Adherence of Mononuclear Cells to Endothelium In Vitro Is Increased in Patients With NIDDM

M. CARANTONI, MD
F. ABBASI, MD
L. CHU, BS
Y.-D.I. CHEN, PHD
G.M. REAVEN, MD
P.S. TSAO, PHD
B. VARASTEH, BS
J.P. COOKE, PHD, MD

OBJECTIVE — To compare the binding to cultured endothelial cells of mononuclear cells isolated from healthy volunteers and patients with NIDDM.

RESEARCH DESIGN AND METHODS — Mononuclear cells were isolated from healthy volunteers (n = 11) and patients with NIDDM (n = 14) and incubated with ECV 304 cells, a human umbilical endothelial cell–derived transformed cell line. Following a period of incubation, the adherence of mononuclear cells to endothelial cells was determined.

RESULTS — Adherence of mononuclear cells from patients with NIDDM was significantly greater ($P < 0.05$) than that of cells isolated from the healthy volunteers, and this difference persisted when adjusted for age, sex, and degree of obesity. Mononuclear cell binding to ECV 304 cells correlated significantly with fasting plasma glucose ($r = 0.52$, $P < 0.01$), insulin ($r = 0.51$, $P < 0.01$), triglyceride ($r = 0.54$, $P < 0.01$), and VLDL ($r = 0.54$, $P < 0.01$) and HDL cholesterol ($r = -0.45$, $P < 0.05$) levels, but not with either total or LDL cholesterol levels or blood pressure.

CONCLUSIONS — Since the adherence of mononuclear cells to the endothelium represents the earliest step in atherogenesis, the observation that mononuclear cells from patients with NIDDM bind more avidly to cultured endothelial cells may help explain why accelerated atherosclerosis occurs in patients with NIDDM. The metabolic abnormality, or abnormalities, present in patients with NIDDM that is responsible for the enhanced adhesiveness of mononuclear cells requires further examination.

Although there is widespread agreement that coronary heart disease (CHD) is the major cause of morbidity and mortality in patients with NIDDM, it is not totally clear why this is so. Patients with NIDDM are known to have abnormalities of lipoprotein metabolism, and the increased prevalence in these individuals of high plasma triglyceride and low HDL cholesterol concentrations (2), accompanied by smaller and denser LDL particles (3), has tended to focus interest on the link between dyslipidemia and CHD. However, the importance of changes in the interaction between circulating blood elements and the vessel wall in the initiation of the atherogenic lesion has received a great deal of recent attention. Indeed, the earliest observable abnormality of the vessel wall in animal models of atherosclerosis is enhanced monocyte adherence to the endothelium (4), mediated by alterations in the expression/activity of adhesion molecules and chemotactic proteins.

In this context, two recent preliminary observations from our research group are of considerable interest. Specifically, we have shown that mononuclear cells from dyslipidemic subjects adhere with increased avidity to cultured endothelial cells (5) and that thoracic aorta isolated from diabetic rats bind monocytes to an increased degree (6). Based on these data and prior evidence that metabolic perturbations can affect mononuclear binding to endothelium (7, 8), the present study was initiated to compare adherence to cultured endothelial cells of mononuclear cells isolated from healthy subjects and patients with NIDDM.

RESEARCH DESIGN AND METHODS

— The study was performed in 25 volunteers: 14 sulfonylurea-treated NIDDM patients and 11 healthy subjects. It can be seen from the data in Table 1 that the patients with NIDDM were somewhat older, more obese as estimated by BMI, and had higher values for systolic blood pressure.

Blood was drawn after an overnight fast for the measurement of glucose, insulin, glycated hemoglobin, and lipid and lipoprotein concentrations (9) and for the isolation of mononuclear cells for adhesion studies (10). The viability of the isolated cells was assessed by trypan blue exclusion, and mononuclear cells isolated in this manner contain ~3–10% monocytes, ~45% T-lymphocytes, and ~45% B-lymphocytes.

Binding assays were performed with ECV 304 cells, a human umbilical vein endothelial cell–derived transformed cell line (11). Endothelial cells were maintained in M199 with 10% fetal calf serum, split into 35-mm diameter wells on six-well plates three days before the adhesion assays, and confluency-confirmed before the binding studies. The adhesion of mononuclear cells to endothelial cells was assessed using previously established methods (12,13). Briefly, freshly isolated human mononuclear cell suspensions (3×10^6/ml final concentration) were added to the wells containing confluent endothelial monolayers; the six-well plates were transferred to a rocking platform, where they were rocked for 30 min at room temperature, turning the six-well plates 90° at 15 min. After 30 min, nonadherent cells were removed and plates rocked for an additional 5 min with fresh binding buffer.

