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Regulation of endothelin synthesis by extracellular matrix in human endothelial cells

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Background. Vascular diseases are characterized by the presence of structural changes and the progressive loss of endothelial function. Although the biochemical basis of these structural changes have started to be outlined, it seems that accumulation of normal extracellular matrix proteins as well as the appearance of interstitial collagens, mainly collagen type I, characterize this process. On the other hand, a role for endothelial vasoactive factors has been proposed in the genesis of endothelial dysfunction, and it is generally accepted that changes in extracellular matrix composition may modify cell behavior.

Methods. Experiments were designed to test the influence of the supporting matrix on endothelin-1 (ET-1) synthesis by endothelial cells. Northern blot experiments were performed to analyze the prepro-endothelin-1 (prepro-ET-1) mRNA expression. ET-1 production was measured by ELISA.

Results. Cells grown on collagen type I (Col I) showed an increase of prepro-ET-1 mRNA level when compared with cells cultured on collagen type IV (Col IV). According to these results, the release of ET-1 to culture medium was also higher in Col I-grown cells than in those cultured on Col IV. Treatment of cells with a peptide that interferes with Col I integrins (D6Y), or with protein tyrosine kinase inhibitors such as genistein and herbimycin, completely abolished the effect of Col I. Moreover, experiments with antibodies against integrins suggest that these cell surface receptors could be involved in the modulation of ET-1 system by extracellular matrix.

Conclusions. These results suggest that the presence of an abnormal extracellular matrix could stimulate endothelin synthesis by human endothelial cells, through integrin activation.

The role of endothelial dysfunction in the pathogenesis of tissue damage in several vascular diseases, including hypertension, atherosclerosis or diabetes has been progressively recognized [1–3]. With regards to tissue perfusion, endothelial dysfunction could be defined as an abnormal pattern of endothelial vasoactive factor synthesis, with the subsequent local hemodynamic derangement. Most of studies in this field have analyzed the vasodilator response to acetylcholine to explore endothelial dysfunction [4, 5]. Acetylcholine induces the synthesis of nitric oxide (NO) by endothelial cells, and a defective vasodilator response after its administration points to an inadequate NO synthesis [6]. With this approach, the concept of endothelial dysfunction in vascular pathology has expanded. However, NO is not the only vasoactive metabolite synthesized by endothelial cells, and an increased endothelin synthesis or a decreased prostacyclin release, for instance, also could be involved in the maintenance of the abnormal endothelial function. In this sense, a deranged synthesis of endothelin has been demonstrated in different vascular diseases [7–9].

The mechanisms responsible for endothelial dysfunction are incompletely known. The importance of mechanical damage in hypertension, oxidized lipoproteins in atherosclerosis or the hyperglycemic environment in diabetes are the more easily understood, and they are supported by wide experimental evidence [10–12]. However, most authors agree that the pathogenesis of endothelial dysfunction is a multifactorial phenomenon, and alternative pathways are being explored. For example, reactive oxygen species (ROS) seem to be one of these pathogenic factors [13–15].

The present experiments analyzed alternative mechanisms in the genesis of endothelial dysfunction. Our hypothesis was based in two major facts. First, damaged vascular structures undergo significant structural changes, with an increased cell proliferation and an accumulation of extracellular matrix (ECM) proteins [16, 17]. Second, the extracellular matrix is one of the main determinants of cell phenotypic expression, and contact of cells with

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abnormal ECM proteins may induce profound effects on cell behavior [18–21].

Extracellular matrix proteins interact with cells through transmembrane heterodimeric proteins named integrins [22, 23]. They are formed by two subunits, alpha and beta chains, which depend on the cell type [24]. As a consequence of the interaction, intracellular kinases are activated and a complex intracellular response develops, inducing profound changes in cell function. The best known kinases involved in cell responses after a matrix to cell interaction include a group of tyrosine kinases, such as focal adhesion kinase (FAK) [22, 24].

Taking this information into account, we tested the hypothesis that the presence of increased amounts of abnormal ECM proteins in vessel walls could induce phenotypic changes in endothelial cells with the subsequent modifications in the patterns of local synthesis of bioactive mediators, that is, endothelial dysfunction and the maintenance of vascular injury. In a more strict sense, we were concerned about the possibility that endothelin synthesis by endothelial cells could change in presence of collagen I, and so we wanted to determine the possible mechanisms involved. As an in vitro experimental approach, human umbilical vein endothelial cells (HUVEC) grown on collagen I and collagen IV coated dishes were used.

