Transforming growth factor-β_1 hyperexpression in African-American hypertensives: A novel mediator of hypertension and/or target organ damage

Manikkam Suthanthiran*, Baogui Li*, Jong O. Song*, Ruchuang Ding*, Vijay K. Sharma*, Joseph E. Schwartz†, and Phyllis August*‡

*Divisions of Nephrology and Hypertension, Department of Medicine, Weill Medical College of Cornell University, and Department of Transplantation Medicine, New York-Presbyterian Hospital, 525 East 68th Street, New York, NY 10021; and †Department of Psychiatry, State University of New York, Stony Brook, NY 11794

Edited by Maria Iandolo New, Cornell University Medical College, New York, NY, and approved January 5, 2000 (received for review October 1, 1999)

Hypertension, a remediable risk factor for stroke, cardiovascular disease, and renal failure, affects 50 million individuals in the United States alone. African Americans (blacks) have a higher incidence and prevalence of hypertension and hypertension-associated target organ damage compared with Caucasian Americans (whites). Herein, we explored the hypotheses that transforming growth factor-β_1 (TGF-β_1) is hyperexpressed in hypertensives compared with normotensives and that TGF-β_1 overexpression is more frequent in blacks compared with whites. These hypotheses were stimulated by our recent demonstration that TGF-β_1 is hyperexpressed in blacks with end-stage renal disease compared with white end-stage renal disease patients and by the biological attributes of TGF-β_1, which include induction of endothelin-1 expression, stimulation of renin release, and promotion of vascular and renal disease when TGF-β_1 is produced in excess. TGF-β_1 profiles were determined in black and white hypertensive subjects and normotensive controls and included circulating protein concentrations, mRNA steady-state levels, and codon 10 genotype. Our investigation demonstrated that TGF-β_1 protein levels are highest in black hypertensives, and TGF-β_1 protein as well as TGF-β_1 mRNA levels are higher in hypertensives compared with normotensives. The proline allele at codon 10 (Pro^{10}) was more frequent in blacks compared with whites, and its presence was associated with higher levels of TGF-β_1 mRNA and protein. Our findings support the idea that TGF-β_1 hyperexpression is a risk factor for hypertension and hypertensive complications and provides a mechanism for the excess burden of hypertension in blacks.

Hypertension, a prevalent and remediable risk factor for stroke, cardiovascular disease, and renal failure, affects 50 million individuals in the United States alone (1). African Americans (blacks) have a higher incidence and prevalence of hypertension compared with Caucasians (whites): 33% of blacks are affected, compared with 25% of whites. Even more striking is the disparity in the prevalence of severe or malignant hypertension; it is estimated to be five to seven times more prevalent in blacks compared with whites (2). Morbidity attributable to hypertension is also more prevalent in blacks. For example, left ventricular hypertrophy was three times more common in blacks compared with whites in the Hypertension Detection and Follow-Up Program (HDFP) (3). After accounting for established baseline risk factors, blacks still have a 38% greater risk of incident ischemic stroke compared with whites, according to the Atherosclerosis Risk in Communities study (4). End-stage renal disease (ESRD), a devastating medical illness of extraordinary personal suffering and societal costs, is four times more frequent in blacks, and ESRD attributable to hypertension is 20 times more frequent in blacks (5). Data from the Multiple Risk Factor Intervention Trial demonstrated that for every level of blood pressure control, blacks are at increased risk for the development of renal disease (6). Finally, and perhaps most disturbing, are the observations that aggressive blood pressure control has not uniformly led to similar renal-protective benefits in blacks and whites (7, 8).

Genetically determined renal and hormonal characteristics as well as several environmental influences have been proposed as contributing to the excess burden of hypertension in blacks. To date there is no unifying hypothesis for the overabundance of hypertensive diseases in the black population.

Transforming growth factor-β_1 (TGF-β_1) is a 25-kDa homodimeric protein secreted by several cell types, including peripheral blood mononuclear cells (PBMC), endothelial cells, vascular smooth muscle cells, platelets, and also renal cells (9, 10). It facilitates extracellular matrix assembly by stimulation of its synthesis and prevention of matrix degradation and is important in tissue repair and remodeling. Overproduction, on the other hand, is an important mechanism for fibrogenesis (9, 10). TGF-β_1 has been implicated in several of the long-term chronic sequelae of poorly controlled hypertension, including left ventricular hypertrophy (11), hypertensive vascular remodeling (12), and ESRD (13). Angiotensin II is a known stimulus of TGF-β_1 production; thus, TGF-β_1 may be an important mediator of angiotensin II-induced hypertensive damage (14). Although much of TGF-β_1's biological action is mediated in an autocrine or paracrine fashion, data from TGF-β_1 transgenic mice have demonstrated that high-circulating levels can mediate renal fibrosis and progressive loss of function (15).

Recent data also suggest that TGF-β_1 may have a direct pathogenic role in elevated blood pressure. TGF-β_1 stimulates the expression of mRNA encoding endothelin-1 in vascular endothelial cells (16) and also increases renin release from juxtaglomerular cells in the kidney (17).

We have demonstrated that circulating levels of TGF-β_1 are higher in blacks with ESRD compared with white ESRD patients and that TGF-β_1 levels are also correlates of blood pressure in patients with ESRD (18, 19). We hypothesized that the increased frequency of hyperexpression of TGF-β_1 may contribute to the excess burden of ESRD in blacks. We also have suggested that hyperexpression of TGF-β_1 might be genetically determined, because DNA polymorphisms in codon 25 of the TGF-β_1 gene have been associated with hypertension (20) and codon 10 polymorphisms have been associated with lung fibrosis (21).

In this study, we explored the hypotheses that, independent of renal disease, TGF-β_1 is hyperexpressed in hypertensives compared

This paper was submitted directly (Track II) to the PNAS office.

