from non-transfected cells, grown in the same rat. The tumors from transfected cells removed, per gm of tissue, 34% ($P < 0.001$) more ^{125}I-labeled LDL than tumors from non-transfected cells in the same animal. The uptake of chylomicron remnants (by tumors from transfected cells) was also modestly enhanced (15 ± 6%, $P < 0.005$). There were no differences in the uptake of ^{125}I-labeled albumin or ^{125}I-labeled asialoglycoprotein. Compared to the liver, the untransfected tumors took up 12%, and the transfected tumors took up about 18% as much LDL per gram of tissue. The uptake of chylomicron remnants compared to liver was far lower. Both types of tumors had about twice as much LDL receptor related protein as the liver. Wild-type tumors had the highest level of LDL receptor, twice hepatic lipase-secreting tumors, and six times that of the liver. Using the novel approach of transfecting transplantable tumor cells with hepatic lipase, the ability of hepatic lipase to facilitate the removal of apoB-containing lipoproteins was demonstrated. The liver still removes low density lipoprotein and especially chylomicron remnants more rapidly than the tumors, suggesting factors in addition to hepatic lipase and LDL receptor level play a major role in hepatic lipoprotein removal.—**Donner, C., S. Choi, M. Komaromy, and A. D. Cooper. **Accelerated lipoprotein uptake by transplantable hepatomas that express hepatic lipase. *J. Lipid Res.* 1998.** 39: **1805–1815.**

Supplementary key words hepatic lipase • LDL • chylomicron remnant • lipoprotein lipase

The enzyme hepatic lipase is a member of a family of proteins that are critical in regulating lipid and lipoprotein homeostasis. Several members of the family, including pancreatic lipase and lipoprotein lipase, have been studied extensively and their functions are well established. The function of hepatic lipase, on the other hand, is less well understood. Based upon studies of its actions in vitro (1) and the lipoprotein pattern in individuals with congenital deficiency of the enzyme (2), it has been established that it plays a significant role in the inter-conversion of lipoprotein species, specifically the transformation of intermediate density lipoprotein (IDL) to low density lipoprotein (LDL) and high density lipoprotein $(HDL)_2$ to HDL_3.

On the other hand, the localization of the enzyme to tissues that have major roles in sterol metabolism suggests it might have a role in delivering lipids, particularly cholesterol, to these tissues. Consistent with this postulate, studies with antibodies in vivo (3, 4) have suggested that the enzyme may play a role in the hepatic removal of chylomicron remnants, particles that transport dietary cholesterol and sterol vitamins to the liver. Previous cell culture experiments by Aviram, Bierman, and Chait (5) suggested that the enzyme could accelerate the removal of LDL by cells in culture. These results were confirmed and extended in our laboratory (6). In addition, we have recently suggested that the presence of hepatic lipase on the surface of a cell may increase the ability of the cell to selectively transport cholesteryl esters from HDL (7). Lauer et al. (8) have reported that expression of the enzyme by McA-RH7777 cells can enhance the uptake of β-VLDL. This plethora of possible functions in lipoprotein transport makes it highly desirable to have a mechanism to evaluate, in vivo, the transport function that can be ascribed to the presence of the enzyme.

In the present work such a system is described. The cDNA encoding the enzyme was transfected into the cells

Abbreviations: IDL, intermediate density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; HRP, horseradish peroxidase; PAGE, polyacrylamide gel electrophoresis; FCS, fetal calf serum; BSA, bovine serum albumin; PBS, phosphate-buffered saline; LRP, LDL receptor related protein; FFA, free fatty acids.

[1] Present address: Department of Internal Medicine, Division of Gastroenterology, University of Iowa, 200 Hawkins Drive, Iowa City, IA 52242.

[2] Deceased March, 1996.

[3] To whom correspondence should be addressed.

of a transplantable hepatoma that had been used to study lipoprotein uptake in this laboratory previously (9). This allows evaluation of the role of the enzyme per se on the removal of lipoproteins and lipid metabolism in vivo. Evidence is presented that the presence of hepatic lipase accelerates the uptake of LDL and, to a lesser degree, chylomicron remnants in cells that express the enzyme as compared to otherwise identical cells that do not express the enzyme. Not only do the tumors derived from transfected cells remove LDL more rapidly but they have the expected metabolic profile of a cell that is receiving more sterol.

MATERIALS AND METHODS

Cells, cell culture, cell transfection, and cloning

HTC 7288c cells were purchased from the University of California at San Francisco. The cells were grown in Swims-77 medium (Sigma, St. Louis, MO) supplemented with l-glutamine (298 mg/L), l-cysteine (13.8 mg/L), phenol red (10 mg/L), tricine (0.05 m), and 10% fetal calf serum (GIBCO, Grand Island, NY). They were maintained at an approximate concentration of 2–3 $\times$ 10^5 cells/ml in roller bottles. In order to facilitate transfection, the cells were adapted to grow in flasks or dishes that were coated with rat tail collagen (Sigma, St. Louis, MO). For transfection, the cells were placed in 100-mm dishes and grown until about 30% confluent.

The preparation of the expression vector was previously described and a diagram was published (10). Briefly, this was composed of a construct containing the full-length rat hepatic lipase cDNA containing 15 bases of the 5′ untranslated region and the entire leader encoding a 3′ untranslated region. This was cloned into the vector pMTSV40polyABam. This vector utilizes the human metalothionine promoter to control transcription. In some experiments, the metalothionine promoter was replaced with the MoMLV-LTR promoter.

The plasmid containing the hepatic lipase cDNA was co-transfected with pSV2neo, a plasmid that encodes a neomycin resistance gene, at a molar ratio of 10:1 by calcium phosphate precipitation with glycerol shock as previously described (10). At 24 h post-transfection, the medium was replaced with one containing the antibiotic G418 (400 μg/ml) (GIBCO). After 2 weeks, the surviving cells were harvested and transferred to 96-well plates at a concentration of 1 cell/well. The medium was as above with 30 μm $ZnSO_4$ and 10 μg/ml sodium heparin. After a 24-h incubation, media were removed for assay for hepatic lipase as described below. Wells containing cells that secreted hepatic lipase were transferred to T-75 flasks and when near confluency, an aliquot was diluted and recloned in 96-well plates as above. Cells from the second cloning that secreted the most hepatic lipase were harvested, expanded, and one high producing cell line was used in further experiments.

