Autoantibodies Against Methylglyoxal-Modified Apolipoprotein B100 and ApoB100 Peptide Are Associated With Less Coronary Artery Atherosclerosis and Retinopathy in Long-Term Type 1 Diabetes

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OBJECTIVE

Methylglyoxal (MGO), a reactive aldehyde forming advanced glycation end products (AGEs), is increased in diabetes and recognized by the immune system, resulting in anti-AGE–specific autoantibodies. The association of these immune responses with macro- and microvascular complications in type 1 diabetes remains unclarified. We investigated associations between MGO-modified apolipoprotein B100 (apoB100) and apoB100 peptide 5 (MGO-p5) autoantibodies and coronary atherosclerosis and retinopathy in type 1 diabetes.

RESEARCH DESIGN AND METHODS

IgM and IgG against MGO-apoB100 and MGO-p5 were measured by ELISA in plasma from 103 subjects with type 1 diabetes and 63 control subjects (Dialong study) and in a replication cohort of 27 subjects with type 1 diabetes (Oslo study). Coronary atherosclerosis was assessed by computed tomography coronary angiography or intravascular ultrasound. Retinopathy was classified by retinal photos.

RESULTS

MGO-apoB100 IgM and MGO-p5 IgM levels were higher in subjects with diabetes with no coronary artery stenosis compared with subjects with significant stenosis (median [interquartile range]: 96.2 arbitrary units [AU] [71–126.8] vs. 54 AU [36.1–85.4], $P = 0.003$ for MGO-apoB100; and 77.4 AU [58–106] vs. 36.9 AU [28.9–57.4], $P = 0.005$ for MGO-p5). MGO-apoB100 IgM and MGO-p5 IgM were associated with less severe coronary stenosis after adjusting for confounders (odds ratio 0.2 [95% CI 0.05–0.6], $P = 0.01$; and 0.22 [0.06–0.75], $P = 0.02$). The inverse association of MGO-p5 IgM and coronary stenosis was confirmed in the replication cohort. Subjects with proliferative retinopathy had significantly lower MGO-apoB100 IgM and MGO-p5 IgM than those with background retinopathy.

CONCLUSIONS

Autoantibodies against AGE-modified apoB100 are inversely associated with coronary atherosclerosis and proliferative retinopathy, suggesting vascular protective effects of these autoantibodies in type 1 diabetes.

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Patients with type 1 diabetes have an accelerated progression of atherosclerosis and increased cardiovascular morbidity and mortality (1). The risk of cardiovascular disease (CVD) in type 1 diabetes is increased twofold (2). Further, the presence of proliferative diabetic retinopathy (PDR) increases the risk for CVD, independently of traditional cardiovascular risk factors (3). An association between retinopathy status and coronary artery calcification (CAC) burden in long-term type 1 diabetes has been described (4). However, the molecular mechanisms behind the increased risk of CVD in individuals with type 1 diabetes and the interconnection between CVD and PDR are not fully understood. One suggested mechanism is the increased production of advanced glycation end products (AGEs) (5). AGEs are formed by nonenzymatical reactions of reducing sugars with amino groups of proteins, which produces a class of irreversibly cross-linked and fluorescent components. Methylglyoxal (MGO) is a precursor to AGE formation, and studies have found elevated serum levels of MGO in subjects with diabetes (6). The MGO-derived hydroimidazalone (MG-H1) is associated with retinopathy in type 1 diabetes (7) and may be involved in deleterious retinal changes, as MGO accumulation can result in an increased angiopoietin 2 expression in endothelial cells thought to play a role in proliferative retinopathy (8). Of particular interest in atherosclerosis is MGO modification of LDL-cholesterol (LDL-c), one of the main components of atherosclerotic plaques. It has been shown that MGO modification of LDL results in the formation of small, dense LDL particles (9), which are considered as particularly atherogenic. In addition, stimulation of macrophages with MGO-modified LDL induces the formation of foam cells (10). Both small, dense LDL particles and foam cells are linked to atherosclerosis and coronary artery stenosis (9,11).

