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Synthesis of α_1-Antichymotrypsin and α_1-Antitrypsin by Human Trophoblast

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ABSTRACT. α_1-Antichymotrypsin (α_1-ACHY) and α_1-antitrypsin (α_1-AT) are closely related glycoprotein protease inhibitors, present in plasma and other extracellular fluids, that neutralize proteases released by leukocytes in response to trauma and inflammatory stimuli. Both inhibitors are synthesized primarily by hepatocytes, although lower levels of synthesis by monocytes and breast and intestinal epithelial cells have been demonstrated. Recently, the immunohistochemical localization of α_1-AT and α_1-ACHY in intrauterine and extrauterine human trophoblastic tissue has been reported. In the present study, we have sought to determine whether human trophoblast is also able to synthesize α_1-AT and α_1-ACHY. Messenger RNA for both inhibitors was found by Northern blotting in chorionic villi obtained from first trimester and term placenta. Substantial differences in messenger levels for both inhibitors among individual placentas were noted. α_1-ACHY and α_1-AT messenger was also present in trophoblast cells in primary culture. Synthesis of α_1-AT and α_1-ACHY protein was demonstrated by SDS-PAGE after immunoprecipitation of [^{35}S]-labeled α_1-AT and α_1-ACHY from conditioned media of trophoblast cells in culture metabolically labeled with [^{35}S]-methionine. It is of some interest that the M_r of the α_1-AT and α_1-ACHY secreted by trophoblast were 50 000 and 49 000, respectively, compared with 54 000 and 68 000 for these proteins in plasma (or secreted by HepG2 human hepatoma and MCF-7 human breast cancer cells). After enzymatic deglycosylation, the M_r of the α_1-AT and α_1-ACHY secreted by trophoblast and HepG2 cells were all approximately 46 000, suggesting incomplete glycosylation of the inhibitors released by trophoblast. (*Pediatr Res* 34: 312–317, 1993)

Abbreviations

α_1-AT, $_1$-antitrypsin
α_1-ACHY, α_1-antichymotrypsin
CBG, corticosteroid-binding globulin
FBS, fetal bovine serum
DMEM, Dulbecco's modified Eagle media
PNGase F, peptide-N-glycosidase F
hCG, human chorionic gonadotropin

α_1-AT and α_1-ACHY are closely related glycoprotein protease inhibitors present in plasma and extracellular fluids that neutralize serine proteases through the formation of stable 1:1 complexes (1). α_1-AT and α_1-ACHY play major roles in the host response to trauma and inflammation. The major function of α_1-AT, which is present at high levels in human plasma (1.5–3.5 mg/mL), is to inhibit elastases released by leukocytes at sites of inflammation. α_1-ACHY, present in plasma at lower levels (0.3–0.6 mg/mL), is thought to neutralize chymotrypsin-like proteases such as cathepsin G also released by leukocytes at inflammatory sites. α_1-ACHY and α_1-AT belong to the serpin supergene family as does CBG, the major glucocorticoid carrier in the circulation (2). CBG is cleaved by elastase at a unique site in its C-terminal region resulting in decreased glucocorticoid binding (3). Because local elastase levels depend to a large degree on local levels of α_1-AT, delivery of glucocorticoids to inflammatory sites may be regulated by a mechanism involving the interaction of elastase and α_1-AT.

α_1-AT and α_1-ACHY are synthesized primarily by hepatocytes and to a lesser degree by monocytes (4). We have shown that MCF-7 human breast cancer cells, a cell line of epithelial origin, can also synthesize these protease inhibitors and that their synthesis can be stimulated by steroid hormones, cytokines, and growth factors (5). Although the amount of α_1-AT and α_1-ACHY produced by nonhepatic cells is small in comparison to that produced by hepatocytes, local synthesis may be important at sites of inflammation, particularly at locations not directly in contact with the circulation where levels of α_1-AT and α_1-ACHY may only be a fraction of that in plasma (6). Implantation of the embryo in the uterus elicits an inflammatory response, the control of which is required for normal implantation and placentation (7). Local synthesis of serpins may be necessary to control the maternal inflammatory response to invasion by trophoblast. Abnormal serpin synthesis by the trophoblast and/or by the endometrium could be associated with spontaneous abortion, gestational trophoblastic disease, and abnormal development of the placenta.

Recently, Earl *et al.* (8) demonstrated immunohistochemically the presence of α_1-AT and α_1-ACHY in early, term, and ectopic trophoblastic tissue. The cytoplasmic localization of these protease inhibitors suggested trophoblastic synthesis as opposed to passive absorption from maternal blood. However, these authors did not provide unequivocal evidence for their synthesis by the placenta. The study described herein was undertaken to provide evidence for placental synthesis of α_1-AT and α_1-ACHY. To this end, *1*) messenger RNA for α_1-AT and α_1-ACHY was identified in first-trimester and term chorionic villi and in human trophoblast cells in primary culture and *2*) after metabolic labeling of cultured trophoblast cells with [^{35}S]-methionine, [^{35}S]-labeled α_1-AT and α_1-ACHY protein was found in the spent media. It may be significant that the α_1-AT and α_1-ACHY released by trophoblast are not glycosylated to the same extent as the α_1-AT and α_1-ACHY found in plasma.