Determination of hepatic lipase

Hepatic lipase in cell media was detected by an ELISA as previously described (10). Media from the cells were transferred to 96-well plates and allowed to dry. Non-specific binding sites on the plate were blocked by incubation with phosphate-buffered saline (PBS) containing 3% bovine serum albumin (BSA). This was followed by incubation with anti-hepatic lipase antibody as previously described (10), followed by incubation with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG. *O*-Phenylenediamine dihydrochloride (OPC) (Sigma, St. Louis, MO) was added, followed by hydrogen peroxide, and color was measured in an automated ELISA reader (Dynatech Laboratories, Inc.).

To detect hepatic lipase in tissues, cells, and tumors, Western blotting was carried out. Protein samples were separated on 6% SDS polyacrylamide gels and after electrophoresis, transferred to nitrocellulose membranes. The membrane was incubated with anti-hepatic lipase IgG, followed by incubation with HRP-conjugated goat anti-rabbit IgG and developed by the addition of 4-chloro-1-naphthol (Bio-Rad, Hercules, CA) and hydrogen peroxide. Hepatic lipase activity was determined by the method of Nilsson-Ehle and Schotz (11) using radiolabeled triolein to measure the generation of free fatty acids (FFA).

Cell binding studies

Control and transfected HTC 7288c cells were cultured in roller bottles in Swims-77 media supplemented with 10% fetal calf serum (FCS) at 37°C in a 5% CO_2 atmosphere until just subconfluent. The medium was then replaced with the same medium containing 30 μm $ZnSO_4$ and 10% FCS and incubated overnight to induce hepatic lipase. HTC 7288c cells were suspended in 0.5 ml of binding buffer containing 30 μm $ZnSO_4$, 3% bovine serum albumin (BSA), and 10 mm N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid (HEPES) in 2 ml Eppendorf microcentrifuge tubes at a concentration of 2 $\times$ 10^6 cell/ml. ^{125}I-labeled LDL (5 μg/ml) or ^{125}I-labeled chylomicron remnants (1 μg/ml) were added. After incubation for 4 h at 37°C, lipoprotein degradation was assessed by measuring the amount of trichloroacetic acid (TCA) and silver nitrate-soluble radioactivity present in the incubation medium. The cells were washed three times with cold PBS containing 0.3% BSA and twice with PBS and counted to determine the amount of the cell-associated lipoproteins. Non-specific binding was determined by the amount of binding in the presence of 100- or 20-fold excess of unlabeled LDL or chylomicron remnants, respectively. Release of free ^{125}I in the absence of cells was negligible.

Preparation of lipoproteins and lipoprotein uptake studies

LDL were isolated from normal human donors by sequential ultracentrifugation in KBr solutions (12). Chylomicrons were collected from rats bearing a lymphatic cannula that were infused with an egg emulsion as previously described (13). Chylomicron remnants were prepared in functionally hepatectomized rats by the method of Redgrave as previously described (14). Radio-iodination was carried out by a modification (15) of the iodine monochloride method as previously described (16). The chemical characterization of the particles has been reported previously (16).

To measure lipoprotein uptake by tumor and liver, the animals were anesthetized with methoxyflurane (Pitman-Moore, Mundelein, IL). The inferior vena cava was exposed, and the radiolabeled lipoprotein, dissolved in PBS (100 μl with about 4 $\times$ 10^6 cpm) was injected. For chylomicron remnant uptake studies, the animals were exsanguinated 10 min later. For LDL uptake studies, the abdominal cavity was sutured and the animals were allowed to recover. The animals were again anesthetized 1 h later for exsanguination. To account for the trapping of plasma in the tissues, the same protocol was used except ^{125}I-labeled albumin (5 $\times$ 10^6 cpm) was injected. At the time of killing, a blood sample was taken and sections of each tumor and of the liver were removed, blotted, dried, weighed, and the radioactivity in the tissue was measured in a gamma counter. The value of trapped fluid was calculated and the tissue specific lipoprotein space was calculated as previously described (9).

Preparation of tumor-bearing animals

Buffalo strain rats were obtained from Charles River Laboratories (Wilmington, MA). Rats of about 300 g were used. Approxi-

mately, 2×10^7 hepatic lipase-expressing cells were injected subcutaneously into one thigh; about 4 days later approximately 1×10^7 wild type HTC 7288c cells were injected into the other thigh. The time and quantity differential was required because of the difference in the growth rate of the two cell types. At the time of the second injection, an ALZET osmotic pump (Alza, Model 2001, Palo Alto, CA), containing 13% Zn gluconate was implanted in the interscalpular region of the rat. The pump delivers about 1 μl per h. About 10–14 days after the implantation of the pump, the tumors were about 1–2 cm in diameter, and the animals were used for experimentation or killed and the tumors were removed.

Immunohistochemistry

Immunohistochemical localization of hepatic lipase was carried out as previously described for the LDL receptor (17). Briefly, sections of fresh tissue were rapidly excised and placed in formaldehyde fixative, cut into 0.5-cm^2 pieces and incubated in a fixative for 3 h. After rinsing with PBS, they were incubated in the refrigerator overnight in 18% sucrose in PBS. The tissues were then quick-frozen in OCT. Tissue sections (5 μm) were collected on gelatin-coated slides and incubated with 0.1% nonfat milk, 15 mm ammonium acetate, 0.15% Triton X-100, diluted (1:50) in one-half strength PBS (pH 7.4), at room temperature for 1 h. Normal goat serum was added to the buffer to reduce nonspecific binding of immunoglobulin. They were then transferred to the above solution, with the addition of either 1–2 μg/ml rabbit anti-hepatic lipase polyclonal IgG (1:2000) or normal rabbit IgG overnight at 4°C. They were rinsed and then incubated overnight at 4°C in fresh buffer and again rinsed to remove unbound immunoglobulin. The rinsed tissue was then incubated with goat anti-rabbit IgG $F(ab')_2$ fragment coupled with horseradish peroxidase and the peroxidase was detected by first incubating with diaminobenzidine, followed by the addition of hydrogen peroxide. The sections were then rinsed, dehydrated, cleared with xylene, and mounted with Permount.

Estimation of mRNA content

The level of mRNA was estimated by Northern blot analysis. After tissue was removed, total cellular RNA was rapidly isolated by the method of Chomczynski and Sacchi (18) using guanidinium thiocyanate, and phenol exactly as described (19). RNA samples were stored in liquid N_2 until used. Samples were separated by 1% formaldehyde agarose electrophoresis and transferred to a nylon membrane. The filter was baked, prehybridized, and hybridized with the appropriate [^{32}P]dCTP-labeled probe of complementary DNA. The filter was then washed, air-dried, and autoradiographed overnight. For quantitation, glucose-3-phosphate dehydrogenase mRNA was also visualized as above and the radioautograph was scanned with a densitometer (Bio-Rad, Richmond, CA).