METHODS

Materials

Collagen types I and IV, endothelial cell growth factor (EGF), salmon sperm DNA, formaldehyde, guanidinium thiocyanate, formamide, genistein, actinomycin D, endothelin-1 (ET-1) and herbimycin were purchased from Fluka-Sigma (St. Louis, MO, USA). M-199 medium, fetal calf serum (FCS), trypsin-ethylenediaminetetraacetic acid (trypsin-EDTA; 0.02%), L-glutamine, HEPES and penicillin-streptomycin were obtained from BioWhittaker (Walkersville, MD, USA). Culture plates were from Nunc (Kamstrup, Denmark). The random prime labeling system (Rediprime II), nylon filters (Hybond N), and deoxy-[^{32}P] cytidine triphosphate were purchased from Amersham Pharmacia Biotech (Buckinghamshire, UK). Endothelin-1 ELISA was from Promega (Ref. G-1250; Madison, WI, USA). X-OMAT films were from Kodak (Rochester, NY, USA). The bicinchoninic acid (BCA) assay kit was from Pierce (Rockford, IL, USA). Human prepro-endothelin-1 cDNA probe was supplied by American Type Culture Collection (Rockville, MD, USA). D6Y peptide (Asp-Gly-Gly-Arg-Tyr-Tyr) was supplied by Neosystem (Strasbourg, France). Specific antibodies against α_1, α_2, α_3 and β_1 integrins were provided by F. Sanchez Madrid (Dept. of Immunology, Hospital de la Princesa, Madrid, Spain).

Cell culture

Human endothelial cells from umbilical vein (HUVEC) were obtained and cultured as described previously [25]. Cells were seeded on dishes coated with gelatin 0.2% at 37°C in a humidified atmosphere of 95°C air and 5% CO_2. Individual clones were established and subcloned to obtain pure cell populations. Clones were characterized by their typical cobblestone morphology, by the presence of factor VIII-related antigen and by the uniform uptake of fluorescent acetylated low-density lipoprotein (LDL), as described [25]. Cells were fed every two days with M-199 medium supplemented with 20% FCS, 100 U/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L L-glutamine, 20 mmol/L HEPES and 300 μg/mL EGF. Cells were passaged every five to seven days with trypsin-EDTA and grown on collagen type IV and collagen type I coated culture plates (12.5 μg/mL), as described (two-dimensional cultures) [26], as well as on gelatin for control purposes. Studies were routinely performed on confluent monolayers five to seven days after passage, at passages 2 to 4. Toxicity was evaluated in any experimental condition by the trypan blue dye exclusion method.

Analysis of mRNA expression

Total cellular RNA was isolated by the guanidinium lysis method in confluent monolayers of HUVEC grown on both collagen types, after 24 hours of serum deprivation under different experimental conditions [27]. For Northern analysis, 10 μg samples of RNA were subjected to electrophoresis in 1% agarose gels containing 0.66 mol/L formaldehyde and transferred to nylon filters as described [27]. Filters were dried at 80°C for two hours and UV cross-linked. Filters were blocked by incubation in a prehybridization solution [50% formamide, 5 × Denhardt's solution, 5 × sodium chloride sodium phosphate EDTA (SSPE), 0.5% sodium dodecyl sulfate (SDS) and 100 to 200 μg salmon sperm DNA solution] at 42°C for three hours and probed overnight at 42°C with ^{32}P-labeled human preproendothelin-1 (1.2 kb fragment; ATCC, Rockville, MD, USA) cDNA probe. Filters then were washed in medium stringency conditions [0.2 × standard sodium citrate (SSC), 0.5% SDS, 30 min at 45°C], and then, exposed to X-OMAT film. Autoradiographs were performed at −80°C using intensifying screens and were developed after 24 hours. Filters were stripped by boiling in 0.1% SDS solution and reprobed with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or 28S cDNA probe, in order to obtain a housekeeping control for both load and specificity of gene expression. Levels of preproendothelin-1 (prepro-ET-1) mRNA were normalized using GAPDH or 28S expression. The densitometric analysis of the films was performed with an Apple scanner and appropriate software (NIH image from the National Institutes of Health).

Measurement of endothelin-1 in incubation media

Confluent monolayers of HUVEC cultured on collagen type IV (Col IV) and collagen type I (Col I) were maintained in serum-free medium for 24 hours under different experimental conditions. After this period, supernatants were collected, lyophilized, and stored at −70°C. Endothelin-1 (ET-1) was measured by ELISA. The intracellular endothelin content was checked by scraping the cells after 24 hours of serum deprivation. Suspensions were sonicated and processed as cellular supernatants.