Abbreviations: ESRD, end-stage renal disease; TGF-β_1, transforming growth factor-β_1; PBMC, peripheral blood mononuclear cells; ARMS-PCR, amplification-refractory mutation system–PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

‡To whom reprint requests should be addressed at: Division of Hypertension, Weill Medical College of Cornell University, 525 East 68th Street, Box 159, New York, NY 10021. E-mail: paugust@mail.med.cornell.edu.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Article published online before print: *Proc. Natl. Acad. Sci. USA*, 10.1073/pnas.050420897. Article and publication date are at www.pnas.org/cgi/doi/10.1073/pnas.050420897

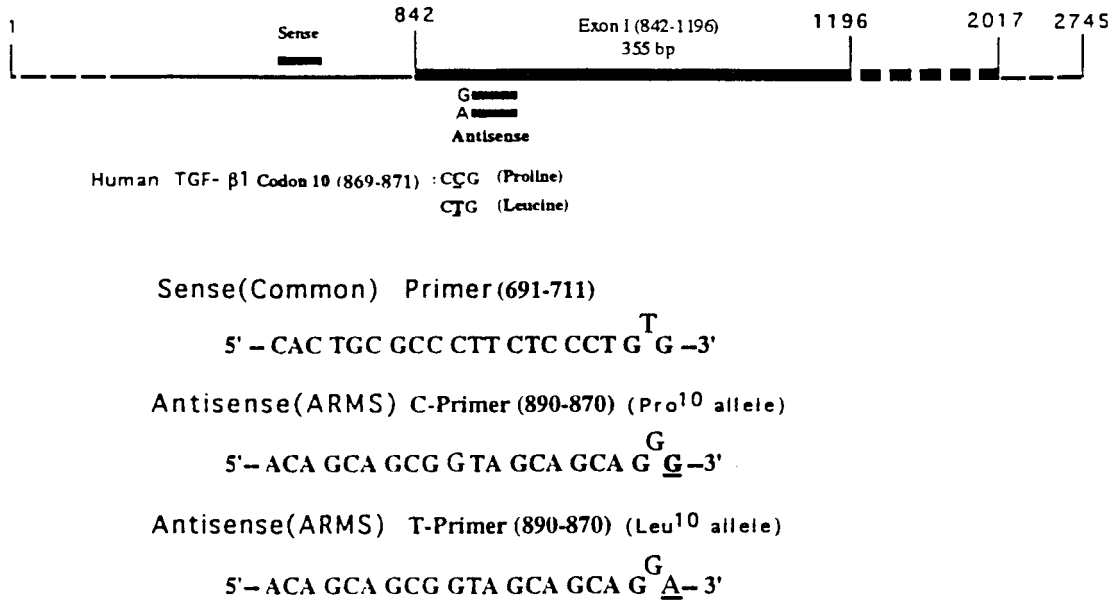

Fig. 1. Design, location, and sequences of the oligonucleotide primers for the detection of codon 10 polymorphisms by ARMS-PCR. A penultimate mismatch was introduced into the sense (common) primer as well as into the allele-specific antisense ARMS primers.

with normotensives and that TGF-β_1 hyperexpression is more frequent in blacks compared with whites. Our postulate was tested by evaluating TGF-β_1 profiles in hypertensive and normotensive subjects. TGF-β_1 profiling included determination of circulating levels of TGF-β_1 protein, quantification of steady-state levels of TGF-β_1 mRNA in PBMC, and analysis of TGF-β_1 DNA codon 10 polymorphisms in hypertensive and normotensive subjects.

Methods

Subjects. Hypertensive subjects were recruited from the outpatient clinic at the Hypertension Division of Weill Medical College of Cornell University. Subjects were included if blood pressure (average of three determinations) was greater than 140/90 mmHg (1 mmHg = 133 Pa) on at least two occasions if patients were off medications. For patients taking antihypertensive medication, records were reviewed to ascertain the validity of the diagnosis of hypertension, and patients were included if blood pressure had been greater than 140/90 mmHg on two occasions in the past, before treatment. Patients with renal insufficiency and/or diabetes (serum creatinine $\geq$2 mg/dl) were excluded. Normal subjects were recruited from hospital employees and were required to have a blood pressure of $<$140/90 mmHg on three determinations. Blood pressure was measured in the seated position according to American Heart Association guidelines (22). Individuals with significant comorbidity (cancer, autoimmune disease, or active infection) were excluded. The protocol was approved by the Committee for Human Rights in Research at Weill Medical College of Cornell University. Oral consent was obtained for collection of venous blood.

Evaluation of TGF-β_1 Profile. TGF-β_1 profiling consisted of measurement of serum TGF-β_1 protein levels, quantification of mRNA in PBMC, and genotyping of codon 10. Peripheral venous blood was obtained and sera were isolated and stored at -70°C until assayed for TGF-β_1 protein. PBM were isolated by Ficoll/Hypaque centrifugation; DNA was extracted from leukocytes for TGF-β_1-genotyping analysis.

TGF-β_1 codon 10 genotyping by amplification-refractory mutation system–PCR (ARMS-PCR). ARMS-PCR is an allele-specific PCR that permits the ready detection of single-nucleotide polymorphisms (23, 24). Our design of ARMS primers for the identification of codon 10 genotypes is based on the principle that oligonucleotides that are complementary to a target DNA sequence except for a mismatched 3′ terminus will not function as PCR primers (Fig. 1). Our primers for codon 10 genotyping also were designed to include a penultimate mismatch. This inclusion enhances further the stringency of ARMS-PCR. Two complementary reactions were established for each allele and consisted of target DNA, allele-specific ARMS primer, and the common primer. PCR products were resolved by agarose gel electrophoresis. A thermal cycler was used, and each experiment had negative and positive controls for the target allele.