Determination of LDL receptor and LDL receptor related protein (LRP) expression

Total cell membranes were prepared from liver or tumors as previously described (20). Membranes were solubilized as previously described (20) and Western blotting was carried out as described above using either rabbit anti-LDL receptor or anti-LRP IgG. The specificity of the two antibodies has previously been described (21–23). The relative amount of protein was quantified by densitometric scanning.

Statistics

Data were analyzed using standard paired or group *t*-test with a Statview statistical program.

RESULTS

Transfection, screening, and cloning of HTC 7288c cells with hepatic lipase cDNA

Two different constructs were used to create transplantable hepatomas that express rat hepatic lipase. In the majority of the experiments the metalothionine promoter was used to control expression. A diagram of this construct has been previously published (10). In a few experiments the MoMLV-LTR promoter was used to exclude any effect of zinc on the results; the cDNA for hepatic lipase was the same in the two constructs. There were never any differences between the metalothionine promoter or the MoMLV-LTR promoter containing cells or tumors (data not shown). After transfection and selection by antibiotic resistance, cell populations were screened for secretion of hepatic lipase with antibody to rat hepatic lipase. Hepatic lipase secreting cells were cloned by endpoint dilution and the resultant colonies were expanded and characterized.

Characterization of secretory products of the transfected cloned HTC cells

Transfected cells were cultured in roller bottles and aliquots of the cells and media were removed. The protein in the media was isolated and examined by immunoblotting with an anti-hepatic lipase antibody (not shown). The media was assayed for hepatic lipase activity (11). The medium of transfected cells produced 2.4 μmol FFA/ml per h. There was neither protein nor lipolytic activity present in the medium of the untransfected cells. The amount of hepatic lipase secretion in even the highest expressing HTC 7288c transfected cells is considerably lower than in transfected CHO cells (10).

Cell association and degradation of LDL and chylomicron remnants by HTC 7288c cells that secrete hepatic lipase

HTC 7288c cells were grown in induction medium as described in Methods. Aliquots containing about 2×10^6 cells were placed in microfuge tubes and incubated with ^{125}I-labeled LDL (5 μg/ml) or ^{125}I-labeled chylomicron remnants (1 μg/ml) with or without 100- or 20-fold excess, respectively, of unlabeled lipoprotein. After 4 h, cells were pelleted, resuspended, and washed three times. The cell association and degradation were determined. As compared to control transfected HTC cells, those expressing hepatic lipase had about 20% more cell association and 30% more degradation of LDL ($P < 0.05$) (**Fig. 1**). The degradation but not the cell association of chylomicron remnants was slightly but significantly ($P < 0.05$) greater in transfected wild-type cells as well (Fig. 1). Although the magnitude of the enhancement of LDL uptake was less than with transfected CHO cells, it was consistent and reproducible ($P < 0.05$).

The enhanced uptake was not due to a difference in the level of LDL receptor expression. This was estimated by immunoblotting of cell membranes. As judged by immunoblotting, there was not a significant difference between the amount of LDL receptor protein in mem-

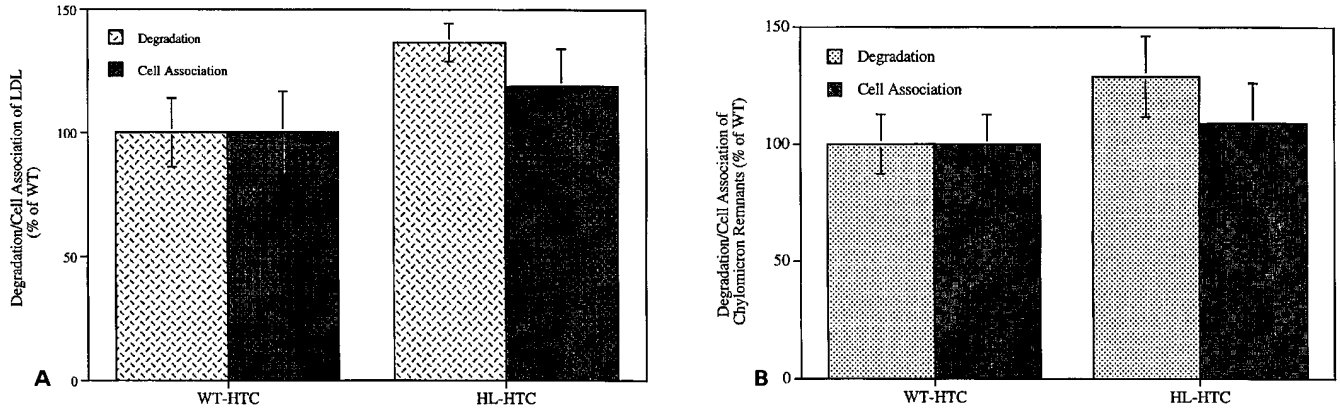

Fig. 1. Cell association and degradation of LDL and chylomicron remnants by transfected and wild-type HTC 7288c cells. Cells were cultured in roller bottles in Swimms 77 medium supplemented with 10% fetal calf serum at 37°C in a 5% CO_2 atmosphere until just subconfluent. The medium was then replaced with the same medium containing 30 μm $ZnSO_4$ and incubated overnight to induce hepatic lipase expression. HTC 7288c cells were resuspended in 0.5 ml of serum-free binding buffer containing ^{125}I-labeled (A) LDL (5 μg/ml) or (B) chylomicron remnants (1 μg/ml) and incubated for 4 h at 37°C. Cell association and degradation were determined as described in Methods, with appropriate correction for non-specific cell association and degradation. In each individual experiment the uptake and binding by transfected cells was greater than that by any wild-type cells, although the absolute values varied with different batches of cells and lipoproteins. Data expressed as mean ± SE (n = 6); HL, hepatic lipase. LDL cell association and degradation and chylomicron remnant degradation were different from control with $P < 0.05$ by group *t* test. Chylomicron remnant cell association was not different from control.

branes from transfected (4.5 densitometric units) compared to non-transfected (5 densitometric units) HTC 7288c cells (**Fig. 2**).

