Immune Response to Glycated and Oxidized LDL in IDDM Patients With and Without Renal Disease

EIJA KORPINEN, MD
PER-HENRIK GROOP, MD
HANS K. ÅKERBLOM, MD
OUTI VAARALA, MD

OBJECTIVE — To study autoantibodies to oxidized and glycated LDL in IDDM patients with and without diabetic nephropathy and in nephropathy-related macroangiopathy.

RESEARCH DESIGN AND METHODS — The study included 101 IDDM patients with a long duration of diabetes and 54 healthy subjects. Patients were divided into two groups according to their median urinary albumin excretion rate (AER); the normoalbuminuric group had AER <20 μg/min and the albuminuric group >200 μg/min. The groups were matched for age and BMI, and the two diabetic groups were matched for duration of diabetes and glycemic control. Antibodies against oxidized LDL (using malondialdehyde-modified LDL as the antigen) and against glycated LDL were measured by enzyme-linked immunosorbent assay (ELISA).

RESULTS — The mean antibody levels against glycated LDL were higher in IDDM patients (0.305 ± 0.399) than in healthy subjects (0.166 ± 0.22 optical density [OD]; $P = 0.019$), but levels did not differ significantly between normoalbuminuric and albuminuric IDDM patients (0.258 ± 0.354 vs. 0.388 ± 0.459, respectively). Among the three groups, antibody levels to oxidized LDL did not differ. IDDM patients showed an inverse correlation between antibodies to oxidized LDL and HbA_1 ($r = -0.211$, $P = 0.04$). The antibody levels to glycated and oxidized LDL did not differ among albuminuric IDDM patients with or without clinical macroangiopathy.

CONCLUSIONS — Antibodies to glycated and oxidized LDL do not seem to associate with diabetic nephropathy or nephropathy-related macroangiopathy.

IDDM is associated with an increased risk of diabetic microangiopathy and atherosclerosis. The predisposition to macrovascular disease is related to microvascular disease, since patients with diabetic nephropathy have a higher risk of getting atherosclerotic disease than patients without nephropathy (1). Diabetic nephropathy is known to be accompanied by changes in the lipid profile favoring atherogenesis (2).

Oxidized LDL plays an important role in the atherogenesis (3). It accumulates in macrophages, which become foam cells found in a fatty streak within the arterial intima. Autoantibodies to oxidized LDL seem to be involved in the atherosclerotic process (3) and their high level has been shown to predict myocardial infarction (4).

Glucose reacts nonenzymatically with proteins forming early glycation products, which further develop to advanced glycation end products (5). LDL may be a target for glycation as well as for oxidation. Antibodies to glycated LDL have been reported in both IDDM (6) and NIDDM (7). In patients with NIDDM, antibodies to glycated LDL or to oxidized LDL did not show any relation to vascular complications (7). The occurrence of antibodies to oxidized and/or glycated LDL in IDDM patients with and without renal disease and their relation to glycemic control and macrovascular complications are not known. We therefore determined the occurrence of antibodies to glycated and oxidized LDL in normoalbuminuric IDDM patients, in albuminuric IDDM patients, and in healthy subjects.

RESEARCH DESIGN AND METHODS

Subjects

The study included 101 IDDM patients with a long duration of diabetes (>15 years) (Guy's Hospital, London and Central University Hospital, Helsinki) and 54 healthy subjects. The patients were divided into two groups according to their median urinary AER of three consecutive timed overnight urine collections. The normoalbuminuric group had urinary AER <20 μg/min and the albuminuric group >200 μg/min. The groups were matched for age and BMI, and the diabetic patients were matched for duration of diabetes and glycemic control. The normoalbuminuric IDDM patients or healthy subjects had not been treated for hypertension, and patients taking diuretics or β-blockers were excluded from the study. All participants had normal renal function determined as normal serum creatinine. The presence of macroangiopathy was defined as a positive history for cardiovascular events (angina, myocardial infarction), elicited and confirmed by the study physician, or as a history of intermittent claudication associated with one or more absent foot pulses. Clinical characteristics of the subjects are shown in Table 1. The study was approved by the ethics committees of the Department of Medicine, University of Helsinki, Finland, and Guy's Hospital, London.

Methods

Antibodies to oxidized LDL were measured by enzyme-linked immunosorbent assay (ELISA) using malondialdehyde (MDA)-modified LDL and native LDL as antigens, as previously described (4). MDA-LDL represents a prominent epitope of oxidized LDL (8). The ELISA for antibodies to glycated LDL was otherwise similar to the ELISA for antibodies to oxidized

From the Children's Hospital (E.K., H.K.Å., O.V.) and Department of Medicine (P.-H.G.), Division of Nephrology, University of Helsinki; and the Department of Biochemistry (E.K., O.V.), National Public Health Institute, Helsinki, Finland.

