

Type 1 Diabetes Increases the Expression of Proinflammatory Cytokines and Adhesion Molecules in the Artery Wall of Candidate Patients for Kidney Transplantation

JAVIER TRIÑANES, MSc¹
 EDUARDO SALIDO, MD, PHD¹
 JULIÁN FERNÁNDEZ, MD, PHD²
 MARGARITA RUFINO, MD, PHD³

JOSÉ MANUEL GONZÁLEZ-POSDADA, MD, PHD³
 ARMANDO TORRES, MD, PHD³
 DOMINGO HERNÁNDEZ, MD, PHD⁴

OBJECTIVE—Diabetes may accelerate atheromatosis in uremic patients. Our aim was to assess the influence of type 1 diabetes on the atheromatosis-related inflammation in patients with chronic kidney disease (CKD).

RESEARCH DESIGN AND METHODS—We analyzed the expression of proinflammatory cytokines and adhesion molecules in the inferior epigastric artery walls of type 1 diabetic patients with CKD ($n = 22$) and compared it with nondiabetic uremic patients ($n = 92$) at the time of kidney transplantation. We evaluated the expression of interleukin (IL)-6, monocyte chemoattractant protein (MCP)-1, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule-1, and the activation of nuclear factor- κ B p65 (NF κ B-p65). Common carotid intima-media thickness (c-IMT) was determined by conventional echography.

RESULTS—IL-6, MCP-1, and VCAM-1 proteins were elevated in type 1 diabetic patients compared with nondiabetic subjects ($P < 0.05$). The nuclear localization of NF κ B-p65 was higher in type 1 diabetic patients ($P < 0.01$) and correlated with the levels of MCP-1 in this group ($r = 0.726$, $P < 0.001$). Arterial fibrosis correlated with IL-6 and MCP-1 levels ($r = 0.411$, $P < 0.001$ and $r = 0.378$, $P = 0.001$). A significant correlation was observed between VCAM-1 levels and both the degree of arterial narrowing and c-IMT.

CONCLUSIONS—Type 1 diabetes produces a proinflammatory state in the arteries of end-stage CKD patients, with increased levels of IL-6, MCP-1, and VCAM-1, as well as a greater degree of p65 activation, which are associated with more severe vascular lesions and higher c-IMT. Although causality is not demonstrated, these findings support the major role of inflammation in type 1 diabetic patients with CKD.

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Vascular inflammation is an important process in the development of cardiovascular disease (CVD), and patients with chronic kidney disease (CKD) represent a subset with a higher risk of CVD (1,2), especially when they also have type 1 diabetes (3). Multiple risk factors

inherent to renal failure and diabetes status involved in the pathogenesis of atheromatosis concur in these patients.

Atheromatosis is an inflammatory disease of the arterial wall mediated by a complex interaction between mononuclear cells, endothelial cells, vascular smooth

muscle cells (SMCs), growth factors, and cytokines. Indeed, the endothelium overlying atherosclerotic lesions expresses vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1, which have been shown to be closely correlated with monocyte/macrophage infiltration and progress of the atherosclerotic lesion. The monocyte chemoattractant protein (MCP)-1 and interleukin (IL)-6 also are key cytokines in this process. In particular, MCP-1 has a crucial role in the attraction, migration, and activation of monocytes (4) and is an important regulator for cell proliferation (5). IL-6 is a central cytokine in the inflammatory process (6), being regulated by high glucose levels (7). This molecule can also activate the expression of different genes, such as MCP-1, contributing to maintaining the inflammatory milieu in the lesion. Finally, the upregulation of these molecules is controlled by transcription factors, such as nuclear factor- κ B (NF κ B) (8) acting as a mediator in the formation of atherosclerotic plaques.

Experimental and clinical studies have demonstrated the significant role of inflammatory molecules in the setting of atheromatosis related to type 2 diabetes (8–10). However, the expression of these molecules and the pathways involved in their pathogenesis barely have been assessed in human tissue, especially in type 1 diabetic patients with CKD.

Common carotid intima-media thickness (c-IMT) is an early marker of atherosclerosis both in the general population and in uremic patients, including kidney transplant recipients (11,12). Moreover, an increase in c-IMT has been observed in nondiabetic patients with prediabetes glucose alterations after kidney transplantation (13), but its relationship with the expression of proinflammatory molecules in the arterial wall in vivo has not been investigated.