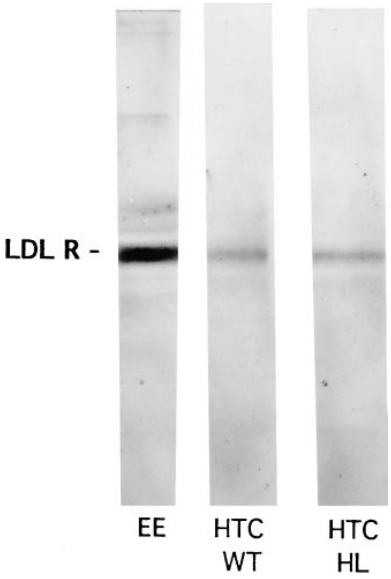

Fig. 2. Immunoblot analysis of the LDL receptor in wild-type and transfected HTC 7288c cells. Cell lysates from wild-type and hepatic lipase-transfected HTC cells were separated on 6% polyacrylamide gels under non-reducing conditions. Protein (300 μg) was transferred to nitrocellulose paper and incubated with 1 μg/ml of anti-LDL receptor IgG for 2 h at room temperature. The paper was then washed and incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG. The HRP-conjugated IgG was developed with the Bio-Rad Immuno-Blot™ assay kit. EE, liver membrane from ethinyl estradiol-treated rats; HTC-WT, wild-type HTC 7288c cells; HTC-HL, hepatic lipase-secreting HTC 7288c cells; LDL-R, LDL receptor. Results of densitometric scanning of duplicates are given in the text.

Characterization of the implanted tumors

The ability of the transfected cells to synthesize and secrete hepatic lipase after implantation and growth as tumors in syngeneic rats was documented. Cells transfected with the plasmid encoding hepatic lipase were injected into one thigh of a Buffalo strain rat and several days later a somewhat smaller number of untransfected cells were injected into the other thigh. It was necessary to carry out the injections on different days and with different amounts of cells because, both in culture and after implantation, the transfected cells grew more slowly. ALZET osmotic pumps containing Zn gluconate were implanted subcutaneously to allow induction of the gene containing the metalothionine promoter because the rats would neither eat food nor drink water supplemented with Zn. When the tumors were about the same size, and between 1 and 2 cm in diameter, the rats were killed and the tumors were removed. Total cell membranes and RNA were isolated. The RNA was subjected to electrophoresis, transferred to nylon paper, and probed with a ^{32}P-labeled DNA fragment corresponding to a portion of the coding region of rat hepatic lipase. The membranes were solubilized, subjected to SDS-PAGE, transferred to nitrocellulose paper, and incubated with polyclonal anti-hepatic lipase antibody. The presence of antibody was detected by HRP-labeled secondary antibody. While growing in vivo the tumors continued to transcribe the genetic information of the plasmid as documented by the presence of mRNA encoding hepatic lipase on the Northern blot (**Fig. 3A**) and continued to translate the mRNA as documented by the presence of immuno-reactive material on the Western blot (Fig. 3B). Neither hepatic lipase mRNA nor protein were detected in tumors derived from the untransfected cells (Fig. 3A, B).

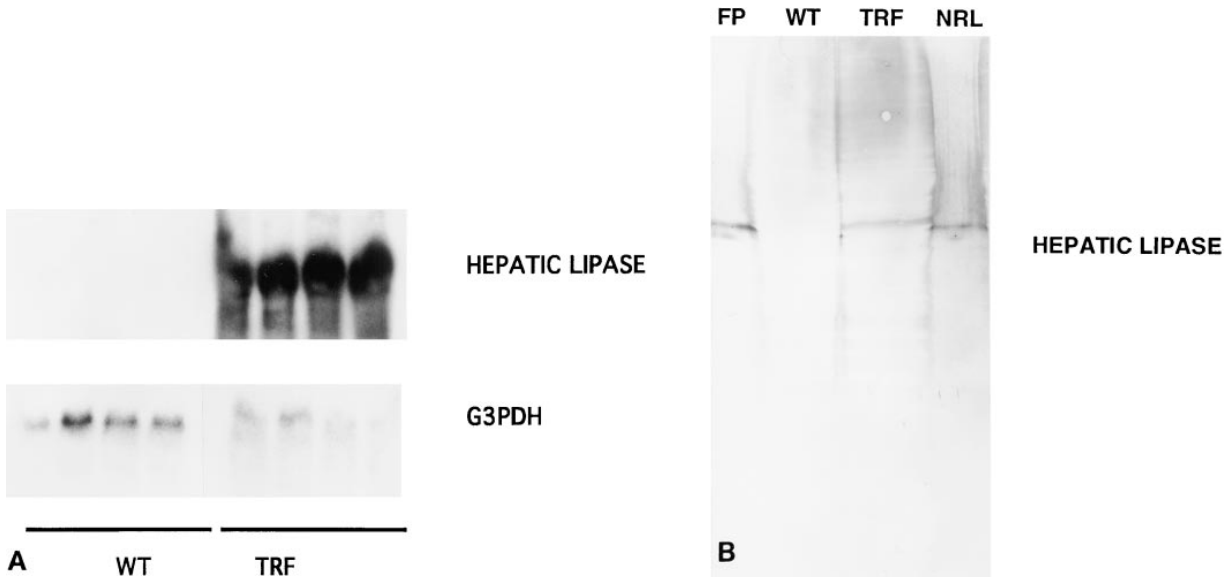

Fig. 3. Expression of hepatic lipase mRNA and protein by tumors derived from HTC 7288c cells. HTC 7288c cells transfected with the hepatic lipase cDNA were injected into one thigh of a Buffalo strain rat. Several days later, a smaller number of non-transfected HTC 7288c cells were injected into the other thigh. When the tumors were about 1 cm in diameter, an osmotic pump containing Zn glucosate was implanted in the interscalpular region of the animals and they were killed 3 days later. Sections of the tumors and liver were removed and RNA and cell membranes were prepared. (A) Northern blotting of the RNA was carried out as described in Methods using a ^{32}P-labeled cDNA fragment of hepatic lipase. (B) Immunoblotting of the membranes was carried out as described in the legend of Fig. 2. WT, tumors derived from wild-type HTC 7288c cells; TRF, tumors derived from hepatic lipase-transfected HTC 7288c cells; FP, pure GST hepatic lipase fusion protein; NRL, normal rat liver. A representative result is shown.