AGE-modified proteins are targeted by the immune system, resulting in the generation of AGE-specific autoantibodies (12). Autoantibodies against modified apolipoprotein B100 (apoB100) may protect against atherosclerosis by blocking the uptake of modified LDL in macrophages (13). We have previously shown that high levels of IgM against MGO-modified apoB100, present in LDL, are associated with less severe and a lower risk of progression of CAC in subjects with type 2 diabetes, as well as a lower risk of developing cardiovascular events in subjects without diabetes (14,15). In patients with type 2 diabetes, those with retinopathy had higher levels of malondialdehyde-modified autoantibodies against apoB100 peptide 45 and peptide 210. In addition, glycated oxidized LDL induced apoptosis in human retinal capillary pericytes (16). This suggests LDL oxidation/glycation to be involved in both micro- and macrovascular complications in type 2 diabetes (17). ApoB100 peptide 5 (p5) (18) is thought to be a novel matrix-binding peptide, which may potentially play an important role in the development of atherosclerosis. Poor glycemic control is found to be associated with cardiac autoimmunity in type 1 diabetes, and clinical studies have shown that the antigenic constitution of immune complexes may have an effect on the development of CVD (19,20). Cardiac autoantibodies are also associated with an increased risk of CVD, suggesting a role for autoimmune mechanisms in the development of CVD in diabetes (21). However, the association of immune responses with macro- and microvascular complications in type 1 diabetes is not known in detail and needs to be further investigated. Identification of factors associated with absence of vascular complications in long-term survival of type 1 diabetes is important for better treatment as well as development of new drugs that can prevent or reduce these complications.

In the current study, our main objectives were to determine if autoantibody levels against MGO-apoB100 or MGO-apoB100 p5 (MGO-p5) were associated with coronary artery stenosis, coronary artery calcium score, and retinopathy in long-term type 1 diabetes and to determine if these autoantibodies are increased between individuals with and without diabetes.

RESEARCH DESIGN AND METHODS

Study Population

The Dialong study is a cross-sectional, controlled study on long-term survivors of type 1 diabetes conducted in 2015. Patients with type 1 diabetes from 1970 or earlier attending a specialized diabetes center were included (22). Out of 136 eligible patients, 103 joined the coronary artery disease substudy (76% of the total population). The control group without diabetes consisted of spouses/friends of patients with type 1 diabetes. First-degree relatives were excluded. The study was approved by the regional ethics committee, and all participants gave informed consent. HbA_1/HbA_{1c} measurements from 1980 to 2015 were collected, and the presence and degree of coronary artery disease were evaluated by clinical history and CAC imaging, and the degree of stenosis was determined by computed tomography coronary angiography (CTCA). The mean HbA_{1c} of 7.4 ± 0.8% (57.8 ± 8.6 mmol/mol) in the group with diabetes was similar to the average HbA_{1c} for all individuals with type 1 diabetes with 45 years' duration registered in the Norwegian Diabetes Register of 7.6 ± 0.9% (59.3 ± 10.4 mmol/mol) ($P = 0.08$). Retinopathy was evaluated with a wide-angle camera based on SLO technique (Optos Daytona). Plasma samples were collected from all participants after an overnight fast and immediately frozen at −80°C (22).

We recruited a second population with long-term type 1 diabetes for a confirmatory analysis of the association between autoantibodies and CAD. This cohort was from the Oslo study originated in 1982, of whom a subpopulation of 27, not significantly different from the total group regarding age and glycemic control, had an invasive coronary angiography with intravascular ultrasound (IVUS) performed (23).

MGO-apoB100 and MGO-p5 Antibody ELISA

MGO-apoB100 and MGO-p5 were generated by incubation of apoB100 (Calbiochem, La Jolla, CA) and p5 (CSFIL KTSQC TLKEV YGFNP) (TAG Copenhagen, Copenhagen, Denmark), respectively, with 100 mmol/L MGO (Sigma-Aldrich, St. Louis, MO) in 0.2 mol/L PBS at 37°C for 24 h. The modified proteins or peptides were subsequently dialyzed against PBS (0.14 mol/L NaCl, 0.0027 mol/L KCl, and 0.010 mol/L phosphate, pH 7.4) before storage at −20°C. AGE modification was verified: *1*) by demonstrating a 10.8-fold increase in AGE-specific fluorescence (excitation 370 nm and emission 440 nm) of MGO-apoB100 and a threefold increase in MGO-p5 relative to native protein or peptide, *2*) by argpyrimidine fluorescence (excitation 320 nm and emission

380 nm) demonstrating an increase in MGO apoB100 and for MGO-p5 relative to native protein or peptide, and *3*) by AGE carboxymethyllysine (CEL) epitopes on MGO-apoB100 and MGO-p5 measured by ELISA using a monoclonal anti-CEL antibody (KNH-30; Cosmo Bio, Tokyo, Japan) (absorbance 405 nm) (Supplementary Fig. 1*A* and *B*). Briefly, MGO-apoB100 or MGO-p5 was coated on microtiter plates, and wells were blocked with SuperBlock (Thermo Fisher Scientific, Rockford, IL) and incubated with anti-CEL (0.2 μg/mL), followed by biotinylated anti-mouse IgG (Vector Laboratories, Burlingame, CA) and alkaline phosphatase–conjugated streptavidin.