The purpose of this study was to assess the influence of type 1 diabetes on the

From the ¹Research Unit, University Hospital of the Canary Islands, Tenerife, Spain; the ²Radiology Department, University Hospital of the Canary Islands, Tenerife, Spain; the ³Nephrology Department, University Hospital of the Canary Islands, Tenerife, Spain; and the ⁴Nephrology Department, University Hospital Carlos Haya, Málaga, Spain.

Corresponding author: Domingo Hernández, domingohernandez@gmail.com.

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atheromatosis-related inflammatory state in CKD patients at the time of kidney transplantation. We analyzed, *in vivo*, the expression levels of atheromatosis-associated inflammatory markers, such as cytokines and adhesion molecules, and compared them with the levels in nondiabetic CKD patients. We also investigated the relationship between subclinical atheromatosis, evaluated by c-IMT measurement, and the molecular expression of these inflammatory markers.

RESEARCH DESIGN AND METHODS

Patients and tissue samples

This cross-sectional observational study initially involved 148 consecutive adult CKD patients (aged ≥ 18 years) who received a single deceased kidney transplant or a simultaneous pancreas-kidney transplant ($n = 4$) between August 2007 and April 2009 at a regional transplant center (Hospital Universitario de Canarias, Tenerife, Spain). For the purpose of the study, we excluded 34 patients with type 2 diabetes. Thus, the final study population comprised 114 CKD patients. We compared type 1 diabetic patients ($n = 22$) with nondiabetic recipients ($n = 92$). Diagnosis of type 1 diabetes was established according to the guidelines of the American Diabetes Association (14), and C-peptide plasma concentrations were determined by immunometric assay (Immulite 2000C-Peptide; Diagnostic Products, Los Angeles, CA). Thus, insulin-dependent individuals with low (<0.9 ng/mL) or undetectable levels of plasma C-peptide were considered to be type 1 diabetic patients. The study was purely descriptive, and no attempts were undertaken to modify any aspect of therapy. Also, the study was approved by the ethics committee of the Hospital Universitario de Canarias and was conducted according to the principles described in the Declaration of Helsinki. Each patient gave written informed consent to participate in the study.

During surgery, a sample of the inferior epigastric artery (IEA) was obtained from all eligible patients. The tissue was quickly dissected in three sections for the different analyses: gene expression, protein quantification, and histological analysis.

Histological, immunohistochemical, and immunofluorescence analyses

Artery segments were fixed and paraffin embedded. Serial sections were cut at 3- μ m thickness, and samples were stained with

hematoxylin-eosin to measure the proportion of arterial lumen reduction and with Sirius Red to determine the degree of fibrosis. The reduction in arterial lumen was calculated as the percentage of lumen lost from the total lumen area measured in the inner elastic lamina, and fibrosis was calculated as the proportion of the total area, including media and intima layers, positive for Sirius Red stain. We also developed a Von Kossa stain to determine the degree of calcium deposits in the artery wall and classified the arteries in four groups according to the severity of calcium deposits.

For immunohistochemistry, serial sections were mounted on silanized slides, and after deparaffination and heat-mediated antigen retrieval, the tissue was permeabilized, blocked, and incubated with the primary antibody overnight. The antibodies used were rabbit polyclonal to MCP-1 (Abcam, Cambridge, U.K.), rabbit monoclonal to ICAM-1 (Abcam), and mouse monoclonal to IL-6 and VCAM-1 (Santa Cruz Biotech, Santa Cruz, CA). The immunoreactivity of the primary antibody was revealed by using a peroxidase-linked antibody against mouse/rabbit IgG and 3'-diaminobenzidine as a chromogen substrate.

All images were taken with an Olympus DX41 microscope (Olympus, Tokyo, Japan) fitted with a Canon DP72 camera (Canon, Tokyo, Japan) and then analyzed using the software ImageJ (National Institutes of Health) and the Wright Cell Imaging Facility (WCIF) plug-in from the Western Research Institute (Toronto, Canada).

For immunofluorescence, we used a rabbit antibody to NF κ B-p65 (Abcam), Alexa-conjugated anti-rabbit IgG as the secondary antibody, and DAPI to stain the nuclei. The images were taken using a Leica confocal microscope (Wetzlar, Germany) and analyzed using the ImageJ software and the procedure developed by Carmona et al. (15) to measure nuclear localization.