Address correspondence and reprint requests to Eija Korpinen, MD, The Children's Hospital, Stenbäckinkatu 11, SF-00290 Helsinki, Finland. E-mail: eija.korpinen@hyks.mailnet.fi.

Received for publication 21 November 1996 and accepted in revised form 19 March 1997.

AER, albumin excretion rate; ELISA, enzyme-linked immunosorbent assay; MDA, malondialdehyde; OD, optical density.

Table 1—*Characteristics of study subjects*

	Normoalbuminuric IDDM patients	Albuminuric IDDM patients	Control subjects
n	64	37	54
Age (years)	37.6 ± 10.1	37.8 ± 8.6	38.6 ± 10.2
BMI (kg/m²)	23.9 ± 2.6	24.8 ± 7.1	24.3 ± 3.5
IDDM duration (years)	22.5 ± 8.6	24.4 ± 7.1	—
HbA_1 (%)	9.4 ± 1.6	9.7 ± 1.5	6.0 ± 0.5
LDL (mmol/l)	2.7 ± 0.8	3.2 ± 0.9	3.1 ± 0.7
AER (μg/min)	7 (0–19)	433 (48–1,756)	4 (0–9)

Data are means ± SD or median (range).

LDL, but glucose-modified LDL was used as the antigen. Glycated LDL was prepared by incubating native LDL in phosphate-buffered saline containing 80 mmol/l glucose in a nitrogen-saturated atmosphere for 7 days at room temperature. The glycated LDL fractions were separated from the glucose-incubated LDL preparation by Sephadex-G25 (Pharmacia) desalting column. Native LDL was treated in the same way in the absence of glucose.

For ELISA, half of a polystyrene plate (Nunc) was coated with native LDL and the other half with either MDA-LDL or glycated LDL (4). Serum samples were studied at dilution 1:50. Results were expressed as optical density (OD) values, and binding to oxidized or glycated LDL was calculated by subtracting OD values for native LDL from those for MDA-modified or glycated antigen, respectively. In both assays, OD values for the modified antigen correlated with the final calculated values (for oxidized LDL, $r = 0.862$; for glycated LDL, $r = 0.861$).

Statistical analysis

The results are expressed as means ± SD, if not otherwise indicated. Comparisons between groups were performed with the Mann-Whitney *U* test. Correlations were analyzed by Spearman's rank correlations.

RESULTS — The mean antibody level against glycated LDL was higher in IDDM patients than in healthy subjects (0.305 ± 0.399 vs. 0.165 ± 0.22; $P = 0.019$). Albuminuric IDDM patients had higher antibody levels to glycated LDL (0.388 ± 0.459) than normoalbuminuric patients (0.258 ± 0.354), although statistically, not significantly (Fig. 1*A*). Antibodies to glycated LDL did not correlate with HbA_1 ($r = 0.037$) or AER ($r = 0.024$).

Antibody levels to oxidized LDL did not differ among the three groups, but IDDM patients as a group had lower antibody levels to oxidized LDL than healthy control subjects (0.140 ± 0.077 vs 0.180 ± 0.105 OD; $P = 0.014$) (Fig. 1*B*). Antibodies to oxidized LDL correlated inversely with HbA_1 in IDDM patients ($r = -0.211$, $P = 0.04$; Fig. 2*A*), the inverse correlation being even stronger in albuminuric patients ($r = -0.458$; $P = 0.007$; Fig. 2*B*). Antibodies to oxidized and glycated LDL did not correlate in any of the groups.

Normoalbuminuric patients had lower LDL concentrations than albuminuric IDDM patients (2.7 ± 0.8 vs. 3.2 ± 0.9 mmol/l, $P = 0.006$) or healthy subjects (3.1 ± 0.7 mmol/l, $P = 0.005$). LDL concentrations did not correlate with antibodies to oxidized LDL or to glycated LDL in IDDM patients ($r = -0.006$ and $r = -0.174$, respectively).

In the albuminuric group, no significant difference was found in antibody levels or in other laboratory or clinical parameters between patients with and without macroangiopathy (Table 2). Only one patient in the normoalbuminuric group had clinical macroangiopathy.

