Tissue specific expression of the human alpha-1-antitrypsin gene in transgenic mice

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ABSTRACT

Normal and mutant human alpha-1-antitrypsin genes were cloned from a PiMz heterozygous individual. Nucleotide sequence comparison demonstrated a T to C transition in exon III and an G to A transition in exon V of the PiZ gene. A 14.4 kb DNA fragment containing the entire PiM gene plus 2 kb of 5' and 3' flanking genomic DNA sequences was introduced into the germ line of mice and five F₀ transgenic lines were established. Transgenic F₁ progeny from F₀ parents exhibited high levels of human alpha-1-antitrypsin protein in their plasma. The human gene was expressed primarily in liver of the transgenic mice as it is in man. However, expression of the human alpha-1-antitrypsin gene was also observed in kidneys of the transgenic mice, which led to the observation that the endogenous mouse gene is also expressed in the kidney. These data indicate that cis-acting elements within or proximal to the human alpha-1-antitrypsin gene are able to direct its *in vivo* transcription with a high degree of tissue specificity.

INTRODUCTION

Alpha-1-antitrypsin (AAT) is the most abundant serine protease inhibitor in plasma. A major physiological function of the protein is to protect elastic fibers in the lung from hydrolytic destruction by excessive polymorphonuclear leukocyte elastase (1,2). Although most individuals are homozygous for the PiM allele, a mutant PiZ allele exists that is associated with decreased levels of AAT in plasma (3) and a concomitant accumulation of AAT in the rough endoplasmic reticulum of hepatocytes (4,5). Homozygosity for the PiZ allele predisposes individuals to development of pulmonary emphysema (6,7).

Partial amino acid sequence comparison of tryptic and CNBr fragments of the PiM and PiZ AAT proteins has demonstrated the substitution of a glutamic acid residue at position 342 in the PiM protein by a lysine in the PiZ protein (8,9). We have previously reported the cloning of a full-length cDNA for normal human AAT and the determination of its nucleotide sequence (10,11). From that data, we hypothesized that the glutamate to lysine substitution at position 342 in the PiZ protein was the result of a G to A

transition in the corresponding position in the AAT gene. Subsequently, specific oligonucleotides capable of distinguishing PiM and PiZ sequences were developed to detect the mutant allele in genomic DNA of carriers and affected individuals (12), and utilized for prenatal diagnosis of AAT deficiency (13). In this report, the normal and mutant AAT genes were cloned from a heterozygous individual in order to verify the mutation in the gene which is the cause of the deficiency syndrome.

In man, AAT is synthesized primarily in hepatocytes (14) and demonstrates a high degree of tissue specificity with regard to its expression. A recent development in the elucidation of tissue-specific regulation of mammalian gene expression has been the identification of cis-acting regulatory elements within or proximal to genes (15-18). Furthermore, the recent development of introducing cloned genes into the germ line of mice has afforded a method for studying tissue-specific expression of mammalian genes *in vivo*. Tissue-specific expression in transgenic mice has been demonstrated for rat elastase I (19), mouse IgG Kappa light chain (20), rat myosin light chain (21), human β -globin (22,23), α -fetoprotein (24) and IgG heavy chain (25). Thus, the normal human AAT gene was introduced into the germ line of mice in order to identify potential cis-acting elements involved in its tissue-specific transcriptional regulation *in vivo*.

MATERIALS AND METHODS

Enzymes

All DNA modifying enzymes and restriction endonucleases were purchased from New England Biolabs and used according to the supplier's specifications.

Construction and screening of cosmid genomic DNA library

High molecular weight DNA (>150 kb) isolated from cultured lymphoblasts obtained from a PiMZ heterozygote (cell line GM 3579 from the Human Genetic Mutant Cell Repository in Camden, New Jersey) was used to construct a genomic DNA library with the cosmid cloning vector pCV107(26) according to a previously reported procedure (27). Bacterial transformants were grown on nitrocellulose filters (3.5×10^4 colonies/137-mm filter) and 7×10^5 individual colonies were screened as previously described (28) using 2×10^6 cpm/ml of a ^{32}P -radiolabeled human AAT cDNA (sp. act. 2×10^8 cpm/ μg) (10) prepared by nick translation (29). Following hybridization and washing at a stringency of 68°C and 2XSSC, filters were autoradiographed. The specific genotype of each cloned gene was determined

by differential hybridization with M-specific and Z-specific oligonucleotide probes according to a previously reported procedure (12).