RNA extraction

The sample of artery used for RNA isolation was quickly frozen in buffer D containing guanidium thiocyanate. Total RNA was isolated by the Chomczynski method (16). The purity and concentration of RNA was determined by Nano-drop 2000 (Thermo-Fisher, Boston, MA).

Real-time PCR

The quantification of relative mRNA abundance was carried out using quantitative PCR and the SYBR green detection method.

Total RNA was reverse transcribed using a kit (Promega, Madison, WI), and the genes of interest were PCR amplified using gene-specific primers. The oligonucleotides used are shown in Supplementary Table 1. The resulting increase in fluorescence during the PCR reaction was detected in the iQ5 system (Bio-Rad, Hercules, CA), and expression of each gene was normalized with the reference genes (18S rRNA and RNase P). The data were analyzed using qBASE software (17).

Protein extraction

The sample of artery used for protein extraction was introduced in radioimmunoprecipitation assay buffer and immediately frozen. For protein extraction, the sample was mechanically homogenized, and after centrifugation the supernatant was collected and total protein concentration was determined using the bicinchoninic acid method (Sigma-Aldrich, St. Louis, MO). Quantification of proteins (IL-6, MCP-1, ICAM-1, and VCAM-1) was determined using xMAP technology (R&D Systems, Minneapolis, MN) and the Luminex platform (Luminex, Austin, TX). The concentration of each analyte was determined for each sample and then corrected by the total protein concentration; the result obtained is expressed as picograms of analyte per microgram of total protein.

c-IMT measurements

After surgery and within the following 2 weeks, c-IMT was determined by an experienced radiologist with no knowledge of the patient's clinical data. Measurements were made according to the standard clinical procedure (13) using an SSA-380 ultrasound transducer (Toshiba, Tokyo, Japan). Either an L6-7 MHz or a linear array transducer L11-7.5 MHz probe (PLM 703AT) was used depending on the depth of the artery. A low intraobserver variability has previously been reported in our radiology section after two repeated measurements (mean intraclass concordance correlation 0.96 [95% CI 0.90 – 0.99]; $P < 0.001$) (13).

Statistical analysis

Descriptive data are expressed as means \pm SD. Comparisons of continuous variables between the two groups were made by means of the Mann-Whitney U test. The χ^2 test and Fisher exact test, when appropriate, were used for between-group analyses of categorical variables. The Spearman correlation coefficient was used to analyze

unadjusted relationships between continuous data. All data were analyzed using the SPSS statistical package 13.0 (SPSS, Chicago, IL). A *P* value <0.05 was considered significant.

RESULTS—No significant differences were found between the two groups in male-to-female ratio, age, BMI, type or time on dialysis, CVD pretransplantation, total cholesterol, HDL cholesterol, triglycerides, smoking status, or use of statins, aspirin, and ACE inhibitors or angiotensin II receptor blockers (ARBs) (Table 1). As expected, the main cause of renal failure in the type 1 diabetic group was diabetic nephropathy. A high prevalence of polycystic kidney disease also was observed in the nondiabetic population. In addition, glucose levels and glycosylated hemoglobin (HbA_{1c}) were significantly higher in type 1 diabetic patients compared with nondiabetic subjects.

Pathological changes of the artery wall

The distribution of the subjects in four classes according to the luminal narrowing (Supplementary Table 2) was age dependent in nondiabetic subjects ($r = 0.332$, $P = 0.003$) but not in type 1 diabetic subjects. Overall histopathologic analysis showed a greater degree of lumen reduction in the epigastric artery from type 1 diabetic patients compared with nondiabetic subjects (17.9 ± 19.5 vs. $6.39 \pm 8.8\%$; $P = 0.014$) as well as a greater proportion of fibrosis in the intima (0.64 ± 0.12 vs. 0.49 ± 0.18 ; $P < 0.001$).

Histological and immunohistochemical analyses also showed that the intima thickening for the IEA was composed, at all the stages observed, of smooth muscle actin (SMA)-positive cells and collagen fibers (Fig. 1A and B), whereas CD68-positive macrophages only were present as isolated cells in the intima and adventitia

or forming aggregates around medial calcifications in the most advanced stages but never as a major cell type in the intima (Fig. 1C and D).