MATERIALS AND METHODS

Materials. FBS, DMEM, and other cell culture materials were obtained from Gibco (Grand Island, NY). [^{35}S]-methionine and

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[^{32}P]-deoxycytidine 5′ triphosphate and the Multiprime DNA Labeling System were from Amersham Corp. (Arlington Heights, IL). PNGase F was purchased from Boehringer Mannheim (Indianapolis, IN). Reagents for PAGE were obtained from Bio-Rad Laboratories (Hercules, CA). Ultra-pure Agarose was from Gibco-BRL (Grand Island, NY) and GeneScreen membrane from DuPont (Wilmington, DE). Rabbit antibodies to human α_1-AT and α_1-ACHY were obtained from Accurate Chemical and Scientific Corp. (Westbury, NY). All other materials were of high purity from commercial sources.

Chorionic villus samples. Small amounts of placental tissue were obtained during pregnancy termination procedures (8–15 wk) or at delivery, immediately after removal from the uterus. The tissue was washed in sterile PBS (0.05 M potassium phosphate, 0.1 M NaCl, pH 7.2) to remove any blood. Villus tissue, identified by its classic fernlike appearance, was dissected and blotted. The tissue was then frozen in liquid nitrogen and stored at −70°C until used for Northern blotting analysis.

Primary trophoblast cultures. Trophoblast cells were isolated from term placentas by a modification of the methods described by Bax *et al.* (9) and Kliman *et al.* (10). Fresh placentas, delivered either vaginally or by cesarean section, were dissected to remove the basal plate. Cotyledons were removed, cut into approximately 4-cm^3 pieces, washed three times with wash buffer (PBS with 5.5 mM glucose, 0.33 mM sodium pyruvate, 20 U/mL heparin, and antibiotics), and then incubated in wash buffer without heparin for 20 min with gentle shaking at 37°C. The tissue fragments were further dissected into 1-cm^3 pieces and incubated in digestion buffer (PBS with 5.5 mM glucose, 0.33 mM sodium pyruvate, 2 mg/mL pancreatin, and 0.25 μg/mL trypsin) for 20–30 min with gentle shaking at 37°C. The digestion buffer was decanted and the digestion process repeated. The digestion mixture was vigorously shaken for 30 s and filtered first through a single layer of sterile gauze, then through two layers, then through three layers. The cells were pelleted by centrifugation and purified by centrifugation through a 50-mL 5–70% Percoll gradient (30 min, 1200 × *g*, room temperature). The trophoblast-containing layer was removed from the gradient, and the trophoblast cells were washed three times with serum-free medium and plated at 2×10^5 cells/cm^2 on Plastek C culture plates (Mattek, Ashland, MA) in medium 199 containing 15% FBS and antibiotics. This procedure yielded a cell population that in these experiments was >95% trophoblast as determined by immunofluorescence with antibodies to the following antigens: cytokeratin (trophoblast), vimentin (fibroblast), and HLA-DR (macrophages). Cells were used 72 h after plating, at which time trophoblast had differentiated into a syncytia-like monolayer resembling trophoblast *in vivo*.

Trophoblast cells were isolated from first trimester placentas using a modification of the method described by Fisher *et al.* (11). Placental tissue obtained by vaginal aspiration from interrupted pregnancies after 8–15 wk gestation was washed in PBS containing antibiotics, 5.5 mM glucose, 0.33 mM sodium pyruvate and 20 U/mL heparin. Chorionic villi were dissected free and incubated for 10–20 min at 37°C in PBS containing 500 U/mL collagenase, 200 U/mL hyaluronidase, 0.2 mg/mL DNase I, and 1 mg/mL BSA. At the first sign of disintegration (after 10–15 min), the villi suspension was centrifuged and the pellet collected. The pellet was resuspended and incubated for 10–20 min in PBS containing 0.25 μg/mL trypsin and 2 mM EDTA. Trophoblast cells were isolated by centrifugation through a Percoll gradient and plated as described above.

α_1-AT and α_1-ACHY synthesis by trophoblast cells in culture. Primary trophoblast cultures in six-well (35-mm diameter) plates were washed three times with methionine-free DMEM media (without FBS) for 60 min and then incubated with fresh DMEM media (without FBS) containing [^{35}S]-methionine (400 μCi/mL) for 6 h. The conditioned medium was centrifuged and the supernatant heated to 100°C and stored at −20°C until used for immunoprecipitation of extracellular proteins. To determine RNA levels, cells were grown in an analogous fashion but without [^{35}S]-methionine. At the completion of the experiment, the medium was decanted and the cells were lysed in homogenization buffer containing guanidine isothiocyanate.