Quantification of TGF-β_1 mRNA. A 347-bp TGF-β_1 competitor DNA template was constructed for quantification of TGF-β_1 mRNA by competitive quantitative PCR. In brief, an oligonucleotide primer pair was designed to amplify a region in the TGF-β_1 gene that contains a *Sau*3A1 restriction site. The *Sau*3A1 digestion of the 255-bp TGF-β_1 PCR product yielded 168- and 87-bp subfragments that were annealed with a 92-bp DNA insert synthesized *in vitro* to have cohesive ends for the *Sau*3A1 restriction site at 5′ and 3′ ends. The phosphorylated 92-bp DNA fragment was ligated with the 168- and 87-bp DNA fragments by using *Escherichia coli* DNA ligase. After ligation, the mixture was run on a 2% low-melting-point agarose gel, and the 347-bp fragment was eluted and purified.

The cDNA for quantification by competitive PCR was synthesized in a reverse transcription reaction mixture containing 1 μg of total RNA (isolated with the Qiagen RNEasy Kit; Qiagen, Chatsworth, CA), 100 ng of random hexanucleotide primers, and 200 units of Moloney murine leukemia virus reverse transcriptase (GIBCO/BRL). A constant amount of the cDNA then was coamplified with known concentrations of the competitor DNA construct for 32–36 cycles by using TGF-β_1 gene-specific primer pairs (0.2 μM each) in a reaction mixture containing 1$\times$ *Taq* buffer, 1 unit of *Taq* DNA polymerase, and 40 μM of each dNTP. The PCR products were resolved by 2% agarose gel electrophoresis, visualized by ethidium bromide staining, and photographed. The negative of the photographs was analyzed by laser densitometry, and the absolute absorbance values of the PCR products were determined. The ratios of the absorbance of the

relevant PCR product pairs (347-bp TGF-β_1 competitor and 255-bp TGF-β_1) were plotted against the concentrations of the competitor DNA used. The concentrations of mRNA thus were determined and expressed as attograms of TGF-β_1 mRNA per μg of total RNA. mRNA encoding a housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (GAPDH), was quantified by using GAPDH-specific competitor template (25).

Measurement of TGF-β_1 protein levels. The biologically active TGF-β_1 protein concentration was determined by using a solid-phase TGF-β_1-specific sandwich ELISA (Promega) as described (26). The sera were activated by acidification and tested at 1:200 dilution. A TGF-β_1 standard curve was constructed by using 1,000, 500, 250, 62.5, 31.25, and 15.6 pg/ml recombinant TGF-β_1 protein, and a curve-fitting software program was used to quantify TGF-β_1 protein concentration in the sera. The minimum level of detection of TGF-β_1 with the sandwich ELISA was 25 pg/ml.

Statistical Analysis. SAS (STATISTICAL ANALYSIS SOFTWARE 6.1) was used for data analysis (27). One-way ANOVA was used for the comparison of multiple groups, and corrected (Bonferroni) P values were calculated to correct for type I experiment-wise error rate. A multiple regression analysis was used to estimate the independent effect of race on TGF-β_1 protein levels controlling for gender and age. A multiple logistic regression analysis was used to estimate the effect of TGF-β_1 protein levels on hypertension status controlling for race, gender, and age. The Mann–Whitney two-sample test was used to compare continuous variables, and categorical variables were analyzed by χ^2 analysis.

Results

TGF-β_1 Protein Concentration. Quantification of TGF-β_1 levels in sera obtained from hypertensive subjects and normotensive controls with a TGF-β_1-specific ELISA demonstrated that TGF-β_1 levels are higher in hypertensives compared with normotensives. The mean ± SEM TGF-β_1 protein concentration was 261 ± 9 ng/ml in the hypertensive subjects (n = 61) and was 188 ± 7 ng/ml in the normotensive controls (n = 90) ($P <$ 0.0001, Mann–Whitney two-sample test, Fig. 2*A*).

Fig. 2*B* compares TGF-β_1 protein levels across diagnosis (hypertensive or normotensive) and race (black or white). TGF-β_1 levels were the highest in black hypertensives, and comparison of the mean TGF-β_1 levels across the four groups demonstrated that the null hypothesis of equal group means should be rejected ($P < 0.0001$, ANOVA, Fig. 2*B*).

Bonferroni P values were calculated to correct for type 1 experiment-wise error rate in view of comparisons across multiple groups. The multiple comparisons showed that the TGF-β_1 protein levels in black hypertensives (322 ± 16 ng/ml, n = 18) were higher than that in white hypertensives (235 ± 9 ng/ml, n = 43, $P < 0.001$), black normals (221 ± 12 ng/ml, n = 39, $P <$ 0.001), and white normals (165 ± 6 ng/ml, n = 51, $P < 0.001$). The analysis also showed that TGF-β_1 protein levels in black normals were significantly higher than those in white normals ($P < 0.001$), but not different from those in white hypertensives ($P > 0.05$).

The frequency distribution of TGF-β_1 levels in the black and white normotensive and black and white hypertensive subjects is shown in Fig. 2*C*. Whereas 66.6% of the black hypertensives had TGF-β_1 levels greater than 300 ng/ml, only 16.2% of white hypertensives had levels greater than 300 ng/ml ($P < 0.00009$, Fisher's exact test).

A multiple regression analysis was used to estimate the independent effects of race (black vs. white) on TGF-β_1 protein levels, controlling for gender and age. Table 1 shows that race is a highly significant predictor of TGF-β_1 levels. In this analysis, neither gender nor age was a significant determinant of TGF-β_1 protein levels.