Immunohistochemistry of the implanted tumors

The architecture of the tumors with respect to the localization of hepatic lipase was examined. Tumors from transfected and non-transfected cells, grown in the same animal, were harvested, frozen, and sections were prepared for immunohistochemistry. The sections were incubated first with anti-hepatic lipase antibody followed by HRP-conjugated second antibody and then developed. The tumors from non-transfected cells did not display immunoreactivity (**Fig. 4A**), while those from transfected cells stained brightly (Fig. 4B). This result excluded the possibility that hepatic lipase secreted by the tumors from transfected cells or the normal liver adhered to tumors from non-transfected cells. Localization of hepatic lipase to the surface of the cells also suggested that the enzyme may exert a local effect in the region of the tumors. The general histology of the tumors was similar regardless of whether they grew from transfected or non-transfected cells. There appear to be two types of architecture: one in which there are sheets of cells and the other in which more open sinusoid-like areas were present (Fig. 4C). Together these results suggested that lipoproteins would be able to gain access to at least a portion of the cells and that in these regions hepatic lipase is present on the cell surface of the tumors that grew from transfected cells but not those from the control cells. Aside from the presence or absence of hepatic lipase on their surface, the cellularity and general histology of the tumors derived from transfected and wild-type cells were quite similar.

Uptake of LDL by the implanted tumors

To ascertain whether the ability of a cell to secrete hepatic lipase affected lipoprotein uptake by the tissue that secreted it, animals bearing tumors composed of cells secreting hepatic lipase in one thigh and not secreting hepatic lipase in the other thigh were studied when the tumors in the two thighs were about the same size. At this time the tumors contained relatively little necrotic tissue (not shown). A trace of ^{125}I-labeled LDL ($\sim 4 \times 10^6$ cpm) was injected into the inferior vena cava and 1 h later the rat was killed and the tumors were removed, weighed, and the amount of radioactivity in the tumors was determined. The amount of trapped plasma was determined in separate experiments by the injection of ^{125}I-labeled albumin as previously described (9). In each experiment ($n = 9$) the tumors that secreted hepatic lipase removed more LDL per gram of tissue than those that did not (**Fig. 5A**). On average the tumors that expressed hepatic lipase removed 34% more lipoprotein than those that did not ($P < 0.02$) (Fig. 5B). There was not a significant difference between the ability of the two types of tumors to accumulate either activated or unactivated α2-macroglobulin (not shown).

The same experiment was repeated with ^{125}I-labeled chylomicron remnants. The time between injection and killing was reduced to 10 min to compensate for the more rapid removal of chylomicron remnants. A similar result was obtained. Although the magnitude of the difference was smaller, $15 \pm 6\%$, it achieved significance ($P < 0.05$) (**Fig. 6**).

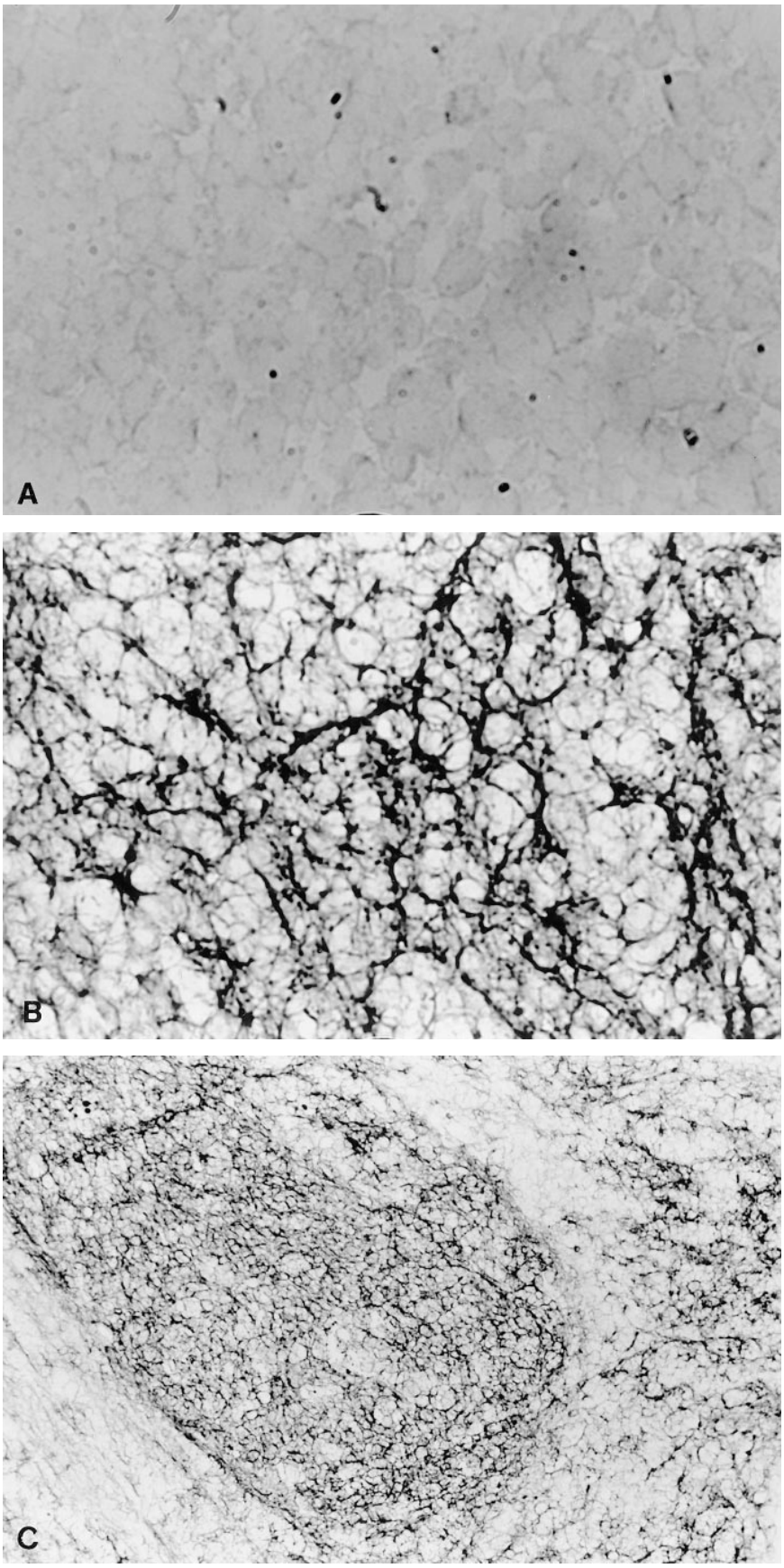

Fig. 4. Immunohistochemical localization of hepatic lipase in tumors derived from HTC 7288c cells. Animals bearing tumors derived from wild-type and hepatic lipase-secreting HTC 7288c cells were prepared as described in the legend of Fig. 3. After killing, portions of the tumors were placed in fixative and immunohistochemical staining using anti-hepatic lipase antibody was carried out as described in Methods. (A) Tumors from wild-type cells; (B) tumors from cells transfected with hepatic lipase cDNA; (C) lower power of "b" to highlight general architecture of tumors.