Enzymatic deglycosylation. Aliquots of spent medium (in 0.1 M $NaPO_4$, 10 mM EDTA, 10 mM β-mercaptoethanol, pH 7.0, containing 0.2% SDS) were heated at 95°C for 10 min to denature glycosylated proteins. After cooling, the reaction mixture was brought to 1% Triton X-100 and incubated with 5 or 10 U/mL of PNGase F at 37°C for 24 h to deglycosylate N-glycoproteins as described in the manufacturer's instructions.

Immunoprecipitation. Aliquots of spent medium were brought to 0.2% in SDS and heated for 2 min at 95°C. Nonspecific rabbit IgG (10 μg/mL spent media) was added and the reaction was incubated for 60 min at 4°C. Protein A-agarose, as a 50% suspension in buffer A (50 mM Tris-HCl, 150 mM NaCl, 6 mM EDTA, pH 7.4, containing 2.5% Triton X-100), was added and the incubation continued for 60 min longer. The Protein A-IgG was removed by centrifugation and four volumes of buffer A was added, followed by antibody to either α_1-AT or α_1-ACHY. The incubation was continued overnight at 4°C. Immune complexes were removed with protein A-agarose as above. The beads were washed six times with buffer A and the labeled inhibitors were released by heating to 95°C in sample buffer containing SDS. Labeled inhibitors were then subjected to PAGE in the presence of SDS under reducing conditions. Prestained molecular mass markers were included on all gels. After electrophoresis, labeled proteins on dried gels were visualized by autoradiography.

Isolation of mRNA and Northern blot analysis. Total cellular RNA was isolated from chorionic villus material or adherent monolayers of trophoblast cells in primary culture (approximately 10^7 cells/sample) by guanidine isothiocyanate extraction and centrifugation through a CsCl gradient (12). Plasmids ph-ACT235, containing a full-length human α_1-ACHY cDNA, and MVR-2, containing a full-length human α_1-AT cDNA, were generous gifts from S. Woo (Baylor University, Houston, TX) and C. Gray (Zymogenetics, Seattle, WA), respectively. A sonicated 2.0-kb human β-actin cDNA probe (pHFβA-1) was a gift from D. Filer (NYU Medical Center, New York, NY). Plasmid DNA was isolated by alkaline extraction, digested with the appropriate restriction endonucleases, and electrophoresed on 1% low melting point agarose gels in TBE buffer (89 mM Tris base, 89 mM boric acid, 2 mM EDTA). Inserts were excised from the gels and purified by phenol/$CHCl_3$ extraction followed by ethanol precipitation. Twenty-five ng of each cDNA insert was labeled with [^{32}P]-deoxycytidine 5′ triphosphate using random hexamers as primers (Multiprime DNA Labeling System, Amersham). Unincorporated nucleotides were removed by gel exclusion chromatography (Push Column, Stratagene, La Jolla, CA). Total RNA samples (25–30 μg) were electrophoresed on agarose/formaldehyde gels and transferred to GeneScreen nylon membrane. Blots were hybridized with [^{32}P]-labeled α_1-AT and α_1-ACHY cDNA under conditions of high stringency (50% formamide, 42°C for 12–18 h) and also washed under conditions of high stringency. After autoradiography, the protease inhibitor cDNA probes were removed and the blots were rehybridized with a [^{32}P]-labeled actin cDNA probe.

RESULTS

Expression of α_1-AT and α_1-ACHY mRNA by chorionic villi. To distinguish between placental synthesis of α_1-AT and α_1-ACHY and contamination by protease inhibitors from the circulation, chorionic villi from first- and third-trimester placentas were examined for the expression α_1-AT and α_1-ACHY mRNA. Total RNA was prepared from placentas of various gestational ages and analyzed by Northern blotting using [^{32}P]-labeled human α_1-AT and α_1-ACHY cDNA probes (Fig. 1). Sample preparation and hybridization were carried out as described in Methods and Materials. Because the amount of total RNA extracted