CONCLUSIONS — It is obvious that continuous hyperglycemia explains elevated antibody levels to glycated LDL in diabetic patients. Although degree of LDL glycation is reported to be proportional to long-standing serum glucose level (9), in our diabetic patients, the antibody response to glycated LDL did not correlate with HbA_1. Thus, the antibody response may not directly reflect the degree of LDL glycation, but could be affected by other factors regulating immune response, such as HLA-type or cytokine profile. Albuminuric patients did not show higher antibody levels to glycated LDL than normoalbuminuric patients and thus it seems unlikely that antibodies to glycated LDL play any crucial role in the pathogen-

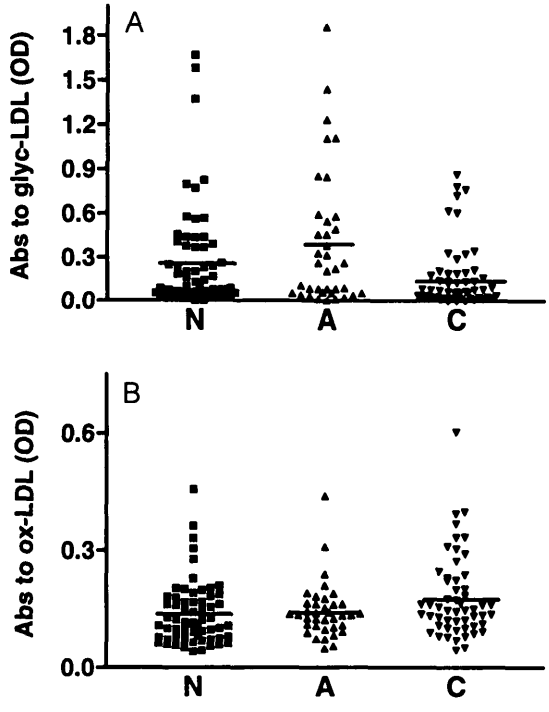

Figure 1—A: *antibodies to glycated LDL as OD units in normoalbuminuric (N) and albuminuric (A) IDDM patients and in healthy control (C) subjects. Means expressed as solid lines.* B: *antibodies to oxidized LDL as OD units in normoalbuminuric (N) and albuminuric (A) IDDM patients and in healthy control (C) subjects. Means expressed as solid lines.*

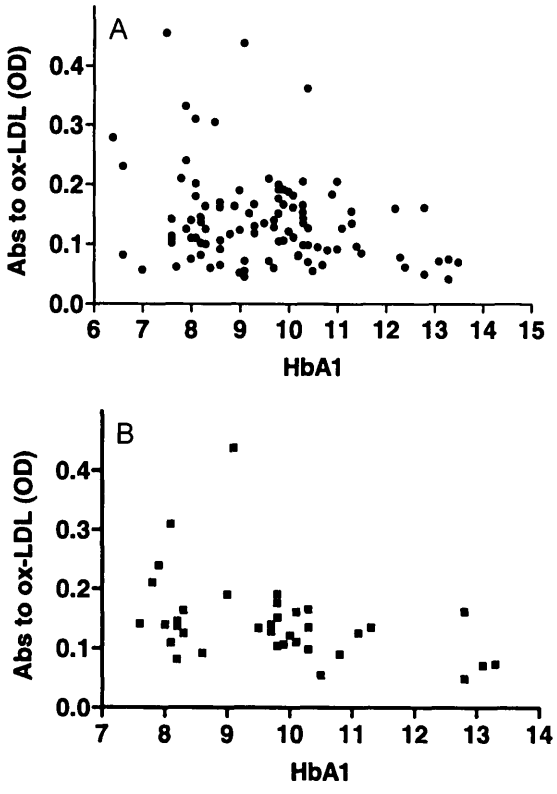

Figure 2—A: *antibodies to oxidized LDL in relation to HbA_1 in IDDM patients* (r = *−0.211*, P = *0.04*). B: *correlation between HbA_1 and antibodies to oxidized LDL in albuminuric IDDM patients* (r = *−0.46*, P = *0.007*)

esis of diabetic nephropathy or nephropathy-related atherogenesis.

We could not find elevated levels of antibodies to oxidized LDL in IDDM patients and the antibodies did not show relation with renal disease or macroangiopathy. Antibody response against oxidized LDL alone, although predictive of atherosclerotic events (4,10), may not be a significant factor in the atherogenesis in diabetic milieu, according to our study and the report by Uusitupa et al. (11).

Interestingly, an inverse correlation between HbA_1 and antibodies to oxidized LDL was found in IDDM patients. In accordance, Uusitupa et al. (11) could not demonstrate elevated levels of antibodies to oxidized LDL in hyperglycemic NIDDM patients. In oxidation and glycation of LDL, lysine residues are conjugated by MDA and glucose, respectively. Therefore, a high degree of glycation may inhibit the formation of MDA-conjugated LDL and, consequently, the development of autoantibodies.