DNA sequencing

The human AAT gene is contained within two adjacent 4.8 and 9.6 kb EcoRI fragments and the nucleotide sequence of the entire PiS allele has been reported (11). Recombinant cosmid DNA containing the PiM and PiZ AAT genes were digested to completion with EcoRI and the respective 4.8 and 9.6 kb AAT fragments were isolated and subcloned into the EcoRI site of pCV107 (26). Subcloned genomic DNA fragments were subjected to further enzymatic digestions to generate smaller fragments containing individual exons, treated with E.coli DNA polymerase I (Klenow fragment) and ligated to SmaI-digested M13mp18 (30). Following transformation of TG-1 cells, single-stranded DNA was prepared and used as templates for sequencing by the dideoxynucleotide chain-termination method (31). Exons I, II and III were sequenced using the M13 universal primer, whereas exons IV and V were primed with synthetic oligonucleotides complementary to adjacent intronic sequences.

Preparation of the human AAT gene for microinjection

An 18.6 kb DNA fragment containing the entire human PiM AAT gene was generated by partial EcoRI digestion of a 45 kb cosmid clone and inserted into the EcoRI site of pCV107 (26). Adjacent EcoRI sites, 50 bp apart, within the first intron of the AAT gene (11) were eliminated by subjecting the plasmid to partial EcoRI digestion followed by fractionation in a 0.5% low-melting agarose gel. Full-length linearized molecules were extracted from the gel and treated with E.coli DNA polymerase I (Klenow fragment). Blunt-ended molecules were re-circularized by ligation at low DNA concentration and used to transform E.coli HB101. Bacterial transformants were selected by growth on nitrocellulose filters and screened with a ³²P-labeled human AAT cDNA clone as described above. Plasmid DNA was isolated (32) from hybridizing colonies and digested to completion with EcoRI. DNA was fractionated in a 0.7% agarose gel and transferred to nitrocellulose (33). Filters were hybridized with a ³²P-labeled human AAT cDNA clone and subjected to autoradiography, as described above. Plasmids containing the human AAT gene in which the internal EcoRI sites had been eliminated were identified by a hybridizing 14.4 kb fragment. This fragment contained the entire human AAT gene plus 2.0 and 2.3 kb of 5' and 3' genomic sequences, respectively, and was isolated by extraction from a 0.5% low-melting agarose gel following fractionation of the EcoRI digested plasmid DNA.

Microinjection and transfer of mouse embryos

All mice were purchased from Harlan Sprague Dawley, Houston, Texas. Superovulated female ICR donor mice (4-5 weeks of age) were mated with 3-12 month old C57Bl/6 males. Single cell embryos were flushed from the oviducts of donor mice and the male pronuclei were microinjected with the human AAT gene. Hormonally synchronized female ICR mice previously mated with 4-12 month old ICR males served as recipients for the transfer of viable injected embryos. All manipulations were performed according to the procedure of Brinster, *et al.* (34).

Isolation and analysis of DNA

Pups born from recipient mothers were weaned at 21 days of age and 1 inch of tail was resected from each. Individual tails were disrupted in 4 ml of a solution containing 0.15 M NaCl, 1mM EDTA and 20mM Tris-HCl (pH7.8) using a Polytron homogenizer at setting 7 for 15 sec. SDS and Proteinase K (Sigma) were added at final concentrations of 1% and 0.8mg/ml, respectively, and samples were incubated for 15 hours at 37°C. Genomic DNA was isolated from the homogenates by extraction in phenol/chloroform/isoamyl alcohol (25:24:1), followed by precipitation in 0.3 M NaOAc and 2.5 volumes of cold ethanol.

Approximately 10µg of DNA from each sample were digested to completion with HindIII, fractionated in a 0.8% agarose gel and transferred to nitrocellulose (33). Filters were hybridized as previously described (35) with a nick-translated (29) human AAT cDNA clone (sp. act. 2×10^8 cpm/µg) (10) at a concentration of 2×10^6 cpm/ml. Filters were washed to a stringency of 0.2 x SSC, 0.5% SDS at 68°C and autoradiographed.