ELISAs measuring IgM and IgG in plasma against MGO-apoB100 and MGO-p5 were performed essentially according to a modified version used by Fredrikson et al. (17). Absorbance values were normalized to a control plasma pool (pooled from 11 individuals without diabetes) and are presented as arbitrary units (AU) (percentage absorbance compared with the plasma control pool). The intra-assay coefficients of variation were 3.6% for MGO-apoB100 IgM, 5.6% for MGO-p5 IgM, 11.7% for MGO-apoB100 IgG, and 10.8% for MGO-p5 IgG. The interassay coefficients of variation were 14% for MGO-apoB100 IgM, 6.5% for MGO-p5 IgM, 16% for MGO-apoB100 IgG, and 11.5% for MGO-p5 IgG. Antibody specificity was determined by a soluble-phase competitive ELISA (Supplementary Fig. 1*C–F*).

CTCA and IVUS

The participants without previous coronary heart disease ($n = 88$) in the group with diabetes and control subjects ($n = 60$) of the Dialong study were referred to CTCA (24). CAC and CTCA imaging were performed using a Dual Source CT scanner (Somatom Definition Flash; Siemens, Erlangen, Germany). CAC levels are given in Agatston units. The CTCA imaging protocol has been previously described (25). Normal coronary arteries were defined as no detected plaque in any of the coronary arteries on CTCA, nonsignificant stenosis as 1–50% lumen diameter stenosis, and significant artery stenosis as having >50% lumen diameter stenosis in any coronary segment.

An IVUS system with an automatic pullback device was used in the confirmatory Oslo study (iLab; Boston Scientific Corporation, Sunnyvale, CA). IVUS images were acquired at a rate of 30 frames/s and pullback speed of 0.5 mm/s. Technically satisfactorily IVUS images of all three coronary segments were performed. By IVUS, an intima thickness >0.3 mm was considered significant. Percent atheroma volume (PAV) was calculated using the equation PAV = (EEMarea − lumen area)/EEMarea × 100 (EEM, external elastic membrane).

Retinopathy

Retinopathy was categorized as none, background, or proliferative (pan-retinal photocoagulation scars or proliferative findings) retinopathy based on retina photos (wide-angle camera based on SLO technique [Optos Daytona]) taken of the group with type 1 diabetes during the study and analyzed by one certified ophthalmologist.

Statistical Analysis

The distributions of continuous variables were assessed using histograms, Q-Q plots, skewness, and kurtosis. Skewed variables were log-transformed before statistical tests. Clinical characteristics, autoantibody levels, and CTCA outcome measurements were compared between the groups using two-tailed Student *t* test or Mann-Whitney *U* test for continuous and χ^2 for categorical data. Spearman correlation analyses were performed to assess correlations between autoantibody levels and the continuous CTCA, CAC score, or IVUS markers. Differences among more than two groups were analyzed by ANOVA for normally distributed and Kruskal-Wallis test for nonnormally distributed variables. Odds ratios (ORs) were calculated using binary logistic regression analyses, which were performed both in univariate analyses and multivariate analyses adjusting for possible confounders. Variables for the model were chosen based on significant univariable associations with anti–MGO-apoB100 or anti–MGO-p5 or previously known to be associated with the outcome measures. There were very few smokers among the participants; therefore, this variable was not included in the model even if smoking is a known risk factor for CVD. Variables found to be best fitted for the model included were age, sex, HbA_{1c}, LDL, systolic blood pressure, estimated glomerular filtration rate (eGFR), and proliferative retinopathy. Persistent albuminuria was not included in the model as we did not find any significant association between persistent albuminuria and anti-apoB100 or p5 and due to the high association with eGFR. Values are presented as mean (SD), median (interquartile range [IQR]), or number of individuals (percentages). *P* values <0.05 were considered significant. All calculations were performed using SPSS Statistics version 25 (IBM Corp., Armonk, NY).