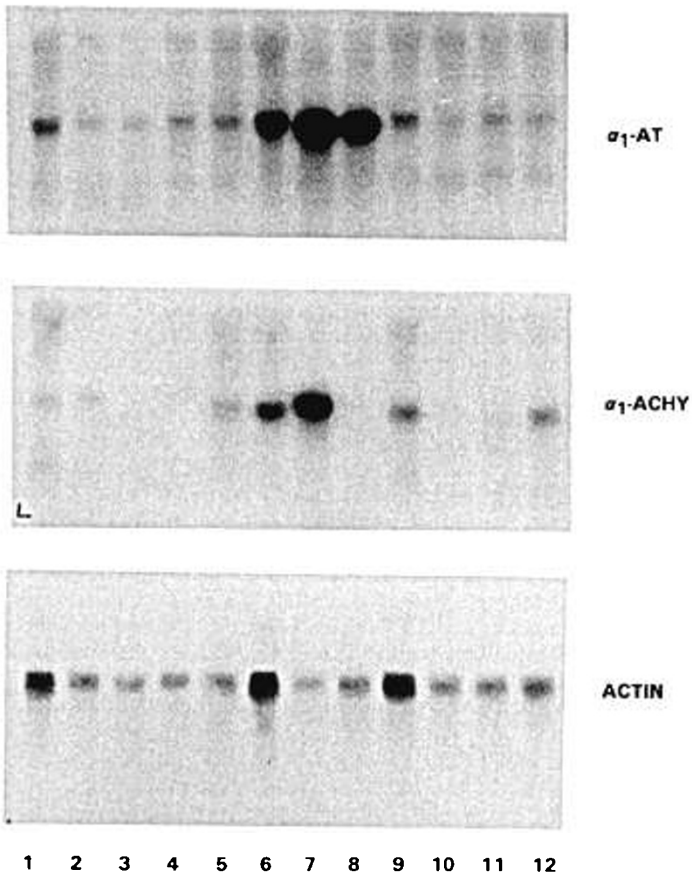

Fig. 1. Northern blot analysis of total trophoblast RNA from chorionic villi of various gestational ages. RNA was electrophoresed on 1% agarose/formaldehyde gels, transferred to nylon filters, and hybridized with [^{32}P]-labeled α_1-AT or α_1-ACHY cDNA probes. The filters were then cleared and rehybridized with a ^{32}P-labeled β-actin cDNA probe. Gestational ages of the samples were as follows: *1–5*, term; *6*, 8 wk; *7–9*, 10 wk; *10*, 11 wk; *11* and *12*, 12 wk.

Table 1. *Placental age and protease inhibitor mRNA levels**

Sample	Age	α_1-AT†	α_1-ACHY†
1	8 wk	0.69	0.19
2	8 wk	3.23	0.48
3	8 wk	1.77	0.49
4	10 wk	8.85	5.32
5	10 wk	4.59	0
6	10 wk	0.52	0.31
7	10 wk	0.43	0.13
8	10 wk	0.14	0.29
9	10.5 wk	0.15	0.08
10	11 wk	0.41	0.43
11	11 wk	0.63	0
12	12 wk	0.90	0.22
13	12 wk	0.68	0.74
14	14 wk	0.27	0.85
15	15.5 wk	0.02	0.17
16	Term	0.85	0.22
17	Term	0.50	0.40
18	Term	0.64	0
19	Term	1.38	0
20	Term	1.61	0.56
21	Term	0.08	0.16

* Autoradiograms of Northern blots of α_1-AT, α_1-ACHY, and actin (Fig. 1) were scanned and integrated with a densitometric scanner (model 910, E-C Apparatus Corp., St. Petersburg, FL).

† Protease inhibitor mRNA levels are normalized with regard to those of actin.

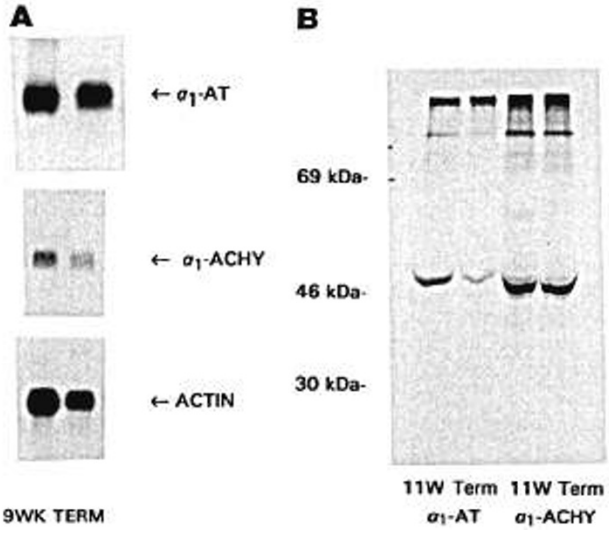

Fig. 2. Synthesis of α_1-AT and α_1-ACHY by first-trimester and term trophoblast after 2 d in primary culture. *A*, Northern blot analysis of total RNA from 9-wk and term placentas. RNA was electrophoresed on 1% agarose/formaldehyde gels, transferred to nylon filters, and hybridized with [^{32}P]-labeled α_1-AT or α_1-ACHY cDNA probes. The filters were then cleared and rehybridized with a [^{32}P]-labeled β-actin cDNA probe. *B*, SDS-PAGE of [^{35}S]-labeled α_1-AT and α_1-ACHY from trophoblast isolated from 11-wk and term placenta and metabolically labeled for 6 h with [^{35}S]-methionine.