From the Department of Medicine (M.C., F.A., L.C., Y.-D.I.C., G.M.R., P.S.T., B.V., J.P.C.), Stanford University School of Medicine, Stanford, and Shaman Pharmaceuticals (G.M.R.), South San Francisco, California.
Address correspondence and reprint requests to Gerald M. Reaven, MD, Shaman Pharmaceuticals, 312 E. Grand Ave., South San Francisco, CA 94080-4812.
Received for publication 23 January 1997 and accepted in revised form 28 May 1997.
Abbreviations: CHD, coronary heart disease; mAb, monoclonal antibody.

Table 1—*Clinical characteristics of control subjects and patients with NIDDM*

	Control subjects	NIDDM patients	*P* value
n	11	14	
Age (years)	50 ± 3	61 ± 2	<0.001
Sex (M/F)	6/5	10/4	NS
Known diabetes duration (years)	—	6 ± 1	—
BMI (kg/m²)	24.5 ± 0.9	28.1 ± 1.3	<0.05
Smokes (yes/no)	1/10	2/12	NS
Systolic blood pressure (mmHg)	118 ± 3	140 ± 5	<0.005
Diastolic blood pressure (mmHg)	74 ± 3	80 ± 3	NS
Glucose (mg/dl)	93 ± 3	198 ± 13	<0.001
Insulin (μU/ml)	9 ± 1	19 ± 6	NS
Glycated hemoglobin (%)	—	12 ± 1	—
Triglyceride (mg/dl)	96 ± 14	247 ± 34	<0.005
Total cholesterol (mg/dl)	202 ± 9	207 ± 14	NS
VLDL cholesterol (mg/dl)	19 ± 3	46 ± 6	<0.001
HDL cholesterol (mg/dl)	52 ± 2	36 ± 2	<0.001
LDL cholesterol (mg/dl)	131 ± 7	121 ± 10	NS

Data are *n* or means ± SE. NS, not significant.

Binding buffer was then replaced with Hank's balanced salt solution containing 2% gluteraldehyde to fix the remaining cells. Adherent cells were quantified by videomicroscopy, using a computer-aided image analysis system (Image Analyst, Automatix, Boston, MA).

In six individuals, three NIDDM patients and three control subjects, additional experiments were performed to identify the subtypes of mononuclear cells found in the bound populations (14,15). After the nonadherent cells were washed away, the remaining cells (bound mononuclear cells and endothelial cells) were detached with EDTA (5 mmol/l) to form single-cell suspensions, and 10% fetal calf serum was added to ensure cell viability. To quantify the relative proportion of different cell types found in the mixed populations, cell suspensions (2×10^5/ml) were incubated for 25 min on ice with anti-human monoclonal antibodies (mAbs) to specify cell surface markers. Specifically, mAbs used were FITC-anti-CD3 (Pharmagen; 1:100) to identify T-lymphocytes and PE-anti-CD14 (Pharmagen, 1:100) to identify monocytes. Cells were then washed of excess antibodies and the mixed populations analyzed by flow cytometry, using a highly modified dual-laser FACS IV (Becton Dickinson, San Jose, CA). Endothelial cells were electronically gated out by their size in comparison to circulating mononuclear cells. The remaining cells (not labeled by specific mAbs and not gated out by size) were considered to be B-lymphocytes. Fluorescently labeled beads were employed as calibrators during cell counting to allow for the quantification of the absolute number of cells.

Data are expressed as mean ± SE. Student's nonpaired *t* test was used to compare the two groups in terms of their demographic and metabolic covariates. Differences in mononuclear cell binding were compared by one-way analysis of variance, before and after adjusting for differences in age, sex, and BMI. Finally, the relationship between mononuclear cell binding and relevant covariates was estimated by Pearson's simple and partial correlation coefficients.

RESULTS — In addition to fasting hyperglycemia and elevated glycated hemoglobin levels, the results in Table 1 show that patients with NIDDM also had significantly higher fasting plasma insulin, triglyercide, and VLDL cholesterol concentrations and lower HDL cholesterol concentrations. However, the two groups had similar values for total fasting cholesterol and LDL cholesterol concentrations.

The binding of isolated mononuclear cells isolated from each group to cultured endothelial cells is illustrated in Fig. 1. The data in the left panel represent the actual experimental results. The adherence of mononuclear cells from patients with NIDDM was significantly higher ($P < 0.005$) than that of cells isolated from the control population. Since patients with NIDDM were older and more obese, the binding data were adjusted for differences in age, sex, and BMI. If anything, the difference between the two groups was greater after adjustment.