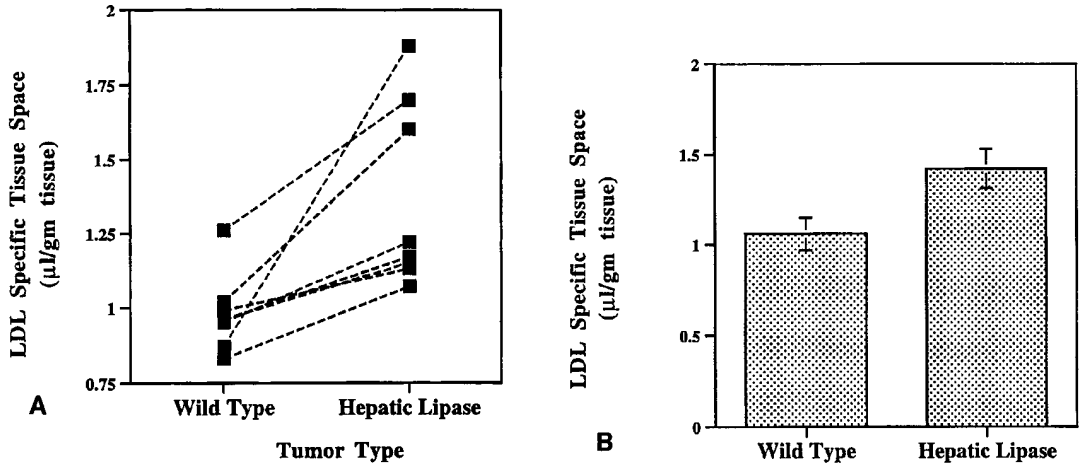

Fig. 5. Uptake of LDL by tumors derived from HTC 7288c cells. Animals were prepared as described in the legend of Fig. 3. When tumors were of approximately equal size, the animals were anesthetized and ^{125}I-labeled LDL was injected into their vena cava. One hour later, they were exsanguinated and samples of tumors from each thigh and of the liver were removed, blotted, weighed, and the amount of radioactivity was determined. The amount of trapped fluid in the liver and tumors was measured using ^{125}I-labeled albumin in separate experiments, and the uptake was corrected for this and the LDL specific tissue space was calculated as described in Methods. (A) Individual experiments; (B) mean and SE of 9 experiments. Hepatic lipase data are different than those from wild-type tumors; $P < 0.001$, n = 9.

Relative to hepatic uptake there were major differences between the two lipoproteins. On a per gram of tissue basis the liver took up about 7.9 times more LDL than the tumors from wild-type cells and 5.9-fold more than the tumors of cells that secreted hepatic lipase (**Fig. 7A**). This suggests that the ability to secrete hepatic lipase could be an important factor in directing LDL to a particular tissue. In contrast, the liver took up 45-fold more chylomicron remnants per gram of tissue than tumors and this was reduced to 40-fold more with the secretion of hepatic lipase (Fig. 7B). The major imbalance between tumors and liver in their ability to take up chylomicron remnants suggests that other local or architectural factors play a critical role in the selective uptake of remnants by liver.

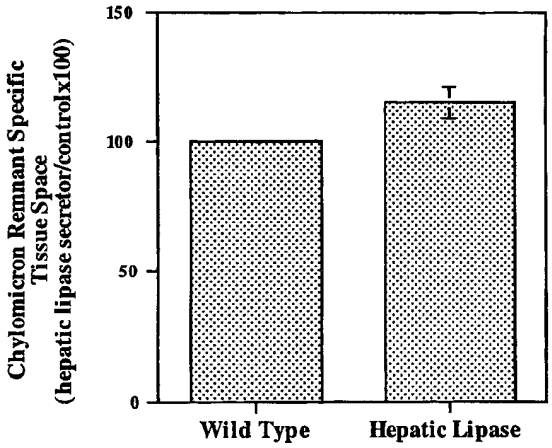

Fig. 6. Uptake of chylomicron remnants by tumors derived from HTC 7288c cells. The same experiment as in Fig. 5 was carried out except that ^{125}I-labeled chylomicron remnants were used. The value of the specific tissue space in hepatic lipase-secreting tumors is plotted as a percent of the specific tissue space in the wild-type tumor; n = 9; $P < 0.005$.

Receptor levels in the implanted tumors

One explanation for a difference in uptake by transfected as compared to untransfected tumors and the difference of both with the liver could be the level of expression of lipoprotein receptors, either the LDL receptor or the LRP. Tumors were harvested and cell membranes were prepared from the tumors and the liver of each animal. The membranes were solubilized; proteins were separated by 6% SDS-PAGE and, after transfer to nitrocellulose, the amount of LDL receptor and LRP was determined by immunoblotting using specific polyclonal antibodies to each of the receptors. Interestingly, the tumors had about twice as much LRP per mg cell membrane protein as the liver (**Fig. 8A**, **B**). There was no difference in the amount of LRP in the tumors derived from hepatic lipase transfected cells compared to the tumors grown from the wild-type cells (Fig. 8 A, B). Thus, expression of LRP cannot explain the difference in chylomicron remnant uptake between tumor and liver. More interesting were the LDL receptor levels. Tumors from both cell types had a greater level of expression of the LDL receptor than the liver from the tumor-bearing animals (Fig. 8C, D). Further, the tumors from the wild-type cells had twice as high a level of LDL receptor protein per mg cell membrane protein as the tumors from the cells transfected to express hepatic lipase (Fig. 8C, D). Thus, lipoprotein uptake was inversely correlated with the level of LDL receptors, suggesting that there was down-regulation of LDL receptors in tissues removing more lipoprotein and that ancillary factors contribute importantly to the net rate of lipoprotein uptake by increasing the amount of uptake per receptor.