Preparation of human AAT-specific antiserum

A polyclonal IgG fraction of goat anti-human AAT was purchased from Cappel. Tests indicated that the antibody preparation had a low level of cross-reactivity with mouse AAT. The cross-reacting immunoglobulins were removed from the antiserum by absorption chromatography over a mouse AAT affinity column prepared by coupling mouse plasma fractions enriched for AAT as described (36) to CNBr-activated Sepharose 4B. The resulting antiserum did not show cross-reactivity with mouse AAT.

Immunoblotting of human AAT protein

Approximately 200µl of blood were collected from anaesthetized mice by orbital vein puncture through heparinized capillaries and centrifuged. Plasma (2.5µl) from each mouse were boiled in 30µl of disruption buffer and electrophoresed in a 10% SDS-polyacrylamide gel (37). Proteins were

electrophoretically transferred to nitrocellulose in 0.025 M Tris, 0.192M glycine and 20% methanol, pH8.3 at 50V for 4 hours. Upon completion of transfer, filters were subjected to immunoblotting (38) using the purified goat anti-human AAT as primary antibody. Swine anti-goat IgG radiolabeled by iodination (39) was used as the secondary antibody.

RNA isolation

Mice were sacrificed by cervical dislocation. Organs were immediately excised, frozen in liquid nitrogen and stored at -80°C until needed. Total RNA was prepared by homogenization of tissues in guanidinium isothiocyanate followed by cesium chloride fractionation as described (40). A final precipitation of the RNA in 2M LiCl_2 was also performed. Human liver was obtained from a renal transplant donor and RNA was isolated as described above.

S1 nuclease assays

Because of the extensive nucleotide sequence homology between coding regions of the human (11) and mouse (41) AAT genes, it was necessary to develop a method that would differentiate human and mouse AAT mRNA transcripts. For the detection of human AAT mRNA, a full-length human AAT cDNA (11) inserted into the EcoRI site of pBR322 was digested with BstEII which cleaved at a unique site within the cDNA. Following treatment with calf intestinal alkaline phosphatase, the 5' termini of the linear molecule were labeled using T4 polynucleotide kinase and $[\gamma\text{-}^{32}\text{P}]\text{ATP}$ (7000 Ci/mmol, ICN). DNA was digested with SalI and a 1.4 kb DNA fragment labeled at the 5' BstEII terminus of the antisense cDNA strand was isolated following fractionation in a 0.8% low-melting agarose gel.

For the detection of mouse AAT mRNA, a 920 bp partial-length mouse AAT cDNA was cloned from a mouse liver cDNA library constructed with $\lambda\text{gt-11}$ (provided by Dr. Hernan Grenett) and inserted into the EcoRI site of pBR322. Following digestion with ClaI and treatment with calf intestinal alkaline phosphatase, the 5' termini of the DNA fragments were labeled as described above. A 1374 bp DNA fragment radiolabeled at the 5' ClaI terminus of the antisense cDNA strand was isolated following digestion with PstI and fractionation in a 0.8% low-melting agarose gel.

For either analysis, 1×10^5 cpm of probe (sp.act, 4×10^6 cpm/ μg DNA) was annealed with total RNA in 40 μl of 1mM EDTA, 0.3M NaCl, 70% formamide and 0.03M Pipes (pH 6.5) by denaturing at 75°C for 15 minutes followed by hybridization at 60°C for 3 hours. Unprotected probe was digested by the addition of 0.32ml of S1 buffer (0.3M NaCl, 2mM ZnCl_2 , 50mM NaOAc, pH6.5),

50µg/ml sheared and denatured herring sperm DNA and 2000 units of S1 nuclease (Miles) followed by incubation at 37°C for 2 hours. Nucleic acids were precipitated with 2.5 volumes of cold ethanol, washed with 70% ethanol and dried under vacuum. Precipitates were dissolved in 20µl of alkali loading dye (0.05 M NaOH, 1mM EDTA, 2.5% Ficoll and 0.025% bromphenol blue) and fractionated in a 1.2% agarose gel in 30mM NaOH and 2mM EDTA at 50V and 4°C. Following electrophoresis, gels were soaked in an excess of 7% TCA for 30 minutes, dried onto Whatman 3MM and autoradiographed.

For quantitation of specific mRNA, radioactive bands were excised and gel pieces were counted by liquid scintillation spectrophotometry in 5 ml of Aquasol (NEN).