RESULTS

The Dialong Study

Clinical and demographic characteristics of the study population and previously reported CTCA data (24,25) are summarized in Supplementary Table 1. The group with type 1 diabetes had significantly higher CAC score, and women with diabetes had significant lower CAC score than men (24). The group with type 1 diabetes had a significantly lower rate of normal coronary arteries than the control group (14.1% vs. 47.6%; $P < 0.001$) and a higher rate of obstructive CAD measured by CTCA and previous coronary events. CTCA also showed significantly higher rates of total plaque volume and calcified plaque volume in the group with type 1 diabetes compared with the control group.

Levels of IgM Against MGO-apoB100 and MGO-p5 and Coronary Artery Stenosis in the Dialong Study

Clinical and demographic characteristics of the subjects in the group with type 1 diabetes with no coronary artery stenosis compared with those with significant stenosis (>50%) measured by CTCA are presented in Table 1. There were significantly higher levels of IgM autoantibodies against MGO-apoB100 and MGO-p5 in subjects with no coronary artery stenosis compared with those with significant stenoses (>50%) measured by CTCA: 96.2 AU (71–126.7) versus 54 AU (36.1–85.2) ($P = 0.003$) for anti–MGO-apoB100; and 77.4 AU (58–106) versus 36.9 AU (28.9–57.4) for anti–MGO-p5 ($P = 0.005$), respectively (Fig. 1). The intermediate group with nonsignificant stenoses also had lower antibody levels than the group with

Table 1—Clinical characteristics of the population with diabetes without or with CAD in the Dialong study

	No CAD ($n = 14$)	Case subjects with CAD ($n = 71$)	*P* value
Demographics			
Age, years	61.4 ± 6.5	62.1 ± 7.2	0.71
Sex, male	9 (64.3)	36 (50.7)	0.36
BMI, kg/m^2	25.5 ± 9.5	25.9 ± 9.2	0.75
Daily smoker	0	4 (5.7)	0.67
Statins	2 (15.4)	34 (47.9)	0.03
ACEi/ARB	5 (35.7)	30 (42.9)	0.77
LDL-c, mmol/L	2.95 (0.85)	2.86 (0.99)	0.99
Systolic blood pressure, mmHg	135 ± 14	147 ± 21	0.05
Diastolic blood pressure, mmHg	75 ± 9.1	75.7 ± 9.0	0.80
eGFR (MDRD), mL/min/1.73 m^2	80.5 ± 15.6	85 ± 19.2	0.42
Diabetes-related factors			
HbA_{1c}, %	6.8 ± 1.1	7.3 ± 0.8	0.1
HbA_{1c}, mmol/mol	51 ± 12	56 ± 9	
Diabetes duration, years	48.4 ± 4.0	50.6 ± 4.8	0.18
Persistent albuminuria	2 (14)	11 (15.7)	0.99
Retinopathy: none or background/proliferative	8 (57)/6 (42.9)	41 (59.4)/28 (40.6)	0.17/0.16
Calcium score >100	3 (21.4)	39 (54.9)	0.034
Autoantibodies MGO-apoB100, p5†			
MGO-apoB100 IgM, AU†	96.2 (71–126.8)	54 (36.1–85.4)	0.003
MGO-apoB100 IgG, AU†	70.8 (55.4–101.1)	58.9 (34.4–75.2)	0.06
MGO-p5 IgM, AU†	77.4 (58–106)	36.9 (28.9–57.4)	0.005
MGO-p5 IgG, AU†	71.3 (48.9–90.6)	65.1 (43.6–82.5)	0.14

Data are mean ± SD, *n* (%), or median (IQR). *P* values indicate differences between case subjects with CAD ($n = 71$) and case subjects without CAD ($n = 14$). ACEi, ACE inhibitor; ARB, angiotensin receptor blocker. †Absorbance units of individual samples as percentage to control plasma pool.

normal coronary arteries ($P < 0.05$) (Fig. 1). There were no significant findings when comparing IgG autoantibody levels and degree of stenosis on CTCA imaging (data not shown). Levels of IgM autoantibodies against MGO-apoB100 and MGO-p5 were negatively associated with the degree of coronary artery stenosis ($r = -0.18$; $P = 0.04$) and ($r = -0.27$; $P < 0.001$), respectively, and MGO-p5 with calcium score ($r = -0.27$; $P = 0.006$) in subjects with type 1 diabetes, whereas IgG for MGO-apoB100 or MGO-p5 did not associate to either degree of coronary stenosis or calcium score (Table 2).