Statistical methods

Results are expressed as the mean ± SEM of a variable number of experiments (defined in the Figure legends). As the number of experiments was under 10 in some cases, non-parametric statistics were used for group comparisons. Friedman test was selected, taking as reference the control sample performed in the same day. A $P < 0.05$ was considered statistically significant.

RESULTS

Endothelial cells grown on Col I showed higher prepro-ET-1 mRNA expression than those in contact with Col IV (Fig. 1). In Figure 1A, the Northern blot results of two different preparations can be observed, as well as the mean values of the Col I-induced changes in the mRNA steady state levels of prepro-ET-1. The increase was over twofold. The release of endothelin-1 to culture medium in the same culture conditions also was evaluated and the results appear Figure 1, B and C. Cells cultured on Col I released more ET-1 to culture medium than those grown on Col IV. No significant ET-1 storing took place in cells grown in the two conditions tested (intracellular immunoreactive ET-1 for Col IV 0.7 ± 0.1, and Col I 0.9 ± 0.2 fmol/well). Moreover, no differences were found in the degree of confluence of cells, in both kind of matrix, as assessed by direct observation and protein measurements (Col IV 187 ± 17, Col I 199 ± 21 μg/well). For control purposes, gelatin-grown cells also were studied. As shown in the Figure 1, the prepro-ET-1 mRNA expression and ET-1 synthesis were comparable in Col I and gelatin cells, with similar degrees of cell confluence (Col I 190 ± 15 μg/well; gelatin 192 ± 11 μg/well).

The relative contributions of an increased gene transcription or a stabilization of the mRNA to the Col I-dependent effects was tested, and the results are in Figure 2. Actinomycin D (Act D), an RNA synthesis inhibitor, completely blunted the prepro-ET-1 mRNA differences observed between both collagens (Fig. 2A). In fact, no changes in the prepro-ET-1 mRNA stability were detected in the two matrices tested (Fig. 2B).

The mechanisms involved in the effects of Col I were

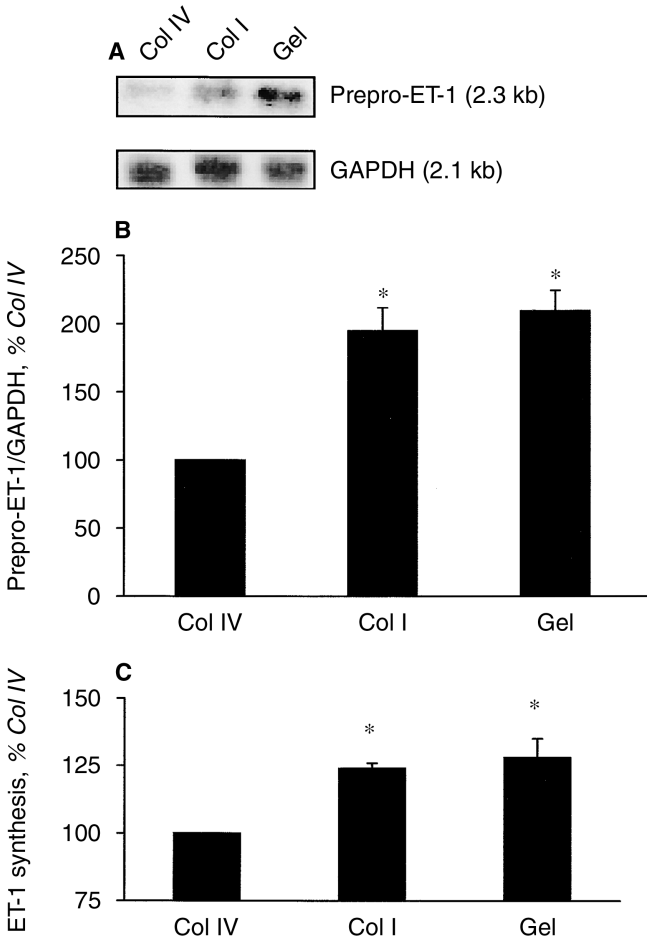

Fig. 1. Effect of cell culture on collagen type I (Col I) on the endothelial-1 (ET-1) system. Analysis of the steady-state prepro-endothelin-1 (Prepro-ET-1) mRNA levels. Results are compared with those from cells cultured on collagen type IV (Col IV) and gelatin (Gel). (*A*) A representative Northern blot experiment is shown. In the lower panel, the ratios between the PreproET-1 mRNA and a housekeeping gene mRNA expression are reflected. Results are expressed as percent of the Col IV values. (*B*) Basal ET-1 production in cells cultured on the two matrices tested, and on gelatin. Results are expressed as percent of Col IV values (132 ± 25 nmol/mg protein). In every case, results are the mean ± SEM of a variable number of experiments (12 for collagens and 4 for gelatin). *$P < 0.05$ vs. Col IV.