Table 2—*The presence of macroangiopathy in the albuminuric IDDM patients, and its relation to clinical and laboratory parameters*

	Macroangiopathy		
	Yes	No	*P* value
n	18	19	—
Sex (M/F)	8/10	9/10	—
Age (years)	39.9 ± 9.2	35.8 ± 7.7	0.153
IDDM duration (years)	25.5 ± 7.6	23.4 ± 6.7	0.316
HbA_1 (%)	9.6 ± 1.7	9.8 ± 1.4	0.557
LDL	3.1 ± 0.9	3.2 ± 0.9	0.090
Anti-glycated LDL (OD)	0.353 ± 0.506	0.421 ± 0.421	0.438
Anti-oxidized LDL (OD)	0.141 ± 0.057	0.150 ± 0.083	0.843

Data are means ± SD or *n*.

Supporting this, Babiy et al. (12) showed that in vitro glycated LDL is more resistant to oxidation than is native LDL. In contrast, high oxidative susceptibility of in vivo isolated LDL and a correlation between LDL glycation and oxidation have been reported in NIDDM patients (13). However, we emphasize that antibody response to oxidized and glycated LDL does not directly reflect the amount of oxidized and glycated LDL in plasma.

In conclusion, elevated levels of antibodies to glycated LDL found in IDDM patients do not seem to be associated with nephropathy or nephropathy-related atherogenesis. Surprisingly, the levels of antibodies to oxidized LDL correlated inversely with HbA_1 levels in IDDM patients. This suggests that hyperglycemia does not contribute to the formation of antibodies to oxidized LDL, despite the increase in degree of LDL glycation in IDDM.

Acknowledgments— The study was supported by grants from the Foundation for Pediatric Research in Finland (E.K.) and the Sigrid Juselius Foundation (P.-H.G.).

We thank Dr. Georg Alfthan for preparing MDA-conjugated LDL.

References

1. Jensen T, Borch-Johnsen K, Kofoed-Enevoldsen A, Deckert T: Coronary heart disease in young type 1 (insulin-dependent) diabetic patients with and without diabetic nephropathy: incidence and risk factors. *Diabetologia* 30:144–148, 1987
2. Groop P-H, Elliott T, Ekstrand A, Franssila-Kallunki A, Friedman R, Viberti GC, Taskinen M-R: Multiple lipoprotein abnormalities in type I diabetic patients with renal disease. *Diabetes* 45:974–979, 1996
3. Witztum JL: The oxidation hypothesis of atherosclerosis. *Lancet* 344:793–795, 1994
4. Puurunen M, Mänttäri M, Manninen V, Tenkanen L, Alfthan G, Ehnholm C, Vaarala O, Aho K, Palosuo T: Antibody against oxidized low-density lipoprotein predicting myocardial infarction. *Arch Intern Med* 154:2605–2609, 1994
5. Brownlee M: Glycation products and the pathogenesis of diabetic complications. *Diabetes Care* 15:1835–1843, 1992
6. Witztum JL,Steinbrecher UP, Kesaniemi A, Fisher M: Autoantibodies to glucosylated proteins in the plasma of patients with diabetes mellitus. *Proc Natl Acad Sci USA* 81:3204–3208, 1984
7. Bellomo G, Maggi E, Poli M, Agosta FG, Bollati P, Finardi G: Autoantibodies against oxidatively modified low-density lipoproteins in NIDDM. *Diabetes* 44:60–66, 1995

8. Palinski W, Rosenfeld ME, Ylä-Herttuala S, Gurtner GC, Socher SS, Butler SW, Parthasarathy S, Carew TE, Steinberg D, Witztum JL: Low density lipoprotein undergoes oxidative modification in vivo. *Proc Natl Acad Sci USA* 86:1372–1376, 1989
9. Lyons TJ, Baynes JW, Patrick JS, Colwell JA, Lopes-Virella MF: Glycosylation of low density lipoprotein in patients with type 1 (insulin-dependent) diabetes: correlations with other parameters of glycaemic control. *Diabetologia* 29:685–689, 1986
10. Salonen JT, Ylä-Herttuala S, Yamamoto R, Butler S, Korpela H, Salonen R, Nyyssönen K, Palinski W, Witztum JL: Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* 339:883–888, 1992
11. Uusitupa MIJ, Niskanen L, Luoma J, Vilja P, Mercuri M, Rauramaa R, Ylä-Herttuala S: Autoantibodies against oxidized LDL do not predict atherosclerotic vascular disease in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 16:1236–1242, 1996
12. Babiy AV, Gebicki JM, Sullivan DR, Willey K: Increased oxidizability of plasma lipoproteins in diabetic patients can be decreased by probucol therapy and is not due to glycation. *Biochem Pharmacol* 43:995–1000, 1992
13. Bowie A, Owens D, Collins P, Johnson A, Tomkin GH: Glycosylated low density lipoprotein is more sensitive to oxidation: implications for diabetic patient? *Atherosclerosis* 102:63–67, 1993