IgM autoantibodies against MGO-apoB100 were significantly associated with the presence of >50% coronary artery stenosis (no stenosis vs. >50% stenosis) nonadjusted (odds ratio unadjusted [OR_{unadj}] 0.5 [95% CI 0.3–0.9]; $P = 0.03$) in type 1 diabetes. This association became stronger when adjusting for age, sex, HbA_{1c}, blood pressure, presence of proliferative retinopathy, LDL-c, and eGFR (OR adjusted [OR_{adj}] 0.21 [95% CI 0.06–0.6]; $P = 0.01$).

Also, MGO-p5 IgM was significantly associated with coronary stenosis (no stenosis vs. >50% stenosis) nonadjusted (OR_{unadj} 0.6 [95% CI 0.3–0.99]; $P = 0.04$) as well as after adjustment for age, sex, HbA_{1c}, presence of proliferative retinopathy, LDL-c, and eGFR (OR_{adj} 0.22 [95% CI 0.06–0.75]; $P = 0.02$) (Supplementary Table 2).

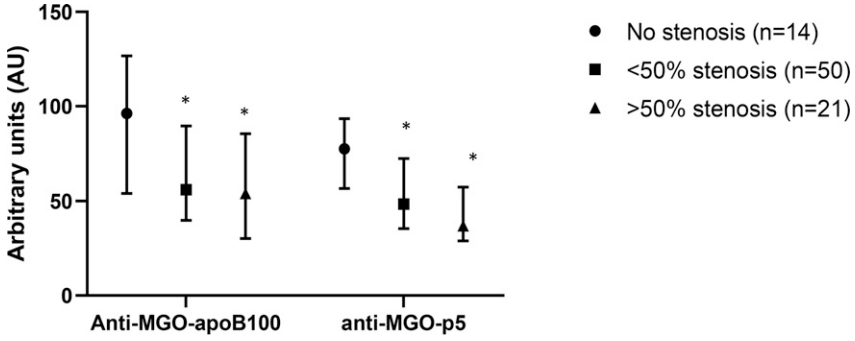

Figure 1—IgM autoantibodies against MGO-apoB100 and MGO-p5 are associated with stenosis of coronary arteries in subjects with type 1 diabetes. Data are presented as median (IQR). Antibody levels are given as AU, absorbance units in percentage compared to control plasma pool. *$P < 0.05$ (Kruskal-Wallis test).

Levels of IgM Against MGO-p5 and Coronary Artery Stenosis in the Oslo Study

Clinical and demographic characteristics of the Oslo study population have previously been reported (26).

By IVUS, 14.8% of all arterial segments (out of 3,263 total) had a normal intima thickness <0.3 mm. Mean PAV was 33.3% (10.9%). There was a significant association between IgM levels of autoantibodies against MGO-modified apoB100 p5 and PAV (Spearman correlation: $r_s = -0.49$; $P = 0.038$). This association was still significant after adjustments for age, sex, and HbA_{1c} (adjusted $r^2 = 0.58$ in linear regression analysis) (Supplementary Table 3).

Levels of IgM Against MGO-apoB100 and p5 and Retinopathy in the Dialong and Oslo Studies

Individuals with type 1 diabetes with PDR had lower levels of IgM MGO-apoB100 (median [IQR] 53.7 [33.8–82.3] vs. 66 [49.6–114.4] AU; $P = 0.03$) and IgM MGO-p5 (36.2 [26.6–73.7] vs. 55.9

Table 2—Correlations of IgM and IgG against MGO-apoB100 and MGO-p5 with coronary artery stenosis and calcium score in the group with type 1 diabetes in the Dialong study

	IgM MGO-apoB100 *r* value	IgG MGO-apoB100 *r* value	IgM MGO-p5 *r* value	IgG MGO-p5 *r* value
Coronary artery stenosis (%)	−0.18*	−0.12	−0.26†	−0.11
Calcium score (Agatston units)	−0.16	−0.15	−0.27†	−0.10

*$P < 0.05$. †$P < 0.01$.