tested at several levels. First, the possible role played by the interaction of Col I with specific membrane proteins of the integrin family was tested with Asp-Gly-Gly-Arg-Tyr-Tyr (D6Y), an oligopeptide that blocks the Col I-dependent effects in some cell types [28]; the results are in Figure 3. Incubation with increasing concentrations of D6Y progressively inhibited the increased prepro-ET-1 mRNA expression elicited by Col I (Fig. 3A). According to this finding, the increase in the ET-1 production induced by Col I also was blocked by the incubation with D6Y (Fig. 3 B, C). In both cases, a complete inhibition was observed from 50 μmol/L D6Y. Lower doses of D6Y did not modify cell adherence, but a slight cell detachment was observed with 100 μmol/L D6Y (pro-

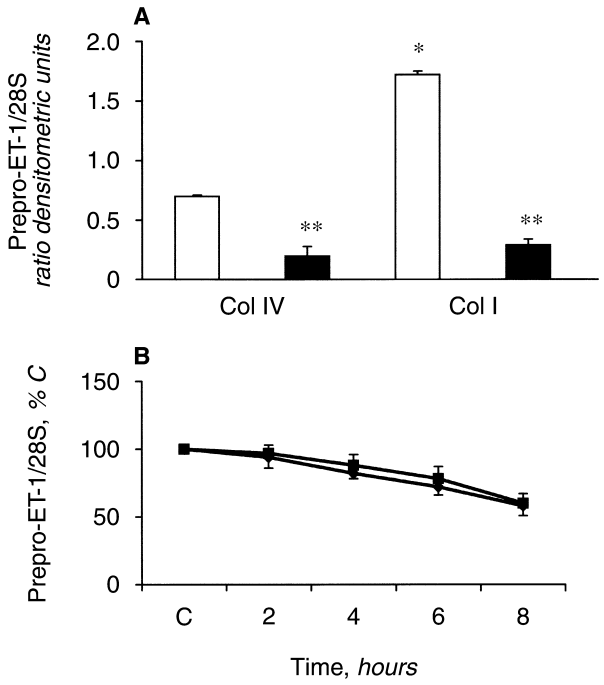

Fig. 2. Analysis of the mechanisms involved in the collagen I-increased steady state prepro-endothelin-1 (Prepro-ET-1) mRNA levels. (*A*) Cultured cells in both matrices were incubated for 24 hours with 10 μg/mL actinomycin D (Act D; ■). $*P < 0.05$ vs. values on Col IV; $**P < 0.05$ vs. values without Act D (□) (Friedman and Wilcoxon tests). (*B*) Prepro-ET-1 mRNA expression of Col IV (◆) and Col I (■) after different incubation times with Act D. In both panels, values are preproET-1/28S RNA ratios after densitometric analysis, and are the mean of 5 experiments expressed as absolute values (panel A) or as percent of control values (C).