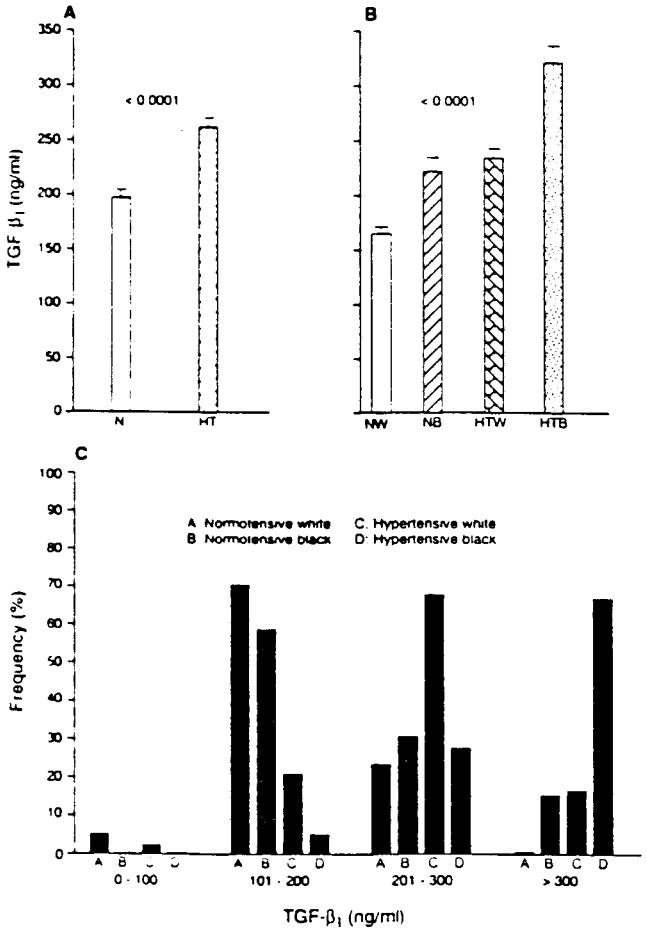

Fig. 2. Serum TGF-β_1 protein levels. Circulating levels of TGF-β_1 protein were quantified by using a TGF-β_1-specific sandwich ELISA. TGF-β_1 protein levels, distinguished by diagnosis (hypertensive vs. normotensive) are shown in *A*. The mean ± SEM TGF-β_1 level was 188 ± 7 ng/ml in normotensives (N) and was 261 ± 9 ng/ml in hypertensives (HT). TGF-β_1 protein levels, distinguished by diagnosis as well as by race, are illustrated in *B*. The mean ± SEM TGF-β_1 concentrations were 165 ± 6 ng/ml, 221 ± 12 ng/ml, 235 ng/ml, and 322 ng/ml in normotensive whites (NW), normotensive blacks (NB), hypertensive whites (HTW), and hypertensive blacks (HTB), respectively ($P < 0.0001$, ANOVA). The frequency distribution of TGF-β_1 levels, distinguished by race and diagnosis, is shown in *C*.

A multiple logistic regression analysis then was used to estimate the effect of TGF-β_1 protein levels on hypertension status controlling for race, gender, and age. This analysis demonstrated that TGF-β_1 protein concentration is a significant predictor of diagnosis (P = 0.004). The estimated odds of an individual at the 75th percentile of TGF-β_1 distribution (285 ng/ml) being hypertensive was 234% greater than that of an individual at the 25th percentile (187 ng/ml).

TGF-β_1 mRNA Steady-State Levels. Quantification of TGF-β_1 mRNA levels in PBMC obtained from hypertensive subjects and

Table 1. Race predicts TGF-β_1 levels

Parameter	Estimate	SE	T	*P*
Intercept	205.86	27.80	7.40	0.0001
Blacks vs. whites	80.46	14.59	5.52	0.0001
Female vs. male	0.84	14.23	0.06	0.95
Age	0.31	0.47	0.65	0.52

R^2 = 0.260.

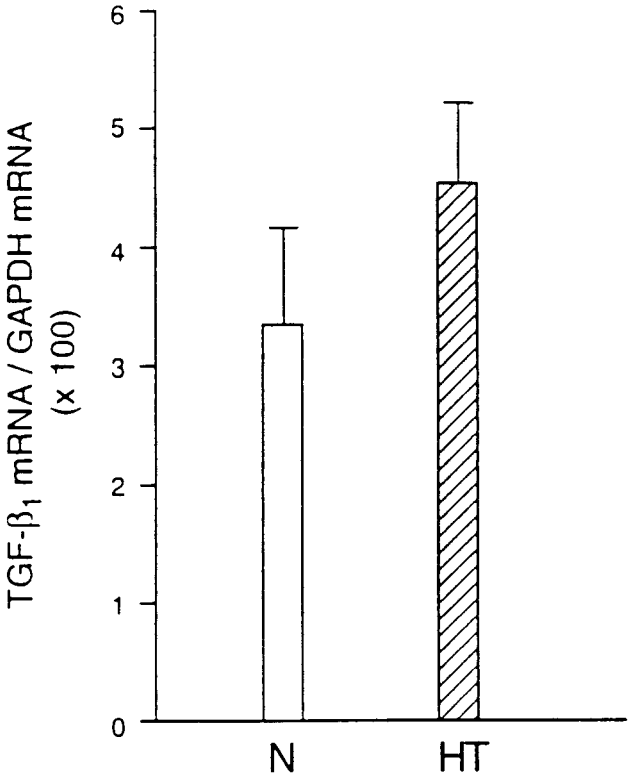

Fig. 3. TGF-β_1 mRNA steady-state levels in hypertensives and normotensives. The mean ± SEM TGF-β_1 mRNA/GAPDH mRNA ratios, calculated after quantification of mRNA levels in PBMC, were 3.35 ± 0.81 and 4.51 ± 0.69 in normotensives (N) and hypertensives (HT), respectively ($P < 0.04$, Mann–Whitney two-sample test).

normotensive controls showed that TGF-β_1 mRNA was expressed at a significantly higher level in PBMC of hypertensive subjects ($n = 55$) compared with PBMC of normotensive controls ($n = 41$) (Fig. 3, $P < 0.04$, Mann–Whitney two-sample test). In a fashion similar to TGF-β_1 protein levels, black hypertensives had the highest level of TGF-β_1 mRNA. However, there was no significant difference in the TGF-β_1 mRNA steady-state levels between black hypertensives and white hypertensives.