[34.2–91.4] AU; $P = 0.04$) than individuals without retinopathy or background retinopathy in the Dialong study (Fig. 2). In the Oslo study, 18 individuals were classified with proliferative retinopathy and 9 with background or no retinopathy. Those with proliferative retinopathy had lower numerical values of IgM MGO-apoB100 (89.7 [39] vs. 102.3 [27] AU; $P = 0.4$) and MGO-p5 (74.9 [21] vs. 92.5 [31] AU; $P = 0.17$), but the differences between the groups were not significant. In the Dialong study, IgM autoantibodies against MGO-apoB100 were significantly associated with the presence of proliferative retinopathy (background/no retinopathy vs. proliferative retinopathy) (OR_{unadj} 0.98 [95% CI 0.97–0.99]; $P = 0.04$) in type 1 diabetes. This association remained when adjusted for blood pressure, LDL-c, eGFR, HbA_{1c}, age, and sex (OR_{adj} 0.98 [95% CI 0.97–0.99]; $P = 0.028$). Also, MGO-p5 IgM was significantly associated with proliferative retinopathy (background/no retinopathy vs. proliferative retinopathy) (OR_{unadj} 0.16 [95% CI 0.03–0.95]; $P = 0.04$) nonadjusted and when adjusted for blood pressure, LDL-c, eGFR, age, and sex (OR_{adj} 0.11 [95% CI 0.01–0.85]; $P = 0.034$). Supplementary Table 4 shows risk factors associated with stages of retinopathy.

Associations of Autoantibodies Against MGO-apoB100 and MGO-p5 With Cardiovascular Risk Factors in the Dialong Study

IgM MGO-apoB100 autoantibodies were significantly associated with systolic blood pressure and HbA_{1c}. IgM MGO-apoB100 autoantibodies did not associate with plasma lipid levels (Supplementary Table 5). The levels of anti–MGO-p5 were significantly higher in females than in males: median 62.4 AU (IQR 38.4–96.4) versus 47.9 AU (IQR 25.9–79.7) ($P = 0.034$). IgM MGO-p5 was significantly associated with eGFR and LDL, but did not show significant associations with HbA_{1c} (Supplementary Table 6) in the group with type 1 diabetes. In the control group, significant associations were found between IgM MGO-apoB100 and systolic blood pressure and between levels of IgM against MGO-p5 and age in the control group, but no associations were observed for LDL or HbA_{1c} (Supplementary Tables 5 and 6). We did not find any significant differences in autoantibody levels between statin and nonstatin users (data not shown). None of the participants used other drugs with immunomodulatory effects that could affect the autoantibody levels.

Levels of Autoantibodies Against MGO-apoB100 in Type 1 Diabetes and the Control Group in the Dialong Study

IgG MGO-apoB100 was significantly lower in type 1 diabetes compared with control subjects, whereas there were no differences in IgM autoantibody levels between the group with type 1 diabetes and the control group (Supplementary Table 1). When combining subjects with type 1 diabetes and control subjects without diabetes, we observed lower autoantibodies against MGO-apoB100 for both IgM and IgG for those who had significant stenosis versus no stenosis on CTCA: MGO-apoB100 IgM values, median 57.5 (IQR 44.2–88.9) versus 72.9 (IQR 51.1–103.9) AU ($P = 0.03$) and for