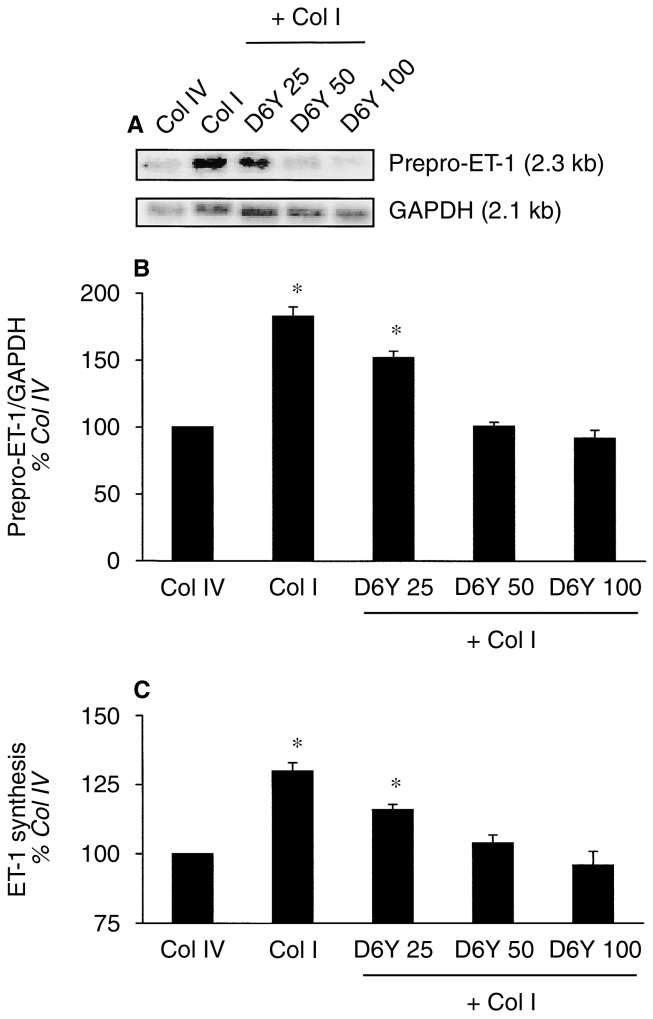

Fig. 3. Modulation of the effects of collagen type I (Col I) on the endothelin-1 (ET-1) system by a peptide that blocks the interactions of Col I with integrins (D6Y). (*A*) Prepro-ET-1 mRNA expression was analyzed in cells cultured on Col I and incubated with different D6Y (Asp-Gly-Gly-Arg-Tyr-Tyr) concentrations for 24 hours. Results are compared with those from cells cultured on Col I and Col IV. A representative Northern blot experiment is shown in the upper gels, and in the lower part, the ratios between the prepro-ET-1 mRNA and a housekeeping gene mRNA expression are reflected. Results are expressed as percent of the Col IV values. (*B* and *C*) Modulation of Col I effects on ET-1 synthesis with D6Y, used as described. Results are expressed as a percent of the Col IV values (151 ± 11 nmol/mg protein). In every case, results are the mean ± SEM of 4 different experiments. $*P < 0.05$ vs. the other values.

tein/well μg/well: Col IV 201 ± 15; Col I 191 ± 14 μg/well; 25 μmol/L D6Y + Col I 205 ± 18; 50 μmol/L D6Y + Col I 196 ± 13; 100 μmol/L D6Y + Col I 167 ± 11*; $*P < 0.05$ vs. the other groups). Second, experiments with antibodies that are able to block different integrins expressed in endothelial cells supported the role of these transmembrane proteins in the effects of Col I on the ET-1 system (Fig. 4). The anti-α3 antibody was the most powerful inhibitor of the stimulatory signal of Col I, whereas anti-α1 only induced a minimal inhibition. Third, the importance of tyrosine kinase activation was analyzed with two pharmacological antagonists of the system: genistein and herbimycin. These tyrosine kinase inhibitors completely prevented the stimulatory effect of Col I on the prepro-ET-1 expression as well as on the ET-1 production (Fig. 5).

DISCUSSION

Our results highlight the importance of an abnormal ECM protein to modulate the pattern of synthesis of vasoactive factors by cultured endothelial cells. Both the prepro-ET-1 mRNA expression and the ET-1 release to the culture media increased in cells seeded on Col I, when compared with cells on Col IV. Moreover, the results suggest that these differences were not the consequence of changes in prepro-ET-1 mRNA stability, and they point to the possibility that different ECM proteins could differentially modulate the expression of this mRNA. By comparing the two cultures, no significant morphological differences were detected by direct cell observation, the protein content was comparable, and no significant cell toxicity was apparent. The control of

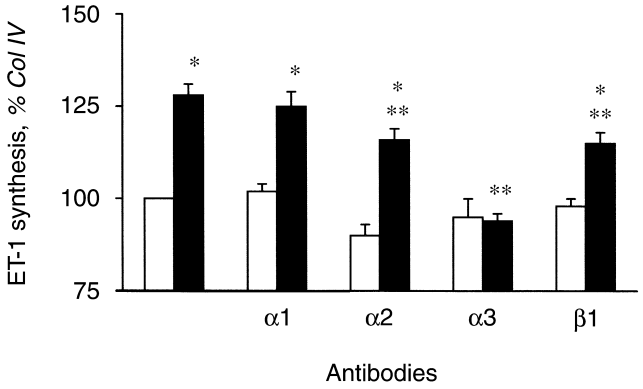

Fig. 4. Modulation of the effects of Col I on the ET-1 system by antibodies against integrins. Confluent HUVEC cultured on both collagen types [(□) Col IV; (■) Col I], were incubated for 24 hours with specific antibodies against α_1, α_2, α_3 and β_1 integrins. After this period, endothelin concentrations in the supernatants were measured by ELISA. Results are expressed as percent of the basal Col IV values (141 ± 9 nmol/mg protein), and they are the mean ± SEM of 5 experiments. *$P < 0.05$ vs. Col IV without antibodies. **$P < 0.05$ vs. Col I without antibodies.