TGF-β_1 Codon 10 Genotypes. A total of 124 individuals were genotyped for TGF-β_1 codon 10 by using the ARMS-PCR primers illustrated in Fig. 1. TGF-β_1 codon 10 genotype of blacks differed significantly from that of whites in that there was an excess of proline allele (C = proline; T = leucine) at codon 10 (Pro^{10}) in blacks compared with whites. The race-based difference in the relative frequencies of T/T, T/C, and C/C genotypes was significant (Table 2, likelihood ratio χ^2 analysis, 8.67, $P =$

Table 2. TGF-β_1 codon 10 genotypes of blacks and whites

Race/diagnosis	(*n*)	T/T	T/C	C/C
Blacks/normal	(23)	13.04% (3)	56.52% (13)	30.43% (7)
Whites/normal	(35)	34.29% (12)	48.57% (17)	17.14% (6)
Blacks/HT	(21)	19.05% (4)	52.38% (11)	28.57% (6)
Whites/HT	(45)	37.78% (17)	51.11% (23)	11.11% (5)

TGF-β_1 codon 10 genotypes were determined by using ARMS-PCR. The race-based difference in the relative frequency of T/T, T/C, and C/C genotypes was significant at $P = 0.01$.

Table 3. Correlation between codon 10 genotypes and TGF-β_1 mRNA levels

Codon 10 genotype	TGF-β_1 mRNA/GAPDH mRNA, mean ± SEM	*P*
C/C + T/C	5.06 ± 0.30	
T/T	3.53 ± 0.46	0.0074

TGF-β_1 mRNA levels and GAPDH mRNA levels were quantified by using gene-specific competitor constructs in competitive PCR and correlated with codon 10 genotype. mRNA steady-state levels (log-transformed) are from 87 individuals (34 white hypertensives, 16 black hypertensives, 26 white normotensives, and 11 black normotensives), who also were genotypes for codon 10. *P* value for the *F* statistic of a one-way ANOVA is shown.

0.017). In blacks as well as whites, no significant deviation in codon 10 frequencies from Hardy–Weinberg expectation was observed.

Correlations Between TGF-β_1 Genotype and Expression. TGF-β_1 mRNA levels were higher in those genotyped as C/C or T/C at codon 10 compared with those genotyped as T/T. In 87 individuals (34 white hypertensives, 16 black hypertensives, 26 white normotensives, and 11 black normotensives) in whom codon 10 genotype and TGF-β_1 mRNA levels were determined, the mean ± SEM TGF-β_1 mRNA level in individuals genotyped as C/C or T/C was 5.06 ± 0.30 and was 3.53 ± 0.46 in those genotyped as T/T at codon 10 (Table 3, $P < 0.0074$, ANOVA).

TGF-β_1 protein concentrations were also higher in those genotyped as C/C or T/C at codon 10 compared with those genotyped as T/T. TGF-β_1 protein levels were 16.25 ng/ml higher in those with the proline allele at codon 10 compared with those homozygous for the leucine allele at codon 10.

Discussion

The findings from this investigation are that TGF-β_1 is overexpressed in hypertensive subjects compared with normotensive controls and that hyperexpression is more frequent in blacks compared with whites. Whereas a cause-and-effect relationship cannot be established from a cross-sectional study, our data regarding TGF-β_1 at the levels of DNA, mRNA, and protein suggest a candidate mechanism for hypertension in humans and for the excess burden of hypertension and hypertensive complications in blacks.

Increased Frequency of Pro^{10} Allele in Blacks. To date, seven single-nucleotide polymorphisms in the human TGF-β_1 gene have been identified (20). These include three single-base substitutions in the upstream region of the TGF-β_1 gene at positions −988 (C → A), −800 (G → A), and −509 (C → T); one in a nontranslated region at position +72 (C insertion); two single-base substitutions in the signal sequence at positions +869 (codon 10, T → C, Leu → Pro) and +915 (codon 25, G → C, Arg → Pro); and one in codon 263 (C → T, Thr → Ile). Strong linkage disequilibrium among six of the seven polymorphisms has been reported. Polymorphisms located in the signal peptide region regulate translation and/or vectorial transport of proteins (28). In view of our findings that TGF-β_1 protein levels distinguished blacks from whites, we explored the hypothesis that codon 10 alleles, located in the signal peptide region of the TGF-β_1 gene, would distinguish blacks from whites. Our focus on the TGF-β_1 codon 10 polymorphism also was informed by the observation that codon 10 polymorphisms are correlates of pulmonary fibrosis, a potential consequence of TGF-β_1 overexpression (21). That codon 10 genotyping would be informative also was suggested by an earlier observation that codon 25 alleles (also located in the signal peptide region) are correlates of blood pressure (20) and by our earlier demonstration that the G/G

genotype at codon 25 is numerically higher in hypertensives compared with normotensives (19). Also, the reported allele frequencies at codon 10 permit appreciation of frequency differences between study populations by using a modest sample size.

The frequencies of T/T, T/C, and C/C genotypes found in our white subjects were similar to the frequencies observed in an earlier study of 1,192 white Europeans (20). The frequencies in our black subjects were different from that in the earlier study. We are unaware of studies of TGF-β_1 DNA polymorphisms in black subjects and, thus, were unable to compare our data with those of others. The number of blacks genotyped in our study for codon 10, however, is small ($n = 44$). A larger study is required to better establish the race-dependent differences in TGF-β_1 allele frequencies.

Our investigation also identified higher TGF-β_1 mRNA steady-state levels in those with a Pro^{10} allele. Whereas a direct cause-and-effect relationship between codon 10 alleles and steady-state levels of TGF-β_1 mRNA remains unproved at this time, it can be hypothesized that codon 10 is important in the regulated expression of the TGF-β_1 gene, because polymorphisms at this location (signal sequence region) can influence protein secretion and TGF-β_1 can regulate its own transcription via AP-1 sites located in its promoter (29). This idea also is supported by our finding that TGF-β_1 protein concentrations are higher in those with the Pro^{10} allele compared with those homozygous for the Leu^{10} allele.