from each sample depended on the quantity of tissue available for analysis, after the first hybridization the blots were cleared and rehybridized with a [^{32}P]-labeled β-actin cDNA probe. Relative protease inhibitor mRNA levels in each sample were determined by densitometric scanning of the autoradiograms and normalized for the amount of actin mRNA (Table 1). In general, all the samples analyzed expressed α_1-AT mRNA, with a wide variation among samples. There were similar variations in the expression of α_1-ACHY mRNA. In some samples, α_1-ACHY mRNA was not detected (Table 1, samples 5, 11, 18, 19). It is possible that these were contaminated by mononuclear cells, which express high levels of α_1-AT. The variability in α_1-AT and α_1-ACHY expression by different placentas is difficult to explain. However, it is consistent with similar interplacental variability in hCG and progesterone secretion as reflected by maternal serum levels. The normal range of hCG and progesterone in maternal serum at 36–40 wk gestation is 2 400–36 000 mIU/mL and 55–255 ng/mL, respectively. No clear-cut pattern emerged relating protease inhibitor levels to gestational age, and because of the relatively small number of samples in this study no conclusions could be drawn.

Expression of α_1-AT and α_1-ACHY mRNA and protein by trophoblast cells in culture. Because of the cellular heterogeneity of the placenta, it was necessary to determine whether the observed results represented synthesis by trophoblast or by contaminating fibroblasts or Hofbauer cells. Therefore, we examined the synthesis of α_1-AT and α_1-ACHY mRNA and protein by trophoblast cells in culture. Trophoblast cells were isolated from placental tissue by protocols that yield a cell population of greater than 95% trophoblast (Esterman AL, unpublished observations; 10). Northern blot analysis of total RNA from trophoblast in primary culture obtained from 9-wk and term placenta demonstrated the presence of α_1-AT and α_1-ACHY mRNA (Fig. 2*A*). As further evidence of the purity of the trophoblast population used in these studies, it should be noted that expression of α_1-ACHY by human peripheral monocytes is <1% of that of MCF-7 cells (or trophoblast), whereas expression of α_1-AT or α_1-ACHY by fibroblasts is not detectable by standard Northern blotting

techniques (Kadner SS, Banerjee S, Cruz MR, Finlay TH, unpublished manuscript).

Synthesis of the specific proteins was investigated using cultured trophoblast cells metabolically labeled with [^{35}S]-methionine. Radiolabeled α_1-AT and α_1-ACHY were found in conditioned media after immunoprecipitation with polyclonal antibodies to α_1-AT and α_1-ACHY and SDS-PAGE (Fig. 2*B*). However, the newly synthesized α_1-AT and α_1-ACHY released by trophoblast cells both appeared to have M_r considerably lower than reported for the same proteins secreted by other cell types (1, 3, 5). The differences were confirmed when the M_r of ^{35}S-labeled α_1-AT and α_1-ACHY released by trophoblast and HepG2 human hepatoma cells were compared using SDS-PAGE (Fig. 3). The α_1-AT or α_1-ACHY synthesized and secreted by trophoblast have M_r of 50 000 and 49 000, respectively, whereas the inhibitors secreted by HepG2 cells and MCF-7 human breast cancer cells (data not shown, but see reference 5) demonstrate M_r of 54 000 and 68 000. To verify the identity of the immunoprecipitated proteins from trophoblast, the immunoprecipitation was repeated with and without the addition of unlabeled protease inhibitor (Fig. 4). In both cases, unlabeled inhibitor blocked the immunoprecipitation of the labeled protein.

Are α_1-AT and α_1-ACHY released by trophoblast glycosylated? To determine whether the lower M_r of the inhibitors released by trophoblast was due to differences in glycosylation or to differences in peptide chain length, the α_1-AT or α_1-ACHY in the spent media from metabolically labeled trophoblast and HepG2 cells were enzymatically deglycosylated with PNGase F and examined by SDS-PAGE after immunoprecipitation (Fig. 3). Incubation with PNGase F, which efficiently cleaves the N-glycan linkage between asparagine and carbohydrate chain in denatured glycoproteins (13), reduced the M_r of the α_1-AT and α_1-ACHY secreted by both trophoblast and HepG2 cells to approximately 46 000, the molecular weight anticipated from the mRNA sequences. In Figure 3*A* and *B*, deglycosylation was incomplete, particularly in the case of the HepG2 cell inhibitors. However, the difference between the untreated and treated trophoblast inhibitors is clear. Complete deglycosylation of the HepG2 cell inhibitors was accomplished after incubation with a higher level of PNGase F (Fig. 3*C*). The α and β subunits of hCG immunoprecipitated from this sample with a rabbit polyclonal antibody to hCG had M_r of 34 000 and 22 000, respectively, suggesting normal glycosylation (data not shown) (14). These results are consistent with the α_1-AT and α_1-ACHY released by trophoblast being only partially glycosylated. If the α_1-AT and α_1-ACHY expressed by trophoblast were incompletely glycosylated, then their transit time through the Golgi apparatus might differ from that in hepatocytes. To test this possibility, the time course for the appearance of α_1-AT and α_1-ACHY in the media after pulse labeling with ^{35}S-methionine was compared for trophoblast and HepG2 cells (Fig. 5). The $t_{1/2}$ for release of α_1-AT and α_1-ACHY from the HepG2 cells were 50 and 80 min, respectively, which agrees with the results of others (15). Consistent with the possibility of incomplete glycosylation, the $t_{1/2}$ for release of both inhibitors from trophoblast is approximately 25 min.