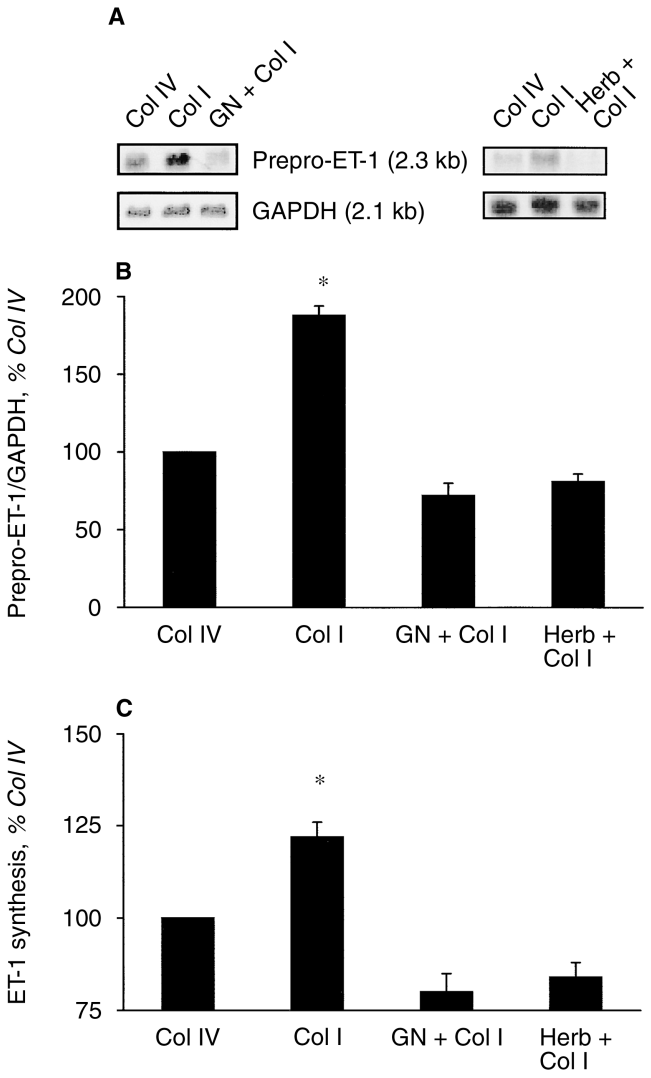

Fig. 5. Modulation of the effects of Col I on the ET-1 system by tyrosine kinase inhibitors. (*A*) Prepro-ET-1 mRNA expression was analyzed in cells cultured on Col I and incubated with 2 μmol/L genistein (GN) or 1 μmol/L herbimycin (Herb) for 24 hours. Results are compared with those from cells cultured on Col I and Col IV. In the upper part of the panel, a representative Northern blot experiment is shown, and the lower part reflects the ratios between the prepro-ET-1 mRNA and a housekeeping gene mRNA expression. Results are expressed as percent of the Col IV values. (*B* and *C*) Modulation of collagen-I effects on ET-1 synthesis with GN or Herb, used as described. Results are expressed as percent of the Col IV values (159 ± 14 nmol/mg protein). In every case, results are the mean ± SEM of a variable number of experiments (8 for GN and 4 for Herb). *$P < 0.05$ vs. the other groups.

cell confluence was especially important, as it has been demonstrated that the mRNA stability of endothelial nitric oxide synthase (eNOS) may be modified by this parameter [29]. Consequently, it seems that the different nature of the culture substrate was responsible for the differences observed.

Significant quantitative differences were observed when considering the Col I-dependent changes in prepro-ET-1 mRNA expression (about twofold) and ET-1 protein synthesis (about 25%). The present experimental design did not extensively evaluate the mechanisms responsible for this difference, but various possibilities could be proposed. For instance, the synthesis of ET-1 from prepro-ET-1 involves two enzymatic cleavages, and extracellular matrix also may regulate these activities.