RESULTS

Sequence analysis of cloned PiM and PiZ AAT genes

In order to identify all mutations in the PiZ AAT gene that might be responsible for the accumulation of the mutant protein in hepatocytes, PiM and PiZ AAT genes were cloned from a cosmid genomic DNA library constructed with lymphoblast DNA from a PiMZ heterozygous individual. Screening the library with a ³²P-labeled human AAT cDNA resulted in five hybridization signals. Restriction endonuclease mapping and Southern blot analysis of the isolated recombinant cosmid DNA demonstrated that all five clones contained a full-length human AAT gene. Clones of the PiM and PiZ alleles were

Fig. 1. Differentiation of cloned PiM and PiZ AAT genes by hybridization to allele-specific oligonucleotides. DNA (5µg) from each of five cosmid clones containing a human AAT gene were digested with HindIII plus XbaI and electrophoresed in duplicate 1% agarose gels (lanes 1-5). Gels were hybridized with either PiM-specific (panel A) or PiZ-specific (panel B) oligonucleotide probes according to a previously reported procedure (12).

distinguished by differential oligonucleotide hybridization (Fig.1). Results demonstrated that two PiM genes (panel A, lanes 2 and 4) and three PiZ genes (panel B, lanes 1, 3 and 5) had been cloned.

Nucleotide sequence comparison of exonic regions and flanking intron/exon junctions of the two genes demonstrated complete homology with two exceptions. We observed a G to A transition in exon V of the cloned PiZ gene, resulting in the substitution of a GAG codon for glutamate by an AAG codon for lysine (Fig. 2A). The data confirm our previous hypothesis that a G to A transition was responsible for the glutamate to lysine substitution at position 342. In addition, we observed a T to C transition in exon III resulting in the replacement of a valine residue at position 213 in the PiM

Fig. 2.(A) Differences in the nucleotide sequence and encoded amino acid sequence of the cloned PiM and PiZ AAT genes. Results of the comparative sequence analysis of the five exons and flanking intron/exon junctions in the cloned genes are shown. Base transitions and the corresponding amino acid substitutions are depicted by vertical arrows. (B) Schematic representation of the cloned human PiM AAT gene. Exonic regions of the gene are shown in black and are designated by Roman numerals. Long vertical arrows show *EcoRI* restriction sites within and flanking the gene. Adjacent *EcoRI* restriction sites within the first intron (short vertical arrow) were destroyed and a 14.4 kb *EcoRI* fragment containing the entire human PiM AAT gene (shown as a horizontal bar) was generated and microinjected into mouse embryos.

Fig. 3. Identification of transgenic mice by Southern blot analysis. Samples (10 μ g) of mouse tail DNA were digested with HindIII, electrophoresed in a 0.8% agarose gel and transferred to nitrocellulose. Following hybridization with a 32 P-labeled human AAT cDNA and autoradiography, the human PiM AAT gene was detected as hybridizing 6.3 and 2.7 kb bands. Lanes 1-15 contain DNA from potential transgenic F_0 mice. Lanes 16-19 contain 1, 5, 10 and 100 haploid gene equivalents of the HindIII digested cloned human PiM AAT gene, respectively.

protein by an alanine residue in the PiZ protein. No mutations were found in the sequence coding for the leader peptide. This indicates that alterations in this region are not responsible for accumulation of the PiZ protein in the rough endoplasmic reticulum of hepatocytes.

Generation of transgenic mice containing the human AAT gene

To determine if elements within or proximal to the human AAT gene can direct its transcriptional regulation in vivo, a 14.4 kb DNA fragment containing the entire PiM AAT gene plus 2.0 and 2.3 kb of 5' and 3' genomic DNA sequences, respectively, was generated (Fig. 2B). Offspring of mice receiving embryos microinjected with the human PiM AAT gene were screened for the incorporation of the gene into their genome. This was accomplished by Southern blot analysis of tail DNA digested to completion with HindIII and hybridized with a full-length human AAT cDNA probe (Fig. 3). The human AAT gene was identified as hybridizing bands at 6.3 and 2.7 kb. Five transgenic F_0 offspring were produced as shown in lanes 2, 4, 6, 9, and 11 and these mice were designated M2.03, M3.03, M5.00, M10.00 and M11.01, respectively. The intensity of the hybridizing 6.3 and 2.7 kb bands relative to that of known standards of the cloned human AAT gene (lanes 16-19) was used to determine the approximate number of gene copies incorporated into the genome of each transgenic mouse.