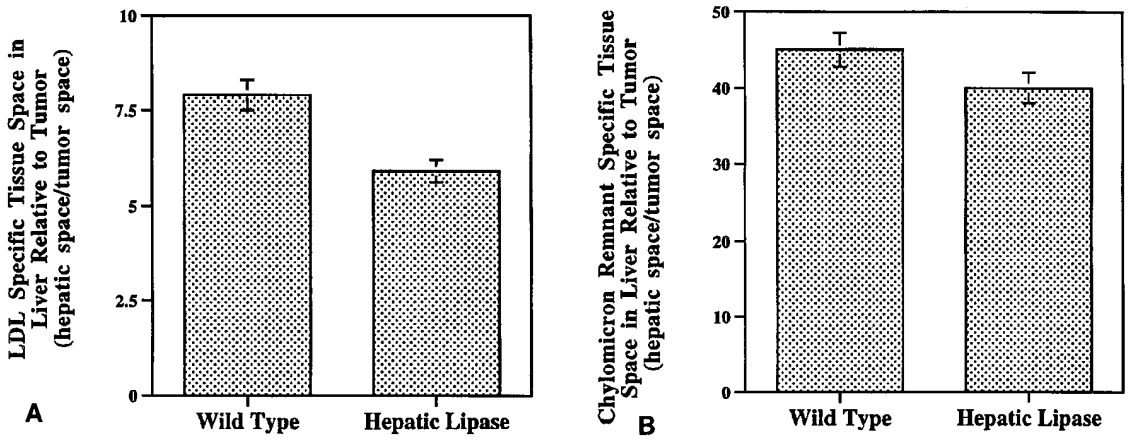

Fig. 7. Uptake of LDL or chylomicron remnants by tumors from wild-type or hepatic lipase-secreting cells compared to the removal by the liver of the same animals. The lipoprotein specific tissue space was calculated for the liver of each of the animals studied, as described in Figs. 5 and 6. The tissue space of the liver relative to that of the tumor was then determined for each animal, and the mean ± SE was plotted. (A) LDL; (B) chylomicron remnants; n = 9 for both types of lipoprotein.

DISCUSSION

In the present study, a novel strategy to assess the role of a molecule in lipoprotein metabolism in vivo was used. The molecule, hepatic lipase, has been proposed to have a number of physiologic functions. These include remodeling HDL and LDL by removing residual triglyceride from these lipoproteins (1). In addition, in recent years support for the possibility that the lipases also play a direct role in lipoprotein uptake by cells has come from results in a variety of cell culture experiments. Studies of a related enzyme, lipoprotein lipase, have provided evidence that the enzyme can serve as a binding site for low density lipoproteins (24, 25) and, perhaps in conjunction with proteoglycans (26), can lead to increased lipoprotein uptake. Both lipoprotein lipase (27) and hepatic lipase (28) may also serve as ligands for the LRP. These studies were done in cell culture and their relevance to in vivo behavior remains to be established. In vivo anti-hepatic lipase antibody infusion results in accumulation of remnant-like particles (29) and delayed removal of chylomicron remnants in rats (3) and mice (4). While these observations are consistent with a role for hepatic lipase in lipoprotein transport in vivo, they are not proof that one exists. The results of the current studies support the hypothesis that this enzyme plays a direct role in the uptake of at least one class of lipoproteins, LDL, in vivo.

The use of a transplantable tumor cell line as a mechanism for studying the effects of a transgene has limited precedent. The ability of a skin graft to express and deliver a transgene has been reported (30). In the current study the tumor continued to express the gene that had been transfected into the parent cell line for as long as the animals bearing the tumors were maintained. The approach is not a substitution for the use of transgenic animals or animals expressing transgenes as the result of infection with adenovirus because it is both transient, in that the tumor eventually kills the animal, and places the gene in a non-physiological location. However, it has the advantage of allowing a direct comparison, in the same animal in vivo, of tissues that are highly similar except for the presence of a single gene product.

For the present studies, it was particularly important that the hepatic lipase remained bound to the tumor. Hepatic lipase is bound to the surface of only a limited number of tissues, most likely due to linkage to a specific heparan sulfate proteoglycan (31). Either the hepatoma used has this proteoglycan or hepatic lipase remains associated with the cell membrane for other reasons. Interestingly, neither the hepatic lipase secreted by the tumor nor that secreted by the normal liver accumulated to any appreciable degree on the surface of tumors from the nontransfected cells. If the tumors have the recognition site this would imply that the amount of hepatic lipase secreted is not enough to result in significant accumulation in the untransfected tumor.

The two tumors were not absolutely identical. It was found that the cells containing the transgene grow more slowly both in culture and in the animal; nonetheless, in culture the cells had similar phenotypes and most importantly had the same level of LDL receptors and LRP.

In vivo, the tumors from the transfected cells consistently took up LDL at a modestly greater rate than the tumors from the non-transfected cells. The magnitude of the difference was less than might have been expected from in vitro studies with CHO cells where the presence of hepatic lipase generally doubles the rate of LDL uptake (6), and with transfected McA-RH7777 cells (8) where there is marked stimulation of chylomicron remnant binding. The effect in cultured HTC 7288c cells was not as dramatic with either ligand. The effect in vivo was, however, consistent with the magnitude of the effect in cell culture. This may have been due in part to the lower level of hepatic lipase expression by HTC 7288c cells as compared to transfected CHO cells. The impact of expression in vivo, however, was probably further understated because the level of LDL receptor expression in tumors from transfected cells was about half that in tumors from