Endothelial cells interact with different ECM proteins through integrins. It seems that the different collagen types are recognized by heterodimeric complexes of α_1, α_2 or α_3 with β_1 [30, 31], whereas $\alpha_V\beta_1$ is used preferentially for fibronectin interactions [32]. The possibility that the differential activation of these integrins could be responsible for the observed differences between the two collagen types was tested by two means. First, a peptide capable of blocking the integrin-mediated Col I-dependent cellular effects but not cell adhesion, D6Y [28], was used. As shown in the **Results** section, this peptide completely abolished the changes in ET-1 elicited by Col I. Second, different specific antibodies were used. Again, the Col I-dependent stimulation on ET-1 synthesis was blocked but differences were detected with respect to the intensity of the blockade. Anti-α_3 antibody was the most powerful inhibitor of the effects of collagen I, followed by anti-α_2 and anti-β_1 antibodies, and finally anti-α_1 antibody. Although the antibody concentration was the same in every case, the interpretation of these quantitative differences must be performed cautiously, as no controls of the antibody blocking activity or the integrin cell surface expression were performed. Nevertheless, the results suggest that the $\alpha_3\beta_1$ integrin could play a significant role in the Col I-dependent increased ET-1 synthesis.

The D6Y and the antibody experiments provide additional information. They stress the specific nature of the

Col I stimulation, discarding that non-specific soluble stimuli may be involved in the differences observed. However, they do not clarify the basis of the differences detected with both ECM proteins. At least two theoretical possibilities exist. The expression of the different integrins could be different in the two culture substrates, as it has been described for other proteins [24, 30, 31]. Alternatively, the affinity of the different integrins for both Col I and Col IV could differ. In both cases, some specificity in signal transduction by a particular integrin might exist, that would explain the detected differences, but this specificity has not been previously described. Additional experiments must be performed to explore these possibilities.

Another interesting aspect of the results is the powerful inhibitory action of genistein and herbimycin on the Col I-dependent ET-1 stimulation. As stated in the introductory comments, tyrosine kinases act as mediators of integrin signaling and significant tyrosine phosphorylation is detected after integrin activation [22, 24]. While our present results support the importance of this pathway in the changes in ET-1 synthesis, they do not completely exclude other alternative intracellular pathways of activation, such as integrin-linked kinase (ILK) [33].

These in vitro findings propose additional explanations for endothelial dysfunction in vascular diseases. As vascular damage develops in hypertension, diabetes or atherosclerosis, increasing amounts of abnormal ECM proteins accumulate in vessel walls [16–18], interacting with endothelial or smooth muscle cells and changing their phenotype. In the case of endothelial cells, an increased local synthesis of endothelin could take place, thus determining an abnormal vasoconstriction. In addition to previously described mechanisms for endothelial dysfunction development, including mechanical forces [10], hyperglycemia [12], oxidized lipoproteins [11], and reactive oxygen species [13–15], we propose that the disease-induced abnormal structure of vessel wall could explain some aspects of the deranged endothelial function. Moreover, alternative therapeutic strategies in the prevention of ischemic tissue damage in vascular diseases are proposed by these results, as the specific blockade of some integrins or tyrosine kinases could improve endothelial dysfunction.