Of the five transgenic F_0 mice produced, M2.03, M5.00 and M10.00 appeared to be mosaic based on the transmission of the human AAT gene to less than 16% of their F_1 progeny and demonstration of a higher human AAT

Fig. 4. Detection of human AAT in plasma from transgenic mice by immunoblotting. Lane 1, 0.5 μ l human plasma; lane 2, 1.0 μ l human plasma; lane 3, 2.5 μ l human plasma; lanes 4 and 10, 2.5 μ l normal mouse plasma. Plasma (2.5 μ l) from transgenic F_0 mice M2.03 (lane 5), M3.03 (lane 6), M5.00 (lane 7), M10.00 (lane 8) and M11.01 (lane 9). Plasma (2.5 μ l) from transgenic F_1 mice of the respective F_0 parent; lane 11, #307 (M2.03); lane 12, #216 (M3.03); lane 13, #350 (M5.00); lane 14, #352 (M10.00); lane 15, #366 (M11.01).

gene copy number in transgenic F_1 offspring than in their respective transgenic F_0 parents (data not shown). The frequency of transmission of the human AAT gene to subsequent generations of mice from these lines has followed a typical Mendelian pattern.

Human AAT protein in plasma of transgenic mice

Since AAT is a major plasma protein in humans and mice, we analyzed plasma from transgenic mice for the presence of human AAT. This was performed by immunoblotting of plasma proteins fractionated in an SDS-polyacrylamide gel and electrophoretically transferred to nitrocellulose. Results of an immunoblotting analysis of plasma from the transgenic F_0 mice and a transgenic F_1 offspring from each, are shown in figure 4. Immunoreactive human AAT was present in the plasma of all transgenic F_0 mice that co-migrated with authentic AAT detected in human plasma (lane 1-3). Although all transgenic F_1 progeny from the five pedigrees exhibited human AAT in their plasma (lanes 11-15), transgenic F_1 progeny (lanes 11, 13 and 14) from mosaic F_0 mice exhibited greater plasma concentrations of human AAT than their parents. Human AAT was not detected in plasma from normal mice (lanes 4 and 10). These data indicated that human AAT was synthesized in tissues of the transgenic mice and properly secreted into the bloodstream. Transgenic F_1 offspring from F_0 parents M2.03, M3.03 and M10.00 (lanes 11, 12 and 14) exhibited plasma levels of human AAT equal to or greater than that observed in human plasma (lane 3).

Fig. 5. (A) Strategy for the specific detection of human AAT mRNA. The S1 nuclease protection analysis was performed with 20 μ g samples of RNA. Lanes 1 and 3, wheat germ tRNA without and with S1 nuclease treatment, respectively; lane 2, blank; lane 4, total RNA from human liver; lane 5, total RNA from a non-transgenic mouse liver. ϕ X-174 DNA digested with *Hae*III and end-labeled with 32 P was electrophoresed in the gel and used as size standards. The strategy for the S1 nuclease protection analysis is depicted below the figure. (B) Detection of human AAT mRNA in tissues of transgenic F_1 progeny from F_0 mice M5.00, M11.01 and M3.03. The S1 nuclease protection analysis was performed with 10 μ g of total RNA isolated from liver (lane 1), pancreas (lane 2), spleen (lane 3), kidney (lane 4), reproductive organs (lane 5) and lung (lane 6) from each mouse.

Expression of the human AAT gene in various tissues of transgenic mice

We wished to determine if regulatory elements within or proximal to the human AAT gene could direct its transcription in mice in a tissue-specific manner. Expression of the human gene was evaluated by detection of human

Table 1. Steady-state level of human AAT mRNA in transgenic mouse livers relative to human liver.

Transgenic F ₀ parent	Transgenic F ₁ progeny†	Human AAT mRNA‡ (cpm/μg RNA)	Relative level*
M2.03	#307	764	1.0
	#325	371	0.5
	#327	651	0.8
M3.03	# 65	741	1.0
	#332	914	1.2
	#216	1490	1.9
	#217	1472	1.9
M5.00	#350	151	0.2
M11.01	#366	943	1.2
	#357	1301	1.7
	#362	1091	1.3
Human liver	--	773	1.0

† Ear tag identification number.

‡ Quantitation of protected fragment in human AAT-specific S1 nuclease assay.

* Numbers represent steady-state levels of human AAT mRNA in transgenic mouse livers relative to that detected in human liver.