TGF-β_1 mRNA Hyperexpression in Hypertensives. A recent study of 22 hypertensive subjects with myocardial hypertrophy found that monocytes obtained from these patients expressed higher levels of mRNA encoding TGF-β_1 compared with normal controls (30). Our observation that TGF-β_1 mRNA steady-state levels are higher in hypertensive subjects without overt target organ damage compared with normotensive controls extends this earlier observation and suggests that TGF-β_1 hyperexpression precedes the development of hypertensive complications.

TGF-β_1 mRNA steady-state levels distinguished hypertensives from normotensives but not blacks from whites, unlike protein levels, which distinguished not only hypertensives from normotensives, but also blacks from whites. One potential explanation for the discordance might be the smaller sample size of the mRNA studies compared with protein determinations. An additional explanation, and one that we favor, is that multiple cell types, in addition to PBMC, contribute to circulating levels of TGF-β_1 protein levels and prevent a strict correlation between PBM mRNA levels and TGF-β_1 protein concentrations. Indeed, in our earlier study of cyclosporine-induced TGF-β_1 hyperexpression we found a similar lack of correlation between circulating levels of TGF-β_1 protein and PBMC TGF-β_1 mRNA (31).

Race Is an Independent Predictor of TGF-β_1 Protein Level, and TGF-β_1 Levels Correlate with Hypertension Status. In our earlier investigation, we found that TGF-β_1 protein levels are higher in black patients with ESRD (18) compared with white patients with ESRD. In that study confined exclusively to ESRD patients, TGF-β_1 levels also correlated with blood pressure. The current investigation extends these observations in a number of important ways and demonstrates, without the confounding variable of ESRD, that race is an independent predictor of TGF-β_1 levels. In the current study, TGF-β_1 levels were higher in blacks compared with whites, and the odds of being hypertensive were significantly higher in those with TGF-β_1 levels in the 75th percentile compared with those in the 25th percentile. In view of the biological attributes of TGF-β_1, a novel mechanism for the higher incidence and prevalence of hypertension and hypertension-associated target damage in blacks, is advanced by our finding that race predicts TGF-β_1 protein levels and TGF-β_1 levels are correlates of hypertension status.

How Might TGF-β_1 Contribute to the Pathogenesis of Hypertension and Cardiovascular and Renal Complications? We propose that TGF-β_1 may contribute to the pathogenesis of hypertension by directly causing elevated blood pressure and/or by causing fibrosis and vascular injury. That TGF-β_1 can raise blood pressure is supported by the observation that TGF-β_1 stimulates the expression of endothelin-1, a potent vasoconstrictor (16). In this regard, it is worth noting that plasma endothelin-1 levels are higher in black hypertensives compared with white hypertensives (32). TGF-β_1 also has been reported to stimulate renin release from juxtaglomerular cells, which may contribute to increased angiotensin II generation and blood pressure (17). An autoamplification loop that involves TGF-β_1, endothelin-1, and the pressor peptide angiotensin II also can be envisioned in view of the observation that angiotensin II stimulates TGF-β_1 expression. The observation that angiotensinogen levels are higher in blacks compared with whites and that the angiotensinogen promoter polymorphism (G/G at position -6), which correlates with a lower transcriptional rate, is more frequent in whites compared with blacks also may be relevant with respect to the interactions between the renin angiotensin system and TGF-β_1. For example, increased angiotensinogen levels may generate excess angiotensin II, which then would lead to increased TGF-β_1.

Hypertensive vascular pathology is a multicellular process, and fibrocellular hyperplasia and vascular smooth muscle proliferation and migration are some of the cardinal histologic features. In this context, it is worth noting that direct transfer of TGF-β_1 gene into arteries results in fibrocellular hyperplasia (33) and that this multifunctional cytokine also can induce vascular smooth muscle cell hypertrophy (34). Transdifferentiation of vascular smooth muscle cells into chondrocytes and intimal growth also have been observed after transfection of the TGF-β_1 gene into murine arteries (35). Differential sensitivity of vascular cells to TGF-β_1 signaling also might be relevant because TGF-β_1 is reported to potentiate platelet-derived growth factor (PDGF)-induced proliferation of vascular smooth muscle cells of the spontaneously hypertensive rat but not the vascular smooth muscle cells of the normotensive Wistar–Kyoto rat (36).

The biologic consequences of TGF-β_1 are most likely realized by its acting in unison with other growth factors such as PDGF and fibroblast growth factor (9). In this context, it is worth noting that endothelin-1 and angiotensin II, each of which is regulated by TGF-β_1, function not only as vasoconstrictors but also as growth factors for vascular cells (37–39).

Angiotensin II as well as other pressor agents also might regulate TGF-β_1 expression via physical forces because shear stress can heighten TGF-β_1 expression (40). TGF-β_1 overexpression has been observed in the aorta of deoxycorticosterone-salt-sensitive, hypertensive rats (12, 41), and cardiac myocytes, subjected to the hypertrophic stimulus of abdominal aortic constriction or norepinephrine infusion, display TGF-β_1 hyperexpression (42). On the other hand, prevention of myocardial hypertrophy with angiotensin-converting enzyme inhibitors is associated with decreased TGF-β_1 expression (43).

A well-documented property of TGF-β_1 is its ability to induce fibrosis and promote accumulation of extracellular matrix (9, 10, 44). TGF-β_1-induced fibroblast proliferation, stimulation of collagen synthesis, and enhanced matrix accumulation contribute to the development of vascular disease as well as progressive renal disease.

Direct evidence that TGF-β_1 expression can engender renal disease exists. In a rat model of glomerulonephritis, intrarenal expression of TGF-β_1 was associated with increased extracellular matrix and anti-TGF-β_1 antibodies prevented matrix assembly (44).