We classified medial calcification in four stages (absence, initial, medium, and advanced) in all subjects. No intimal calcification was detected in any sample, whereas medial calcification was present in the arteries of both groups, with a higher incidence and severity in the type 1 diabetic group (Supplementary Fig. 1). Calcification in the media layer was present in 22.9% of nondiabetic subjects and in 86.4% of type 1 diabetic patients ($P < 0.001$) (Supplementary Fig. 1). The variables significantly associated with the stage of medial calcification in nondiabetic subjects were time on dialysis ($r = 0.213$, $P = 0.05$) and age ($r = 0.416$, $P < 0.001$). On the other hand, fasting glucose levels ($r = 0.469$, $P = 0.037$) seemed to be the most relevant factor in type 1 diabetes. Immunohistochemical analysis showed no relationship between the expression of molecules under study in the intima and media layer calcification.

Table 1—Clinical characteristics of both groups

	Type 1 diabetic patients	Nondiabetic subjects	<i>P</i>
<i>n</i>	22	92	
Sex (male/female)	15/7	69/23	0.619
Age (years)	50.32 ± 12.15	46.47 ± 14.29	0.167
BMI (kg/m ²)	25.83 ± 5.25	24.31 ± 4.09	0.141
Dialysis type (% hemodialysis)	77.3	81.5	0.743
Time on dialysis (months)	29.38 ± 38.47	26.61 ± 27.57	0.705
Pretransplant CVD (%)	22.7	10.9	0.124
Ischemic heart disease	8.7	7.6	
Peripheral arterial disease	4.3	1.1	
Transient ischemic attack	9.7	2.2	
Use of ACE inhibitors/ARBs (%)	11.0	8.7	0.751
Use of statins (%)	17.4	15.4	0.816
Use of aspirin (%)	31.8	23.9	0.533
Hypertension (%)	95.5	92.4	0.579
HDL (mg/dL)	39.23 ± 14.88	42.9 ± 12.12	0.353
Total cholesterol (mg/dL)	133.14 ± 36.33	136.15 ± 33.39	0.592
Triglycerides (mg/dL)	153.52 ± 59.55	139.55 ± 72.51	0.481
Smokers (%)			0.256
No	59.1	72.8	
Former	27.3	14.1	
Current	13.6	13.1	
Cause of CKD (%)			<0.001
Diabetes	81.8	—	
Glomerulonephritis	—	21.7	
Polycystic kidney disease	4.5	21.7	
Hypertension	—	4.4	
Nephroangiosclerosis	—	8.7	
Other	13.6	43.5	
Fasting glucose (mg/dL)	138.15 ± 33.36	100.12 ± 14.32	<0.001
HbA _{1c} (%)	6.97 ± 0.62	5.31 ± 0.99	<0.001

Data are means ± SD and proportions, unless otherwise indicated. Ischemic heart disease includes myocardial infarction, coronary artery bypass, and stent.

Expression of proinflammatory cytokines and adhesion molecules in the artery wall at the time of transplantation

Figure 2A shows the gene expression in the artery wall of proinflammatory cytokines and adhesion molecules in the two groups of patients. A significantly higher expression of MCP-1 and IL-6 was observed in type 1 diabetic patients compared with the nondiabetic group. In addition, a trend toward higher mRNA levels from the adhesion molecules (ICAM-1 and VCAM-1) also was seen in type 1 diabetic patients.

As a result, significant differences between both groups also were observed for protein quantification of IL-6, MCP-1, and VCAM-1 but not for ICAM-1 (Fig. 2B). Moreover, a significant correlation between mRNA expression and protein levels was evidenced for IL-6 ($r = 0.263$, $P = 0.003$) and MCP-1 ($r = 0.238$, $P = 0.008$) but not for the adhesion molecules, indicating that posttranscriptional processes could be responsible for the differences between the mRNA expression of adhesion molecules and protein levels.