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REFERENCES

1. Luscher TF: The endothelium and cardiovascular disease—A complex relation. *N Engl J Med* 330:1081–1083, 1994
2. Tsao PS, Cooke JP: Endothelial alterations in hypercholesterolemia: More than simply vasodilator dysfunction. *J Cardiovasc Pharmacol* 32:48–53, 1998
3. De Vriese AS, Verbeuren TJ, Van de Voorde J, *et al*: Endothelial dysfunction in diabetes. *Br J Pharmacol* 130:963–974, 2000
4. Lahera V, Salom MG, Fiksen-Olsen MJ, *et al*: Effects of N-monomethyl-L-arginine and L-arginine on acetylcholine renal response. *Hypertension* 15:659–663, 1990
5. Tolins JP, Palmer RMJ, Moncada S, Raij L: Role of endothelium-derived relaxing factor in regulation of renal hemodynamic response. *Am J Physiol* 258:H655–H662, 1990
6. Kugiyama K, Ohgushi M, Motoyama T, *et al*: Nitric oxide-mediated flow-dependent dilation is impaired in coronary arteries in patients with coronary spastic angina. *J Am Cell Cardiol* 30:920–926, 1997
7. Miyauchi T, Masaki T: Pathophysiology of endothelin in the cardiovascular system. *Annu Rev Physiol* 61:391–415, 1999
8. Barton M, Luscher TF: Endothelin antagonists for hypertension and renal disease. *Curr Opin Nephrol Hypertens* 8:549–556, 1999
9. Cardillo C, Kilcoyne CM, Cannon RP, Panza JA: Increased activity of endogenous endothelin in patients with hypercholesterolemia. *J Am Cell Cardiol* 36:1483–1488, 2000
10. Safar MC: Pulse pressure, arterial stiffness and cardiovascular risk. *Curr Opin Cardiol* 15:258–263, 2000
11. Catapano AL, Maggi RM, Tragni E: Low density lipoprotein oxidation, antioxidants and atherosclerosis. *Curr Opin Cardiol* 15:355–363, 2000
12. Cosentino F, Luscher TF: Effects of blood pressure and glucose on endothelial function. *Curr Hypertens Rep* 3:79–88, 2001
13. Aviram M: Review of human studies on oxidative damage and antioxidant protection related to cardiovascular diseases. *Free Radic Res* 33(Suppl):S85–S97, 2000
14. Zalba G, Beaumont J, San Jose G, *et al*: Vascular oxidant stress: molecular mechanisms and pathophysiological implications. *J Physiol Biochem* 56:57–64, 2000
15. Laight DW, Carrier MJ, Anggard EE: Antioxidants, diabetes and endothelial dysfunction. *Cardiovasc Res* 18:457–464, 2000
16. Raines EW: The extracellular matrix can regulate vascular cell migration, proliferation, and survival: Relationship to vascular disease. *Int J Exp Pathol* 81:173–182, 2000
17. Tunon J, Ruiz-Ortega M, Egido J: Regulation of matrix proteins and impact on vascular structure. *Curr Hypertens Rep* 2:106–113, 2000
18. Streuli C: Extracellular matrix remodelling and cellular differentiation. *Curr Opin Cell Biol* 11:634–640, 1999
19. Miralem T, Whiteside CI, Templeton DM: Collagen type I enhances endothelin-mediated contraction and induces nonproliferating phenotype in mesangial cells. *Am J Physiol (Renal Fluid Electrol Physiol 39)* 270:F960–F970, 1996
20. He CJ, Striker LJ, Tsokos M , *et al*: Relationships between mesangial cell proliferation and types I and IV collagen mRNA levels in vitro. *Am J Physiol (Cell Physiol 38)* 269:C554–C562, 1995
21. Marx M, Perlmutter RA, Madri JA: Modulation of platelet-derived growth factor receptor expression in microvascular endothelial cells during in vitro angiogenesis. *J Clin Invest* 93:131–139, 1994
22. Giancotti FG, Ruoslahti E: Integrin signaling. *Science* 13:1028–1032, 1999
23. Ruoslahti E, Engvall E: Perspectives series: Cell adhesion in vascular biology. *J Clin Invest* 100(Suppl):S53–S56, 1997
24. Boudreau N, Jones P: Extracellular matrix and integrin signalling: The shape of things to come. *Biochem J* 339:481–488, 1999
25. Jaffe EA, Nachman RI, Becker CG, Minick RC: Culture of human endothelial cells derived from umbilical veins. *Circulation* 46:211–253, 1972
26. Iglesias-De La Cruz MC, Ruiz-Torres MP, De Lucio-Cazaña FJ, *et al*: Phenotypic modifications of human mesangial cells by extracellular matrix: The importance of matrix in the contractile response to reactive oxygen species. *Exp Nephrol* 8:97–103, 2000

27. Chomczynski P, Sacchi N: Single step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem* 162:156–159, 1987
28. Garnotel R, Monboisse JC, Randoux A, *et al*: The binding of type I collagen to lymphocyte function-associated antigen (LFA) 1 integrin triggers the respiratory burst of human polymorphonuclear neutrophils. Role of calcium signaling and tyrosine phosphorylation of LFA 1. *J Biol Chem* 17:27495–27503, 1995
29. Flowers MA, Wang Y, Stewart RJ, *et al*: Reciprocal regulation of endothelin-1 and endothelial constitutive NOS in proliferating endothelial cells. *Am J Physiol* 269:H1988–H1997, 1995
30. Dickeson S, Mathis N, Rahman M, *et al*: Determinants of ligand binding specificity of the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins. *J Biol Chem* 274:32182–32191, 1999
31. Kern A, Eble J, Golbik R, Kuhn K: Interaction of type IV collagen with the isolated integrins $\alpha_1\beta_1$ and $\alpha_2\beta_1$. *Eur J Biochem* 215:151–159, 1993
32. Bourdoulous S, Orend G, MacKenna DA, *et al*: Fibronectin matrix regulates activation of RHO and CDC42 GTPases and cell cycle progression. *J Cell Biol* 143:267–276, 1998
33. Hannigan GE, Leung-Hagesteijn C, Fitz-Gibbon L, *et al*: Regulation of cell adhesion and anchorage-dependent growth by a new beta 1-integrin-linked protein kinase. *Nature* 4:91–96, 1996