In a diabetic mouse model, anti-TGF-β_1 antibodies constrained TGF-β_1 mRNA hyperexpression and prevented glomerular hypertrophy (45). *In vivo* transfection of the TGF-β_1 gene resulted in glomerulosclerosis (46), and a TGF-β_1 antisense oligomer reduced intrarenal expression of TGF-β_1 and suppressed extracellular matrix accumulation (47). Of particular relevance to our observations of higher levels of TGF-β_1 in black hypertensives, high-circulating levels of TGF-β_1 protein have been reported to lead to fibrotic disease of kidneys and other organs in an experimental glomerulonephritis model. In TGF-β_1 transgenic mice, high-circulating levels were associated with mesangial expansion, glomerular immune deposits, extracellular matrix accumulation, interstitial fibrosis, and progressive renal disease (15). Indeed, 25% of the transgenic mice died of renal failure.

Increased Frequency of TGF-β_1 Hyperexpression as a Pathogenetic Mechanism for Excess Hypertension in Blacks. Genetic as well as socioenvironmental factors have been proposed to explain the heightened incidence of hypertension and hypertensive complications in blacks, and both are likely to be important. Polymorphisms in several candidate genes for hypertension such as angiotensinogen, angiotensin-converting enzyme, α_2-adrenergic receptor, and polymorphisms in genes coding mitochondrial proteins all have been reported to be more frequent in blacks compared with whites (48). Social, psychological, and cultural variances also have been explored, but to date, there is no consensus regarding the relative importance of any of these factors.

We hypothesize that TGF-β_1 hyperexpression is a pathogenetic mechanism for the excess burden of hypertension and hypertensive complications in blacks compared with whites. The biological attributes of TGF-β_1 and the reported association between TGF-β_1 overexpression and cardiovascular and renal pathology support the postulate that the increased frequency of TGF-β_1 overexpression in blacks is a candidate mechanism for hypertension and hypertensive complications, particularly the progression of hypertensive renal disease to ESRD.

An intriguing aspect of hypertension and progression to renal failure in blacks is that aggressive blood pressure control does not always lead to similar renal benefits in blacks and whites. Rostand *et al.* (7) have reported that renal function deteriorated more rapidly in hypertensive black patients compared with hypertensive whites despite similar levels of blood pressure control. Perry *et al.* (8) have noted a 2-fold higher risk of ESRD among black hypertensives compared with their white counterparts. The Multiple Risk Factor Intervention Trial study has shown that for every level of blood pressure control, blacks are at an increased risk for the development of renal disease compared with whites with the same blood pressure (6). These observations together suggest that other risk factor(s), in addition to hypertension, contribute to hypertension-associated renal disease in blacks.

In view of the experimental evidence that TGF-β_1 hyperexpression results in renal disease, and in light of our data, we suggest that the increased frequency of TGF-β_1 hyperexpression contributes to the excess burden of ESRD in blacks and, particularly, to ESRD attributed to hypertensive disease.

In summary, our cross-sectional studies have identified that TGF-β_1 is overexpressed in hypertensive subjects compared with normotensive controls and that hyperexpression is more prevalent in blacks compared with whites. The protean biological activities of TGF-β_1 and the experimental and clinical data suggest that TGF-β_1 hyperexpression is a candidate mechanism for hypertension. Our observations support the idea that the heightened frequency of TGF-β_1 overexpression in blacks contributes to the excess burden of hypertension and hypertensive complications in blacks. Further exploration of the contribution of TGF-β_1 to hypertension and hypertensive complications in a prospective investigation might provide new insights into mechanisms of hypertension as well as suggest novel therapeutic targets.

We are grateful to Ms. G. Helseth for her diligent efforts toward sample collection and Ms. L. Stackhouse for meticulous assistance in the preparation of this manuscript. This work was supported in part by an award from the National Institutes of Health (R01 DK54943).