Comparison of α_1-AT and α_1-ACHY mRNA from trophoblast and other cell types. α_1-AT has been shown to be transcribed from at least four different initiation sites in monocytes, enterocytes, and HepG2 cells (16, 17). This results in a series of mRNA of from 1.6 to 2.0 kb, the level of each depending on cell type and degree of up-regulation. To determine whether synthesis of α_1-AT and α_1-ACHY mRNA by trophoblast matched the pattern by one of the above cell types, Northern blot analysis was performed on total RNA obtained from trophoblast, HepG2 cells, and MCF-7 cells (Fig. 6). It is clear that MCF-7 and trophoblast α_1-ACHY mRNA have similar mobilities, which are different from that of HepG2 cells. However, although it is difficult to distinguish between the HepG2 cell and trophoblast α_1-AT mRNA, they both appear to be smaller than the α_1-AT mRNA from MCF-7 cells.

DISCUSSION

The immunohistochemical identification of α_1-AT and α_1-ACHY in the human placenta has recently been described (18). The location of these protease inhibitors in syncytial trophoblast but not in cytotrophoblast suggests that they play a role in the regulation of proteolytic activity around the invading component of trophoblastic tissue. However, both α_1-AT and α_1-ACHY are present at high levels in the blood and have been shown to be synthesized by breast and intestinal epithelial cells, monocytes, and resident macrophages. Thus, it is possible that their presence in the placenta results from contamination by proteins derived from the circulation. In addition, many cell types have cell surface receptors able to bind and internalize complexes of α_1-AT and α_1-ACHY with proteases (so-called serpin-enzyme-complex or SEC receptors) (17). Although the identification of SEC receptors in the placenta has not yet been reported, it is possible that the presence of α_1-AT and α_1-ACHY antigens in this tissue represents internalization rather than local synthesis because polyclonal antibodies to protease inhibitors can also recognize protease inhibitor-protease complexes. Our objective in this study was to determine whether the α_1-AT and α_1-ACHY in the placenta resulted, at least in part, from synthesis by trophoblast.

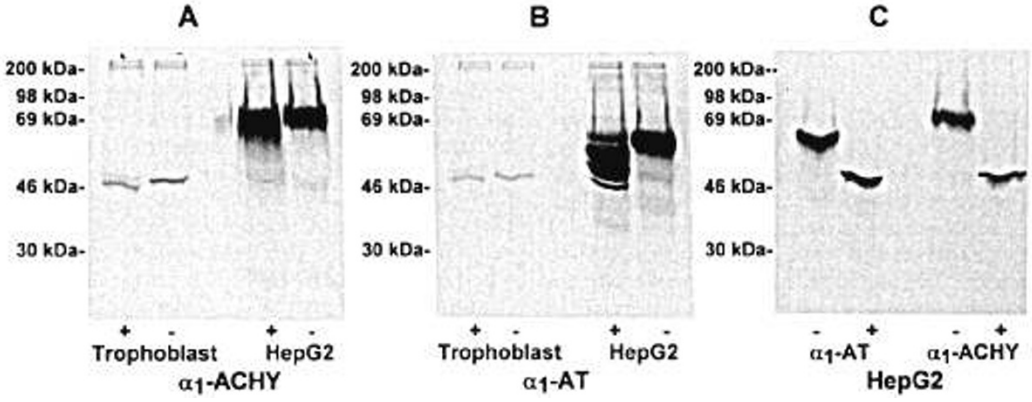

Fig. 3. Effect of enzymatic deglycosylation on apparent molecular masses of α_1-AT and α_1-ACHY synthesized and secreted by HepG2 cells and trophoblast cells. Spent medium from trophoblast and HepG2 cells metabolically labeled with [^{35}S]-methionine was deglycosylated with either 5 U/mL (*A* and *B*) or 10 U/mL (*C*) PNGase F as described in Materials and Methods. The [^{35}S]-labeled α_1-AT and α_1-ACHY in the treated medium were then immunoprecipitated and subjected to SDS-PAGE. +, Inclusion of PNGase F in the incubation; −, enzyme-free control. *A*, medium from trophoblast and HepG2 cells immunoprecipitated with antibody to α_1-ACHY. *B*, medium immunoprecipitated with antibody to α_1-AT. *C*, medium from HepG2 cells immunoprecipitated with antibody to α_1-AT and α_1-ACHY.