Of note, NFκB-p65 nuclear localization measured along the intima-media was significantly higher in type 1 diabetic patients compared with nondiabetic subjects ($P = 0.019$) (Fig. 3) and correlated with the MCP-1 protein levels in the type

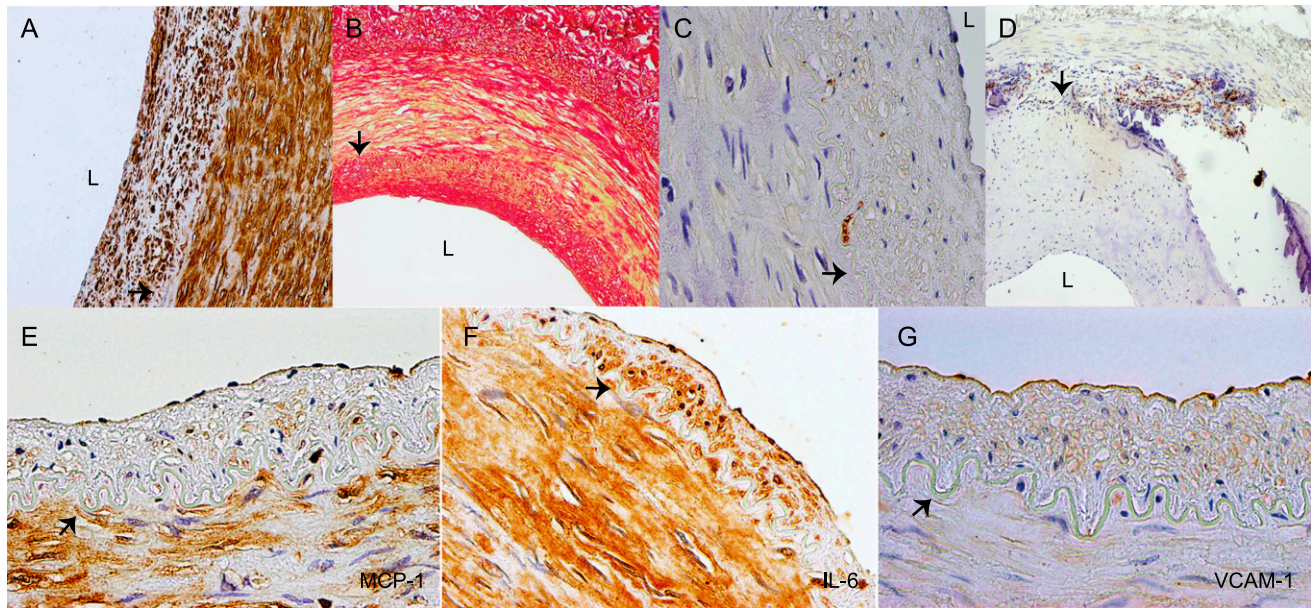


Figure 1—Histological and immunohistochemical characteristics of the IEA. The intima thickening is composed of SMA-positive cells (magnification $\times 200$) (A) and collagen fibers positive for Sirius Red (magnification $\times 100$) (B). Macrophages only are detectable as isolated cells in the intima (magnification $\times 400$) (C) or forming aggregates around medial calcifications (magnification $\times 100$) (D). Abundant localization of MCP-1-positive cells (E), as well as IL-6-positive cells (F), is present in both the intima and muscular layers. Strong VCAM-1 expression is observed in the endothelial layer (G) of the artery wall (magnification $\times 400$). Note that endothelial and SMCs, as well as SMA-positive cells in the neointima, are positive for the three molecules. A and B represent contiguous sections of the same artery; note the fibrous cap formed by SMA-positive cells and collagen. Arrow, internal elastic lamina; L, lumen. (A high-quality digital representation of this figure is available in the online issue.)

1 diabetic group ($r = 0.797, P = 0.001$) but not in nondiabetic subjects.

Finally, the degree of lumen reduction in the epigastric artery correlated with the protein levels of MCP-1 and VCAM-1 ($r = 0.275, P < 0.05$ and $r = 0.258, P < 0.05$, respectively), and it also was associated to abundant SMA-positive cells in the neointima (Fig. 1E and G), whereas the degree of fibrosis correlated with the protein levels of IL-6 and MCP-1 ($r = 0.41, P < 0.01$ and $r = 0.378, P < 0.01$, respectively) produced also by

the SMA-positive cells in both intima and media layers (Fig. 1E and F).

Similar to the differences found in the IEA between both groups, a higher c-IMT was seen in type 1 diabetic patients compared with nondiabetic subjects (0.70 ± 0.21 vs. 0.58 ± 0.20 mm; $P = 0.05$), and this parameter correlated significantly with VCAM-1 protein levels in the epigastric artery ($r = 0.220, P = 0.046$). Finally, the c-IMT also showed a significant correlation with fasting glucose levels ($r = 0.283, P = 0.009$) and the degree of

arterial lumen reduction ($r = 0.307, P = 0.02$).