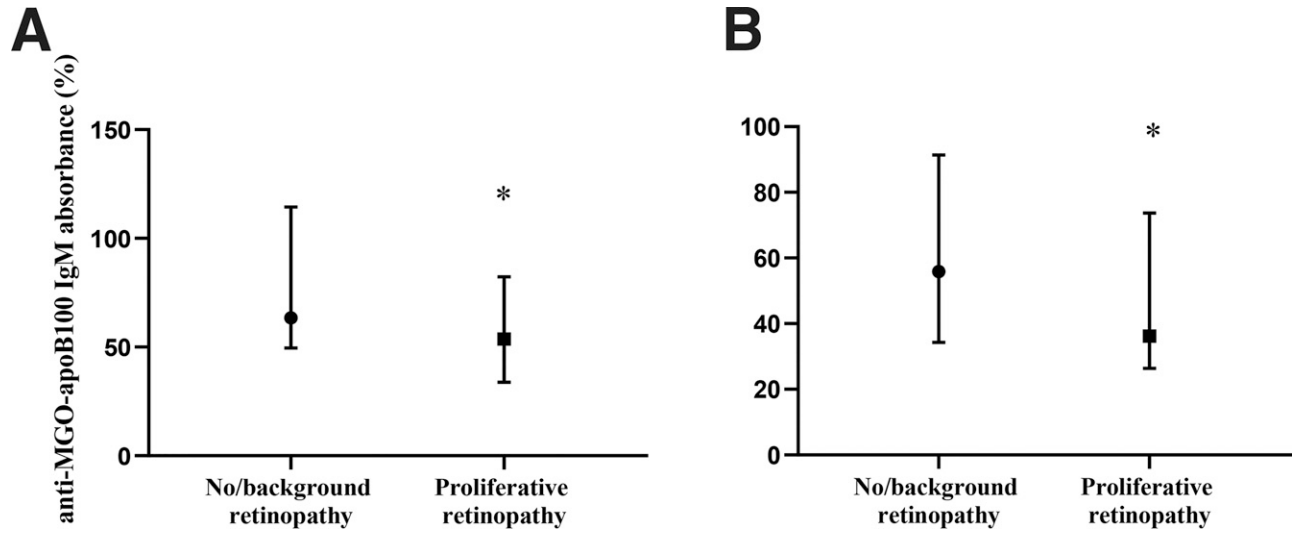

Figure 2—Levels of anti–MGO-apoB100 (*A*) and anti–MGO-p5 (*B*) in type 1 diabetes with no/background retinopathy ($n = 49$) and proliferative retinopathy ($n = 34$). The values are presented as median (IQR). Absorbance was measured at 405 nm and is presented as absorbance units in percentage compared to control plasma pool. *$P < 0.05$ (Kruskal-Wallis test).

MGO-apoB100 IgG, 53.2.3 (IQR 36.1–84.5) vs. 76.1 (IQR 47.9–91.8) AU ($P = 0.005$).

CONCLUSIONS

In the current study, we show that individuals with long-term type 1 diabetes and high plasma levels of IgM against MGO-modified LDL protein apoB100 and apoB100 p5 are characterized by 88% and 78%, respectively, reduced risk of having coronary artery disease and also that high autoantibody levels were associated with less proliferative retinopathy in the Dialong study. There was a nonsignificant tendency to lower levels of anti–MGO-apoB100 and MGO-p5 in the group with diabetes versus the control group. Our findings suggest a possible protective role of MGO-apoB100 IgM and MGO-p5 IgM against vascular complications in autoimmune diabetes.

There are several possibilities for how these autoantibodies could play a protective role in the vasculature of type 1 diabetes. An increased atherogenicity of the lipids due to MGO modification may explain some of the elevated cardiovascular risk in diabetes (9). Previous studies have shown that MGO-induced modification of LDL alters the three-dimensional structure of the apoB100 protein, making the LDL particles more prone to bind to the vessel wall matrix (9). The p5 peptide sequence of apoB100 is suggested to be involved in the binding of the matrix of the vessel wall (R. Khamis, personal communication). Furthermore, modified LDL particles are highly proinflammatory and can act as a ligand for macrophage pattern recognition receptors, including Toll-like receptors, and modified LDLs can also be engulfed by macrophages, causing cellular cholesterol accumulation amplifying Toll-like receptor signaling when stimulated (27). Autoantibodies against AGE-modified proteins and molecules may have a function by facilitating the removal of antigens or by blocking the binding of AGE epitopes to the AGE receptor or binding of MGO–LDL to matrix molecules in the vessel wall. They may also induce impairment of inflammasome-mediated innate immune response in macrophages (28,29). Lastly, IgM antibodies may retard atherosclerosis by blocking the scavenger receptor–mediated uptake of modified LDL into macrophages (30).

In addition, we found lower levels of MGO-apoB100 IgM and MGO-p5 IgM in individuals with proliferative retinopathy. However, most individuals with diabetes over 45 years' disease duration have some background retinopathy. Nevertheless, our findings may implicate a protective role of autoantibodies in severe microvascular complications represented as proliferative retinopathy that clinically matters the most. This is in accordance with data from the Joslin study, which found that absence of proliferative retinopathy in subjects with type 1 diabetes and chronic kidney disease were associated with a decreased prevalence of CVD, suggesting common protective factors for PDR and CVD (31). Furthermore, data from the ACCORD trial and Finnish Diabetic Nephropathy Study (FinnDiane) suggest tight interconnections between micro- and macrovascular disease progression in both type 1 and type 2 diabetes (32,33).