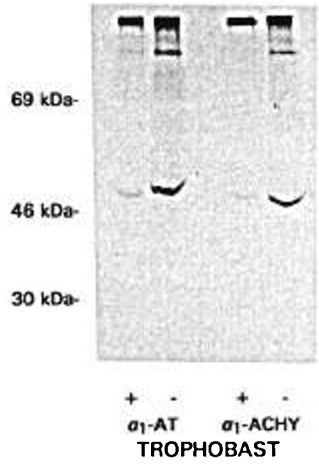

Fig. 4. Effect of excess unlabeled α_1-AT and α_1-ACHY on the immunoprecipitation of [^{35}S]-labeled α_1-AT and α_1-ACHY from metabolically labeled trophoblast. Term placental trophoblast in primary culture was incubated for 6 h with [^{35}S]-methionine. The medium (500 μL) was immunoprecipitated with polyclonal antibodies to either α_1-AT or α_1-ACHY in the presence (+) or absence (−) of 10 μg of unlabeled α_1-AT or α_1-ACHY purified from human serum. The immunoprecipitated proteins were collected on immobilized protein A and subjected to SDS-PAGE.

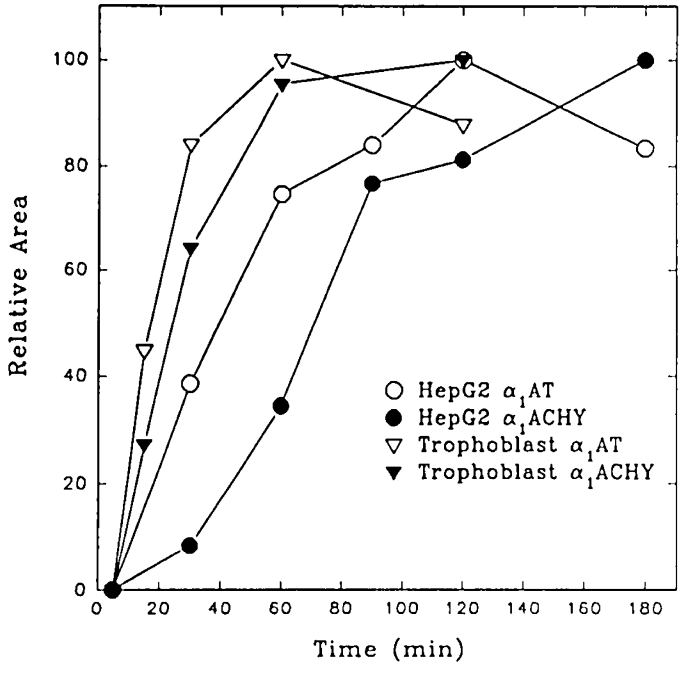

Fig. 5. Comparison of α_1-AT and α_1-ACHY secretion by trophoblast and HepG2 cells. Cells, in 9.6-cm^2 wells, were washed with methionine-free medium and then incubated with this same medium containing 250 μCi/mL ^{35}S-methionine for 60 min. Cells were washed with medium containing 1 mM cold methionine, and 1 mL of medium containing 1 mM methionine was added to each well. At the intervals indicated, levels of α_1-AT and α_1-ACHY in the medium from individual wells was determined by immunoprecipitation and electrophoresis as described in Materials and Methods. Bands in the autoradiogram were quantitated by densitometry. In each experiment, 100% was taken at the time point showing maximum incorporation.