CONCLUSIONS—This cross-sectional study demonstrates, for the first time, that type 1 diabetes produces a proinflammatory state in the artery wall of uremic patients, evidenced by the expression of cytokines and adhesion molecules as well as a greater degree of NFkB-p65 activation, which are related to more severe vascular lesions and higher c-IMT. To better elucidate the role of type 1 diabetes in atherosclerosis, we excluded patients with type 2 diabetes and used as a control group of uremic patients without diabetes. We focused on candidate patients for transplant to avoid including patients with a higher cardiovascular morbidity and who were therefore not eligible for transplantation. Thus, our results highlight the role of inflammatory pathways in the development of atheromatosis in type 1 diabetic patients with uremia.

Atheromatosis is the result of a complex inflammatory process in the artery wall where important risk factors, such as diabetes and uremia, may play a crucial role via different pathogenetic mechanisms. The relative contribution of each factor to the progression of atheromatosis when both pathological situations concur

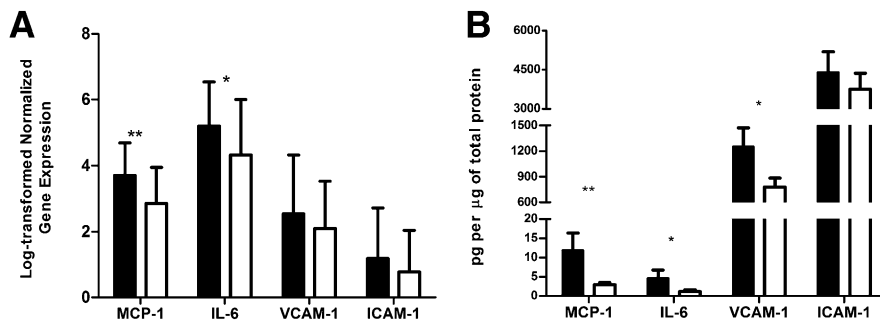


Figure 2—A: Differences in the gene expression of proinflammatory markers in the artery wall of type 1 diabetic patients (■) and nondiabetic patients (□). B: Differences in the quantification of proinflammatory proteins between type 1 diabetic patients (■) and nondiabetic patients (□). * $P < 0.05$; ** $P < 0.01$.