The data in Fig. 1 present the binding of the entire population of mononuclear cells. In a subset of the two experimental groups (three control subjects and three NIDDM patients), FACS analysis of the bound cells was performed. There tended to be an enrichment of monocytes in the NIDDM group (NIDDM patients vs. control subjects, 15 vs. 18%), although this did not reach statistical significance, possibly due to the small size of the experimental groups. However, an increase in monocyte binding cannot entirely explain the increase

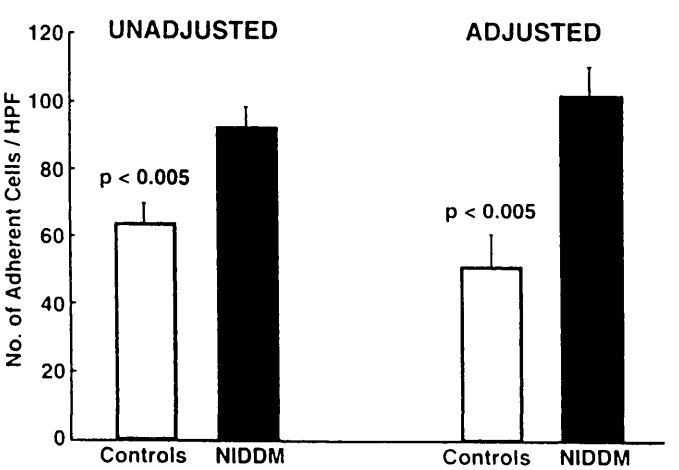

Figure 1—*Comparison of the number of mononuclear cells isolated from the control subjects and NIDDM patients adherent to cultured endothelial cells per high power field (HPF). Data in the left panel present the actual values, and data in the right panel present the comparison between the two groups adjusted for differences in age, sex, and BMI.*

Table 2—*Correlation coefficients (r) between mononuclear cell binding and metabolic variables*

Variable	Simple		Partial	
	r	*P*	*r*	*P*
Systolic blood pressure	0.35	NS	0.24	NS
Diastolic blood pressure	0.26	NS	0.14	NS
Fasting glucose	0.52	<0.01	0.39	0.09
Fasting insulin	0.44	<0.05	0.44	<0.05
Triglyceride	0.54	<0.01	<0.46	<0.05
Total cholesterol	0.33	NS	0.32	NS
VLDL cholesterol	0.54	<0.01	0.46	<0.05
LDL cholesterol	−0.08	NS	0.25	NS
HDL cholesterol	−0.45	<0.05	<0.42	0.07

NS, not significant.

in bound mononuclear cells. It is possible that NIDDM induces a generalized activation of mononuclear cells, in which case other determinants of adhesion and infiltration determine the preferential accumulation of monocytes and T-lymphocytes in vascular lesions.

The results in Table 1 indicate that there were multiple metabolic differences between the two groups of patients. To gain insight into the association between these changes and mononuclear cell binding, simple correlation coefficients were calculated between metabolic variables and binding to endothelial cells. The results in Table 2 demonstrate that significant relationships were seen in the entire population between mononuclear cell binding and fasting plasma glucose, insulin, triglyceride, and VLDL and HDL cholesterol concentrations. However, significant correlations were not seen between binding and either total cholesterol or LDL cholesterol concentrations or blood pressure. Partial correlations were also calculated for all these variables, adjusting for differences in age, sex, and BMI. These results are also shown in Table 2, and although decreased in magnitude, the general association between mononuclear cell binding and metabolic variables remained.

CONCLUSIONS — Evidence has been previously published showing increased adherence to endothelium of mononuclear cells isolated from nondiabetic subjects with hypercholesterolemia, with and without associated hypertriglyceridemia (5,7,8). More recently, enhanced binding of monocytes to endothelial cells has also been demonstrated in patients with NIDDM who were both hypercholesterolemic and hypertriglyceridemic, compared with control subjects (16). In contrast to our results, Hoogerbrugge et al. (16) found that the degree of monocyte binding was related to both plasma triglyceride and LDL cholesterol concentrations but not glycemia. Our results differ from their findings in that we could discern a relationship between mononuclear cell binding and plasma triglyceride but not LDL cholesterol concentrations. We also found a relationship of marginal statistical significance ($r = 0.39$, $P = 0.09$) between the degree of binding and the level of glycemia, even when the correlation coefficient was corrected for differences in age, sex, and BMI. The difference between our results and those of Hoogerbrugge et al. (16) regarding the effect of glycemia on mononuclear cell binding may be due to the fact that the vast majority of their patients were insulin-treated. The disparity with respect to LDL cholesterol concentrations could be explained by the fact that our patients were not hypercholesterolemic. On the other hand, we believe that these differences in detail between the two studies are not as important at this juncture as the fact that the results of both show that mononuclear cells from patients with NIDDM bind to endothelial cells with enhanced avidity. Taken together, their results and ours focus on the view that this phenomenon may help explain the accelerated atherogenesis seen in these patients. Given the importance of mononuclear cell binding to endothelium in the initiation of atherogenesis and the uncertainty as to why the risk of CHD is accentuated in patients with NIDDM, we believe these observations to be potentially of great importance. Consequently, we plan to initiate additional studies aimed at defining the metabolic abnormality (or abnormalities) in patients with NIDDM that may account for this phenomenon.

Acknowledgments — This study was supported by National Institutes of Health Research Grants (HL-08506, RR-00070, and HL-2HLC 641) and a Mentor Award from the American Diabetes Association.

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