Using whole tissue as well as cultured trophoblast cells obtained from first- and third-trimester placentas, we demonstrated the presence of α_1-AT and α_1-ACHY mRNA by Northern blot analysis. Consistent with this, culture medium from first- and third-trimester trophoblast grown in the presence of [^{35}S]-methionine was found to contain labeled, immunoreactive α_1-AT and α_1-ACHY. These observations provide firm evidence for the synthesis and secretion of α_1-AT and α_1-ACHY in the placenta. In this respect, trophoblast differs from monocytes, which express very low levels of α_1-ACHY and resemble MCF-7 cells (*i.e.* breast epithelial cells). It is noteworthy that the α_1-AT and α_1-ACHY secreted by trophoblast have M_r of 50 000 and 49 000, respectively, whereas the α_1-AT and α_1-ACHY secreted by hepatocytes, monocytes, and epithelial cells and present in the circulation have M_r of 54 000 and 68 000, respectively (1, 3, 5). The circulating forms of α_1-AT and α_1-ACHY are highly N-glycosylated (13% and 24% carbohydrate in three and four glycan chains, respectively) and the N-glycans are easily removed with PNGase F (19). Our finding that after deglycosylation the M_r of the α_1-AT and α_1-ACHY produced by trophoblast and HepG2 cells all appear to be the same suggests that the lower M_r of the inhibitors secreted by the trophoblast results from a reduced level of glycosylation. Although there is no evidence for the existence of more than one peptide sequence for either α_1-AT or α_1-ACHY in humans, different RNA splicing patterns in trophoblast resulting in altered peptide sequences cannot be ruled out. The relationship between glycosylation and rate of secretion of α_1-AT and α_1-ACHY by trophoblast and HepG2 cells is consistent with the fact that glycosylation patterns are in many instances tissue-specific (20) and that transit time through the Golgi apparatus can increase with increased glycosylation (21). It is confusing, however, that inhibitors of carbohydrate processing have been shown to retard secretion of α_1-AT and α_1-ACHY in HepG2 cells (22). α_1-AT mRNA has been shown to be transcribed from at least four different initiation sites (16, 17). This apparently can result in different levels of expression under different conditions of up-regulation in different cell types. It is unclear from our data whether α_1-AT transcription in trophoblast follows the hepatocyte or epithelial cell model, although the sizes of the HepG2 and trophoblast mRNA are both smaller than mRNA from MCF-7 cells. Our data suggest that an analogous situation exists for α_1-ACHY. In this instance, α_1-ACHY mRNA from trophoblast and MCF-7 cells are similar in size and smaller than the mRNA from HepG2 cells.

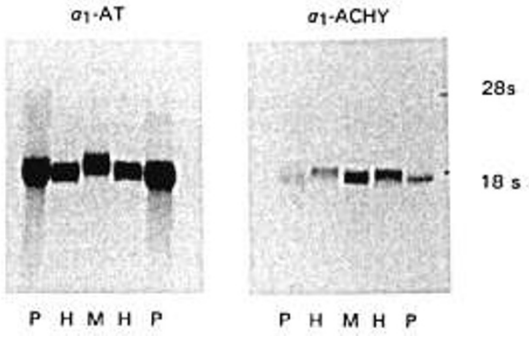

Fig. 6. Comparison of α_1-AT and α_1-ACHY mRNA from trophoblast, HepG2, and MCF-7 cells. Total RNA was electrophoresed on 1.5% agarose/formaldehyde gels, transferred to nylon filters, and hybridized with [^{32}P]-labeled α_1-AT or α_1-ACHY cDNA probes. On the blot hybridized with the α_1-AT probe, lanes contained: *P*, 19.6 μg of trophoblast RNA; *H*, 0.1 μg of HepG2 cell RNA; and *M*, 4.7 μg of MCF-7 cell RNA. On the blot hybridized with the α_1-ACHY probe, lanes contained: *P*, 37.4 μg trophoblast RNA; *H*, 0.33 μg HepG2 cell RNA; and *M*, 16.4 μg MCF-7 cell RNA.

Serpins such as α_1-AT and α_1-ACHY modulate physiologic responses by inhibiting physiologically active proteases. For α_1-AT and α_1-ACHY, these are primarily the proteases released by leukocytes at sites of inflammation. It has been proposed that one such protease, polymorphonuclear neutrophil elastase, releases glucocorticoids from CBG and thus α_1-AT may be involved in the regulation of local glucocorticoid levels. α_1-AT and α_1-ACHY may also regulate local levels of proteases involved in

the activation and release of growth factors, a second feature of the inflammatory response. Possible candidates are an unidentified, elastase-like enzyme that cleaves the 50-amino acid form of transforming growth factor-α from its membrane-bound precursor on the surface of many cell types (23) and a kallikrein-like protease, inhibited by α_1-ACHY *in vitro*, that has been proposed to effect the release of IGF-I from its binding protein (24). In the rat, serum levels of IGFBP-3, the principal IGF-I binding protein, decrease as pregnancy advances because of such a proteolytic cleavage (25).

The ability of the trophoblast to regulate its own growth and the maternal inflammatory response may be important for proper implantation as well as continued growth and function of the placenta. It is possible that synthesis of α_1-AT and α_1-ACHY by the invading trophoblast blocks the maternal defense mechanism and facilitates establishment of a normal placenta. Continued production of α_1-AT and α_1-ACHY may be necessary for modulation of growth factor activity as well as suppression of local inflammation at the decidual cell and vascular interfaces with trophoblast. It may be significant that wide variations in α_1-AT and α_1-ACHY mRNA levels were found among different placentas. Possible correlations between synthesis of α_1-AT and α_1-ACHY and underlying inflammatory conditions or the existence of infection are presently under investigation. Most intriguing is the possible regulation of this process by serpin/protease pairs. Because levels of placental proteases appear to vary during pregnancy (23, 24), protease inhibitors synthesized locally as well as circulating inhibitors produced by the liver may be required for coordinated growth and differentiation of the placenta.

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