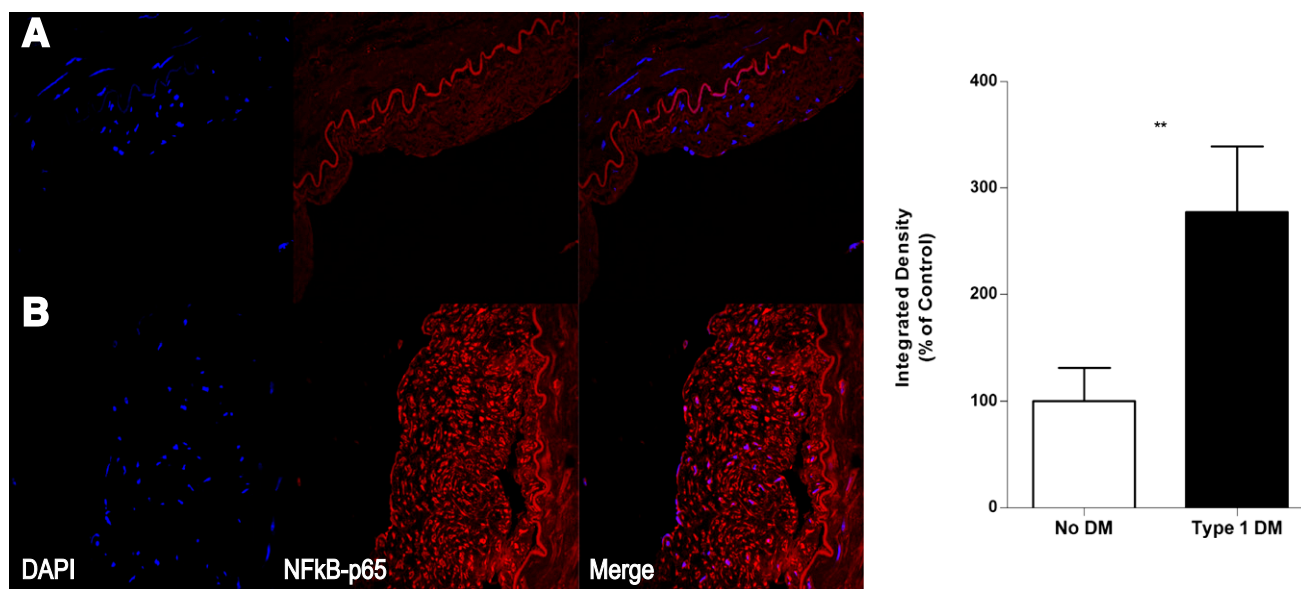


Figure 3—Immunofluorescence images showing the difference in the expression and nuclear localization of NFκB-p65 in the artery wall of the IEA from a nondiabetic patient (A) and a type 1 diabetic patient (B) with a similar grade of intima thickening and CKD. The nuclei were stained with DAPI (blue), and NFκB-p65 was revealed using an Alexa-conjugated antibody (red). The third column shows the colocalization. Original magnification $\times 600$. The histogram represents the increase in percentage of colocalization referred to the control (** $P < 0.001$). DM, diabetes. (A high-quality digital representation of this figure is available in the online issue.)

is, however, undetermined. CKD is associated with increased generation of cytokines and adhesion molecules, suggesting an early endothelial dysfunction and up-regulation of these molecules in the artery wall (18,19). Growing evidence indicates that proinflammatory cytokines play a determinant role in the development of vascular diabetes complications, especially in the presence of high glucose levels (9). Thus, it is plausible that the negative effects of both risk factors on the artery wall can be enhanced. In other words, diabetes may accelerate the development of atherosclerosis in the presence of uremia by triggering inflammation pathways.

We focused our attention on the study of the gene expression and protein transcription of proinflammatory cytokines (IL-6 and MCP-1) and adhesion molecules (ICAM-1 and VCAM-1), factors potentially involved in the pathogenesis of atherosclerosis. Indeed, a higher expression of IL-6, MCP-1, and adhesion molecules was observed in the artery wall of type 1 diabetic patients compared with nondiabetic subjects. Activation of these inflammatory molecules could translate to more severe vascular lesions (fibrosis and luminal narrowing) and a higher c-IMT, as was observed in our diabetic patients. In fact, overexpression of IL-6 and MCP-1 was related with more advanced atherosclerotic lesions, endorsing the role of MCP-1 both

for attracting monocytes to the inflammation area and inducing collagen production by SMA-positive cells (20). In addition, c-IMT measurements correlated with VCAM-1 protein levels and the degree of arterial lumen reduction, which suggests that this parameter may be an early surrogate marker of atherosclerosis in diabetic patients who are candidates for kidney transplantation.

NFκB is a ubiquitous transcription factor that regulates the inflammatory response and whose overexpression seems to be related to type 1 diabetes (18,19). Thus, a greater NFκB expression in the arterial wall of diabetic patients seems likely. Accordingly, a significantly higher nuclear localization of p65 was seen in our type 1 diabetic patients, supporting the role of this important transcription factor in the atherosclerotic process through the increase of proinflammatory cytokines. In consonance with this finding, we also observed a significant correlation between p65 nuclear translocation and levels of MCP-1 in the type 1 diabetic group but not in nondiabetic subjects. This suggests that, although MCP-1 may be regulated in an NFκB-dependent fashion in the presence of a proinflammatory state like type 1 diabetes (21), other cytokines may be under the control of more complex interactions, especially when diabetes is absent.

Of note, histological analysis of our patients showed that intimal thickening of the IEA was mostly composed of SMA-positive cells and collagen fibers but not of CD68-positive macrophages. Thus, a pathogenic mechanism accounting for the role of proinflammatory cytokines produced by resident and infiltrating cells, in patients with diabetes and uremia, seems plausible. In early stages of atherosclerosis, intima cells recruit SMCs and SMC precursors by the release of multiple factors (cytokines and inflammatory markers). Later, these cells may switch from a contractile phenotype to a synthetic phenotype, generating *in situ* cytokines (IL-6 and MCP-1) and adhesion molecules (VCAM-1). Finally, these molecules induce the generation of fibrosis and arterial lumen reduction, contributing to perpetuating the inflammatory environment in the lesion, as previously documented (22). The fact that the production of MCP-1, IL-6, and VCAM-1 correlated with more severe vascular lesions in the presence of abundant SMA-positive cells and collagen fibers in the intima layer of our patients supports this view.