1. Burt, V. I., Whelton, P., Rocella, E. J., Brown, C., Cutler, J. A., Higgens, M., Horan, M. J. & Labarthe, D. (1995) *Hypertension* **25,** 305–313.
2. Lackland, D. T. & Keil, J. E. (1996) *Semin. Nephrol.* **16,** 63–70.
3. Taylor, J. O., Borhani, N. O., Entwisle, G., Farber, M. & Hawkins, C. M., on behalf of the HDFP Cooperative Group (1983) *Hypertension* **5,** Suppl., IV-44–IV-50
4. Rosamond, W. D., Folsom, A. R., Chambless, L. E., Wang, C. H., McGovern, P. G., Howard, G., Copper, L. S. & Shahar, E. (1999) *Stroke* **30,** 736–743.
5. U. S. Renal Data System (USRDS) (1998) *1998 Annual Data Report* (The National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, Bethesda, MD).
6. Klag, M. J., Whelton, R. K., Randall, B. L., Neaton, J. D., Brancati, F. L. & Stamler, J. (1997) *J. Am. Med. Assoc.* **277,** 1293–1298.
7. Rostand, S. G., Brown, G., Kirk, K. A., Rutsky, E. A. & Dustan, H. P. (1989) *N. Engl. J. Med.* **320,** 684–688.
8. Perry, H. M., Miller, J. P., Fornoff, J. R., Baty, J. D., Sambhi, M. P., Rutan, G., Moskowitz, D. W. & Carmody, S. E. (1995) *Hypertension* **25,** 587–594.
9. Roberts, A. M. & Sporn, M. B. (1988) *Adv. Cancer Res.* **51**, 107–145.
10. Massagne, J. (1990) *Annu. Rev. Cell Biol.* **6,** 597–641.
11. Villarreal, F. J. & Dillmann, W. H. (1992) *Am. J. Physiol.* **262,** H1861–H1866.
12. Sarzani, R., Brecher, P. & Chobanian, A. V. (1989) *J. Clin. Invest.* **83,** 1404–1408.
13. Ketteler, M., Noble, N. A. & Border, W. A. (1994) *Curr. Opin. Nephrol. Hypertens.* **3,** 446–452.
14. Border, W. A. & Noble, N. A. (1998) *Hypertension* **3,** 181–188.
15. Bottinger, E. P., Letterio, J. J. & Roberts, A. B. (1997) *Kidney Int.* **51,** 1255–1360.
16. Kurihara, H., Yoshizumi, M., Sugiyama, T., Takaku, F., Yanigisawa, M., Masaki, T., Hamaoki, M., Kato, H. & Yazaki, Y. (1989) *Biochem. Biophys. Res. Commun.* **159,** 1435–1440.
17. Antonipillai, I., Hoang Le, T., Soceneantu, L. & Horton, R. (1993) *Am. J. Physiol.* **265,** F537–F541.
18. Suthanthiran, M., Khanna, A., Cukran, D., Adhikarla, R., Sharma, V. K., Singh, T. & August, P. (1998) *Kidney Int.* **53,** 639–644.
19. Li, B., Khanna, A., Sharma, V., Singh, T., Suthanthiran, M. & August, P. (1999) *Hypertension* **33,** 271–275.
20. Cambien, R., Ricard, S., Troesch, A., Mallet, C., Generenaz, L., Evans, A., Arveiler, D., Luc, G., Ruidavets, J.-B. & Poirer, O. (1996) *Hypertension* **28,** 881–887.
21. Awad, M. R., El-Gamel, A., Hasleton, P., Turner, D. M., Sinott, P. J. & Hutchinson, I. V. (1998) *Transplantation* **66,** 1014–1020.
22. Perloff, D., Grim, C., Flack, J., Frohlich, E. D., Hill, M., McDonald, M. & Morgenstern, B. Z. (1993) *Circulation* **88,** 2460–2470.
23. Newton, C. R., Graham, A., Heptinstall, L. E., Powell, S. J., Summers, C., Kalsheker, N., Smith, J. C. & Markham, A. F. (1989) *Nucleic Acids Res.* **17,** 2503–2516.
24. Little, S. (1995) in *Current Protocols in Human Genetics, Unit 9.8,* eds. Dracopoli, N. C., Haines, J. L., Korf, B. R., Moir, D. T., Morton, C. C., Seidman, C. E., Seidman, J. G. & Smith, D. R. (Wiley, New York), pp. 9.8.1–9.8.12.
25. Strehlau, J., Pavlakis, M., Lipman, M., Shapiro, M., Vasconcellos, L., Harmon, W. & Strom, T. B. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 695–700.
26. Khanna, A., Li, B., Stenzel, K. H. & Suthanthiran, M. (1994) *Transplantation* **57,** 577–582.
27. SAS Institute, Inc. (1992) SAS User's Guide: Statistics (SAS Institute, Cary, NC), Version 6.07.
28. Benson, S. A., Hall, M. N. & Silhavy, T. J. (1985) *Annu. Rev. Biochem.* **54,** 101–134.
29. Van Obberghen-Schilling, E., Roche, N. S., Flanders, K. C., Sporn, M. B. & Roberts, A. B. (1988) *J. Biol. Chem.* **263,** 7741–7746.
30. Porecca, E., Di Febbo, C., Mincione, G., Reale, M., Baccante, G., Domenica Gugliemli, M., Cuccurullo, F. & Coletta, G. (1997) *Hypertension* **30,** 134–139.
31. Shin, G. T., Khanna, A., Ding, R., Sharma, V. K., Lagman, M., Li, B. & Suthanthiran, M. (1998) *Transplantation* **65,** 313–315.
32. Ergul, S., Parish, D. C., Puett, D. & Ergul, A. (1996) *Hypertension* **28,** 652–655.
33. Nabel, E. G., Shum, L., Pompili, V. J., Yang, Z. Y., San, H., Shu, H. B., Liptay, S., Gold, L., Gordon, D., Derynck, R., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* **90,** 10759–10763.
34. Saltis, J., Agrotis, A. & Bobil, A. (1996) *Clin. Exp. Pharmacol. Physiol.* **23,** 193–200.
35. Schulick, A. H., Taylor, A. J., Zuo, W., Qiu, C. B., Dong, G., Woodward, R. N., Agah, R., Roberts, A. B., Virmani, R. & Dichek, D. A. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 6983–6988.
36. Agrotis, A., Saltis, J. & Bobik, A. (1994) *Clin. Exp. Pharmacol. Physiol.* **21,** 145–148.
37. Kirchengast, M. & Munter, K. (1999) *Proc. Soc. Exp. Biol.* **221,** 312–325.
38. Battistini, B., Challer, P., D'Orleans-Juste, P., Briere, N. & Sirois, P. (1993) *Peptides* **14,** 385–399.
39. Matsubara, H. (1998) *Circ. Res.* **83,** 182–119.
40. Ohno, M., Cooke, J. P., Dzau, V. J. & Gibbons, G. H. (1995) *J. Clin. Invest.* **95,** 1363–1369.
41. Tamaki, K., Okuda, S., Nakayama, M., Yanagida, T. & Fujishima, M. (1996) *J. Am. Soc. Nephrol.* **7,** 2578–2589.
42. Takahashi, N., Calderone, A., Izzo, N. J., Maki, T. M., Marsh, J. D. & Colucci, W. S. (1994) *J. Clin. Invest.* **94,** 1470–1476.
43. Zimmermann, R., Kastens, J., Lilnz, W., Wiemer, G., Scholkens, B. A. & Schaper, J. (1999) *J. Mol. Cell. Cardiol.* **8,** 1447–1456.
44. Border, W. A. & Nobel, N. A. (1994) *N. Engl. J. Med.* **331,** 1286–1292.
45. Sharma, K., Jin, Y., Guo, J. & Ziyadeh, F. N. (1996) *Diabetes* **45,** 522–530.
46. Isaka, Y., Fujiwara, Y., Ueda, N., Kaneda, Y., Kamada, T. & Imai, E. (1993) *J. Clin. Invest.* **92,** 2597–2601.
47. Akagi, Y., Isaka, Y., Arai, M., Kaneki, T., Takenada, M., Moriyama, T., Kaneda, Y., Ando, A., Orita, Y., Kamada, T., *et al.* (1996) *Kidney Int.* **50,** 148–155.
48. Grim, C. E. & Robinson, M. (1996) *Semin. Nephrol.* **3,** 83–93.