AAT mRNA in total RNA isolated from various tissues of the transgenic mice. This was accomplished by a specific S1 nuclease protection assay using an end-labeled human AAT cDNA. The specificity of the analysis is shown in figure 5A. Human AAT mRNA was detected as a radiolabeled 700 nt DNA fragment in human liver RNA (lane 4), but was not detectable in RNA from mouse liver (lane 5). Using this analysis, expression of the human AAT gene was analyzed in tissues from transgenic F₁ progeny produced from F₀ mice that exhibited low, medium or high levels of human AAT in their plasma. Results of this analysis are shown in figure 5B. Human AAT mRNA was detected in liver and kidney from a transgenic F₁ offspring from mouse M3.03 (lanes 1 and 4). In addition to these organs, a female transgenic F₁ offspring from mouse M11.01 exhibited a detectable level of human AAT

Fig. 6. (A) Strategy for the specific detection of mouse AAT mRNA. The S1 nuclease protection analysis was performed with 20 μ g samples of RNA. Lanes 1 and 3, wheat germ tRNA without and with S1 nuclease treatment, respectively; lane 2, blank; lane 4, total RNA from human liver; lane 5, total RNA from a non-transgenic mouse liver. ϕ X-174 DNA digested with *Hae*III and end-labeled with 32 P was electrophoresed in the gel and used as size standards. The strategy for the S1 nuclease protection analysis is depicted below the figure. (B) Detection of endogenous mouse AAT mRNA in tissues of the transgenic F_1 offspring from F_0 mouse M5.00 (figure 5B). The S1 nuclease protection analysis was performed with 80 μ g of total RNA isolated from liver (lane 1), pancreas (lane 2), spleen (lane 3), kidney (lane 4), reproductive organs (lane 5) and lung (lane 6). The film was overexposed for the detection of mouse AAT mRNA in kidney.

mRNA in its reproductive organs (lane 5). Expression of the human AAT gene in this organ was limited to offspring from M11.01. Steady-state levels of human AAT mRNA in the transgenic F_1 offspring from mouse M5.00 appeared to be less than that detected in the other mice examined. Although expression of the human AAT gene appeared to be limited to the liver in this mouse, a

longer exposure of the film revealed the presence of a 700 nt fragment in RNA from the kidney (data not shown).

In order to compare the steady-state levels of human AAT mRNA in livers from transgenic F_1 mice to that in human liver, a quantitative S1 nuclease analysis for human AAT mRNA was performed on 5 μ g of liver RNA from several transgenic F_1 progeny and from human liver. Results of this analysis are shown in Table 1. Steady-state levels of human AAT mRNA in livers of transgenic mice were shown to range from 0.2 - 1.9 fold of the level detected in human liver, indicating that the human AAT gene was efficiently expressed in the proper organ of the transgenic animals.

Expression of the endogenous AAT gene in kidneys of mice

The unexpected observation that the human AAT gene was also expressed in the kidneys of transgenic mice led to the investigation of the expression of the endogenous mouse AAT gene in this organ. For the specific detection of mouse AAT mRNA, a 1374 bp mouse AAT cDNA fragment was generated which was labeled at the 5' terminus of the antisense strand of the cDNA. Using this DNA fragment as a probe in an S1 nuclease analysis of mouse liver total RNA, mouse AAT mRNA was detected as a radioactive 620 nt DNA fragment (Fig. 6A, lane 5). No radioactive band was detected in human liver RNA (lane 4), demonstrating that the analysis was specific for mouse AAT mRNA. The S1 nuclease analysis of RNA from tissues isolated from the F_1 transgenic offspring from mouse M5.00 are shown in figure 6B. Overexposure of the film revealed that the endogenous mouse AAT gene was expressed abundantly in liver (lane 1) and to a lesser extent in kidney as shown by a faint band at 620 nt (lane 4). Radioactive bands of smaller size present in all lanes were due to degradation of the probe and were only observed upon overexposure of the film. These data indicate that the endogenous mouse AAT gene was expressed in the same tissues as the human AAT gene.

DISCUSSION

AAT is the major plasma serine protease inhibitor in humans (14). Its function of protecting elastic fibers in the lung from hydrolysis by polymorphonuclear leukocyte elastase is well documented (12). The PiZ AAT allele codes for a mutant protein that accumulates within the rough endoplasmic reticulum of hepatocytes, resulting in reduced levels of AAT in the circulation (3). Homozygosity for the PiZ AAT gene predisposes individuals to the development of pulmonary emphysema (6,7).