Previous histological reports from autopsies of elderly individuals who died of noncardiac diseases have documented that coronary arteries and the IEA undergo progressive narrowing with age as a result of intimal thickening, with migration of

myocytes from the media and duplication of the internal elastic lamina. These features were present in all the muscular arteries studied, and the internal thoracic artery, a mainly elastic vessel, was the only artery resistant to atherosclerosis (23,24). Although this evidence favors the interpretation that our findings in the IEA are relevant to the understanding of the vascular lesions present in other territories with predominantly muscular walls, we cannot be sure that they are generalizable to all arterial territories. A certain degree of controversy remains, because older studies had proposed the IEA as a good source for coronary artery bypass grafts based on its relative resistance to atheromatosis (25). Although no intimal calcification of the IEA was found in our study, most diabetic patients had a significant arterial lumen reduction (Supplementary Table 2). By contrast, a higher proportion of the nondiabetic patients had minimal luminal narrowing (<5%). Our molecular and cell biology studies of the IEA suggest that local vascular injury induced by the combination of diabetes and uremia may promote the overexpression of proinflammatory molecules, leading to more advanced atheromatous lesions in the IEA, which agrees with the carotid artery thickening detected by ultrasounds.

A higher prevalence and severity of medial calcification also was observed in type 1 diabetic patients. In agreement with previous reports (26), we found no relationship between the molecules studied and these lesions. Of interest, fasting glucose levels were associated with medial-layer calcification in these patients. Thus, advanced glycation end products and hemodynamic abnormalities related to type 1 diabetes may trigger signaling pathways involved in the appearance of vascular calcification (27). Glycemic control was acceptable ($HbA_{1c} < 7\%$) in 60% of our diabetic patients before starting dialysis, but we cannot rule out that a longer duration of diabetes could contribute to more severe vascular lesions. The fact that the diabetic patients were older in our study supports this argument.

Whether modulation of the inflammatory response by anti-inflammatory drugs may slow down the atheromatous process in type 1 diabetic patients, as observed in other diabetes complications (28), needs to be tested in future studies.

This study has some limitations. First, we did not determine the plasma levels of the cytokines studied. Plasma cytokine levels may vary greatly under certain clinical

conditions, such as diabetes and uremia. Thus, direct assessment of proinflammatory gene activation in vascular tissue may better reflect the pathways of inflammatory-related atheromatosis, especially in diabetic patients. In addition, we conducted a rigorous and accurate molecular and histological analysis, which affords reliability to our findings. Second, we did not assess proinflammatory cytokine expression in the artery wall of healthy subjects, but our objective was to evaluate the effect of type 1 diabetes on the atheromatosis-related inflammatory state in kidney transplantation candidates, and uremia was present in all the patients. In addition, we cannot rule out that the other molecules undetermined in this study, such as adipokines, angiotensin II, or coagulation factors, may have a relevant role in the pathogenesis of atheromatosis. Nevertheless, this was not the scope of our study, and it deserves additional research to elucidate alternative mechanisms of disease. Finally, because the sample size may be considered small, the results of this study should be taken with caution, and additional studies are required to enhance the understanding of atheromatosis-related inflammation in the presence of diabetes and uremia.

In conclusion, the findings of this study show that proinflammatory cytokines, adhesion molecules, and NF κ B may play a pivotal role in the atheromatous process in type 1 diabetic patients with CKD. These results provide potential mechanistic insights for the increased inflammation and accelerated atherosclerosis in this particular population. The understanding of these mechanisms may lead to new therapeutic strategies in this high-risk population.

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J.T. and E.S. contributed to writing the manuscript, obtained the research data for gene and protein expression, and contributed to the histological and immunohistochemical analysis. J.F. conducted the carotid echography. M.R.

and J.M.G.-P. obtained all the clinical data. A.T. contributed to the critical review of the manuscript. D.H. is the guarantor for this article, participated in the study design, performed the statistical analysis, and wrote the manuscript.

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