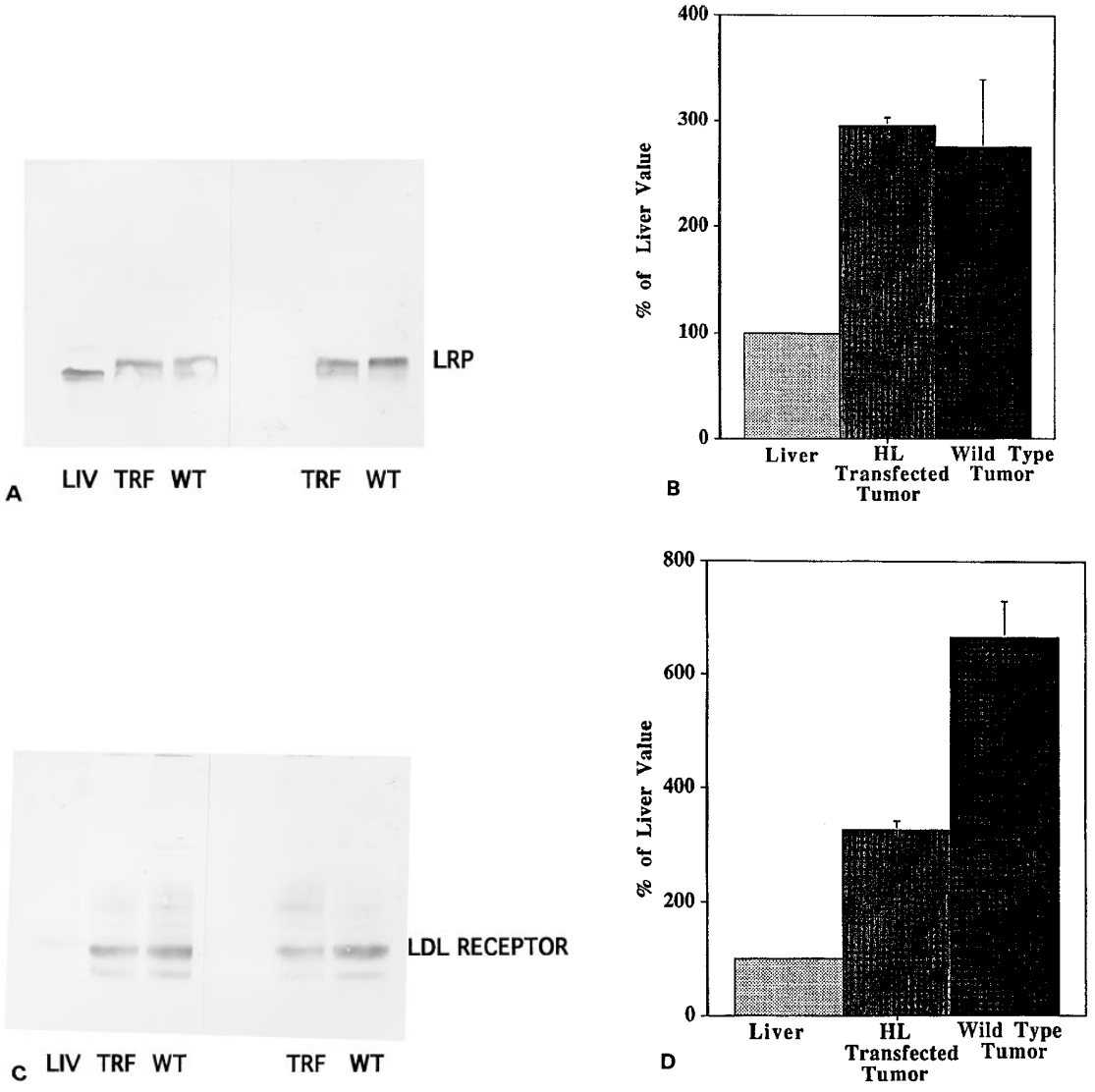

Fig. 8. LDL receptor and LRP protein level in tumors from HTC 7288c cells and liver. Animals were prepared as described in the legend of Fig. 3. When the tumors in the thighs were about the same size, the animals were killed, sections of tumors and liver were removed, and membranes were prepared. Immunoblotting was carried out as described in the legend to Fig. 4, and protein cross-reactivity to either the LDL receptor or the LRP was identified. Representative blots of (A) LRP and (C) LDL receptor; quantitation of relative density of staining as determined by scanning densitometry of three separate experiments (B) LRP, (D) LDL receptor. Liver, membrane from normal liver; TRF, membrane from tumor of HTC 7288c cells transfected to express hepatic lipase; WT, tumor from wild-type HTC 7288c cells.

nonhepatic lipase-producing cells. Thus, the transport of LDL per LDL receptor was essentially more than doubled in the hepatic lipase-containing tumors. Because in culture the two types of cells had a similar level of LDL receptor, the lower level of LDL receptors found in vivo would be expected in a tissue that has a greater ability to take up lipoproteins, and thus cholesterol, than a comparable tissue that takes up less lipoprotein-derived cholesterol. Interestingly, we have recently reported (7) that the presence of hepatic lipase in a cell enhances the transport of cholesteryl esters from HDL by the selective uptake pathway; thus, some of the cholesterol that induces the lower level of LDL receptors may enter the tumor by this pathway. This makes the transplantable hepatoma an attractive model for future studies of the role of hepatic lipase in conjunction with molecules such as SR-B1 in selective cholesteryl ester uptake. Normal liver had an even lower level of LDL receptors and a greater uptake of LDL per unit of weight. The more rapid LDL uptake in normal liver could be due to the hepatic architecture which allows greater blood flow and contact of lipoproteins with the cell surface or to a higher concentration of molecules that enhance lipoprotein uptake, such as hepatic lipase or, most likely, to both of these factors. These considerations may be even more important in explaining the results obtained with chylomicron remnants. First, there have been inconsistent results in experiments when binding of chylomicron remnants to hepatic lipase-transfected cells has been measured. Lauer et al. (8) observed increased binding of β-VLDL to McA-RH7777 cells, a differentiated

hepatoma line, after expression of hepatic lipase. In contrast our laboratory (6) did not observe an increase in chylomicron remnant binding to hepatic lipase-expressing CHO cells, even when the hepatic lipase was anchored to the cell surface (7). The difference can be ascribed to either the particles used or to the relatively undifferentiated cell surface of the CHO cells. Subsequent studies suggest both appear to contribute to this (S. Choi and A. Cooper, unpublished observation). The hepatoma cell line used in this study was intermediate in this regard in that the hepatic lipase-transfected cells degraded remnants slightly better than untransfected cells. In vivo, there was also slightly more uptake by the hepatic lipase-containing tumors. However, consistent with our previous report (32), compared to liver neither tumor took up significant amounts of remnants despite the abundance of LDL receptors, LRP, and hepatic lipase in the tumors. The simplest interpretation is that with particles of the size of remnants, the ability to gain access to the cell surface is an essential initial determinant as suggested by Fraser, Dobbs, and Rogers (33). It remains possible, however, that another molecule expressed by the mature liver contributes to this difference.

In summary, the present experiments used a transplantable tumor, transfected with the gene for a protein whose functions are complex and not fully elucidated, to help establish one of the functions of the protein in vivo. The data provide significant support for a role of hepatic lipase in facilitating the uptake of apoB-containing lipoproteins by tissues to whose surface the enzyme is localized. Other studies from this laboratory suggest that the effect of hepatic lipase is mediated by a direct interaction between apoB-48 and hepatic lipase (34). This function of hepatic lipase may help to explain the high uptake of LDL by the liver, adrenal gland, and ovaries, the three tissues where hepatic lipase is normally found, and suggest that the level of LDL receptor and the LRP are not the only determinants of the rate of removal of apoB- and apoE-containing lipoproteins by a tissue.

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