In order to determine the cause of the intracellular accumulation of

the PiZ AAT protein, the nucleotide sequence of cloned normal (PiM) and mutant (PiZ) AAT genes were compared. A T to C transition in exon III of the cloned PiZ gene resulted in a conservative valine to alanine substitution at position 213 in the mutant protein. Recently, it was demonstrated that this mutation destroyed an MaeIII restriction site in the AAT gene (42). Using a 6.5 kb AAT genomic DNA fragment as probe, an RFLP analysis that detected the loss of this MaeIII site in the AAT gene was developed. Application of this RFLP analysis to genomic DNA from individuals of known AAT genotype demonstrated that the T to C transition in exon III was not limited to the PiZ AAT gene but was also detected in 13% of PiM1 AAT alleles examined (42). This indicates that the conservative valine to alanine substitution at position 213 in the PiZ AAT protein is not responsible for its accumulation within hepatocytes. In our study, the only other mutation found in the PiZ gene was a G to A transition in exon V which we had previously postulated (12) as the cause of the glutamate to lysine substitution at position 342 in the PiZ protein. Our findings confirm that this mutation is responsible for the PiZ type of AAT deficiency.

A variety of proteins are synthesized primarily in specific cell types in eukaryotes, and little was known about the molecular mechanisms involved in the tissue-specific expression of these genes. Recently however, cis-acting elements within or proximal to mammalian genes have been identified which direct the tissue-specific transcription of these genes (15-18). This finding serves as an important step in understanding the molecular basis of differential gene expression in higher organisms. AAT is synthesized most abundantly in liver (14), indicating that the AAT gene has a high degree of tissue specificity with regard to its transcription. It was recently demonstrated in transient expression assays that DNA elements within 700 bp upstream of the transcription initiation site of the human AAT gene, when fused to the bacterial chloramphenicol acetyltransferase (CAT) reporter gene, directed the expression of CAT activity in human hepatoma Hep3B, but not in HeLa cells (43). This suggests that liver-specific transcription elements for the human AAT gene are located immediately 5' of the gene. Although the transient expression assay using cultured cells as recipients of genetic material functions as a powerful tool for the identification of potential cis-acting elements responsible for liver-specific AAT gene transcription, it fails to provide information concerning the negative regulation of AAT gene expression in a variety of non-hepatic cell types in vivo.

By introducing the cloned human AAT gene into the genome of mice, we were able to determine whether cis-acting elements within the injected DNA fragment would regulate the transcription of the gene in a tissue-specific manner in vivo. The results of our study indicated that the human AAT gene was transcribed, primarily in the livers and kidneys of the transgenic mice, which was identical to the tissue specificity of the endogenous mouse AAT gene. In the mouse M. musculus, AAT mRNA accumulates to a steady-state level of 6000 molecules/cell in liver and only 15-25 molecules/cell in kidney (44,45). These figures correlate well with our observation of low-level expression of the endogenous AAT gene in kidneys of transgenic mice. These data suggest that putative trans-acting elements which normally regulate the transcription of the mouse AAT gene in tissues of mice must also recognize cis-acting elements within the genomic DNA fragment containing the human AAT gene. Furthermore, these putative trans-acting factors may be common to hepatocytes and unique cell types within the kidney. Although it was reported (43) that cis-acting elements upstream from the human AAT gene produced a positive influence on its transcription in human hepatoma cells, these or additional elements within the gene may function as positive or negative transcriptional signals in other cell types in vivo. Additional experiments using the transgenic mouse model should help to elucidate the mechanisms involved in the tissue-specific expression of the human AAT gene.

Detection of human AAT mRNA in kidneys of transgenic mice suggests that the gene may be expressed in the human kidney as well. Comprehensive tissue-specificity studies of AAT gene expression in humans have not been reported. Further experiments must be conducted to determine the possible physiological role of AAT synthesized in the kidney.

The human PiZ AAT gene codes for a protein that accumulates within the rough endoplasmic reticulum of hepatocytes. Now that the human PiZ AAT gene has been cloned, it too can be introduced into the germ line of mice. Results from our present study indicate that the human PiZ gene will also be expressed in the livers of the transgenic mice. If the mutant PiZ AAT protein accumulates within the rough endoplasmic reticulum of hepatocytes in these animals, as it does in man, the molecular basis of its apparent secretory block can be studied.

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