The levels of MGO-p5 IgM were found to be significantly higher in female than in male subjects. Women were also characterized by less severe coronary disease as assessed by the CAC score (24). This is in accordance with previous findings of IgM against MGO-apoB100 in type 2 diabetes (14) and may reflect differences in the immune response during atherosclerosis in men and women, resulting in different disease phenotypes (34).

We did observe a significant inverse correlation between HbA_{1c} and IgM anti–MGO-apoB100 in the group with diabetes, but not in the control group. This relates to similar findings in a study of patients with type 2 diabetes and control subjects in whom glycated apoB100 was positively associated with HbA_{1c} and fasting glucose concentration (35). We did not find any correlation between LDL-c and anti–MGO-apoB100 IgM or IgG, but this was observed for anti–MGO-p5 IgM in type 1 diabetes, supporting a link between LDL-c and anti–MGO-p5 in diabetes (36).

While IgM antibodies against both MGO-apoB100 and MGO-p5 were associated with coronary artery stenosis in subjects with type 1 diabetes, similar associations were not present for IgG against the same antigens in the group with type 1 diabetes. This is in accordance with our previous studies, in which IgM, but not IgG, against MGO-apoB100 was associated with lower CAC in type 2 diabetes (14). In addition, IgM, but not IgG, against the apoB100-derived peptide MGO-p220 was associated with increased risk for cardiovascular events (15). Previous studies by other groups have also found differences between IgM and IgG antibodies against oxidized LDL and an association with CVD (20,37). The DCCT/EDIC study found high levels of IgG immune complexes for AGE–LDL to be associated with CVD (19). Previous data from mouse models have reported decreased IgG titer, increased formation of immune complex formation with modified LDL, and larger atherosclerotic lesions in the absence of circulating IgM (38). These studies support that both circulating IgM and IgM immune complexes may be players in the complex development of atherosclerosis. Another possible explanation of the differences in IgM versus IgG associations with CVD could be that IgM antibodies against MGO-apoB100 and MGO-p5 are T-cell independent, whereas most IgG subtypes are T-cell dependent and may reflect different pathways in atherosclerosis development (39).

The limitations of this study are: *1*) the cross-sectional design, which makes this study hypothesis-generating and, thus, conclusions of causality cannot be drawn; and *2*) the sample size, which increases the risk for false-negative findings and limits the possibility to adjust for all possible confounders. Multiple testing rounds in a relatively small population may weaken the statistical significance, so the results must be interpreted with caution. However, the lack of association of IgG against MGO-apoB100 or MGO-p5 to CVD presented in this study is in accordance with previous studies from our group, which have investigated apoB100 autoantibodies in larger cohorts of type 2 diabetes. The strengths of the current study are: *1*) the control group, consisting of spouses/friends of the patients; *2*) the strong associations found for IgM MGO-apoB100 and macro- and microvascular disease in type 1 diabetes even after adjusting for confounders; and *3*) a confirmation of the results in a replication cohort of type 1 diabetes showing the same association between anti–IgM-MGO-p5 and coronary atherosclerosis, the latter assessed by invasive angiography with IVUS as a gold-standard measurement.

In summary, we demonstrate that high levels of IgM antibodies against MGO-apoB100 and MGO-p5 are associated with absence of coronary artery disease and proliferative retinopathy in patients with long-term type 1 diabetes. These observations propose a potential role of an immune response against AGE-modified self-antigens in vascular complications of autoimmune diabetes and suggest that IgM antibodies against MGO-apoB100 and MGO-p5 may have a protective function and are potential biological treatments of macro- and microvascular complications in type 1 diabetes.

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References

1. Laing SP, Swerdlow AJ, Slater SD, et al. Mortality from heart disease in a cohort of 23,000 patients with insulin-treated diabetes. Diabetologia 2003;46:760–765
2. Lind M, Svensson AM, Kosiborod M, et al. Glycemic control and excess mortality in type 1 diabetes. N Engl J Med 2014;371:1972–1982
3. Pongrac Barlovic D, Harjutsalo V, Gordin D, et al.; FinnDiane Study Group. The association of severe diabetic retinopathy with cardiovascular outcomes in long-standing type 1 diabetes: a longitudinal follow-up. Diabetes Care 2018;41:2487–2494
4. Lovshin JA, Bjornstad P, Lovblom LE, et al. Atherosclerosis and microvascular complications: results from the Canadian Study of Longevity in Type 1 Diabetes. Diabetes Care 2018;41:2570–2578
5. Retnakaran R, Zinman B. Type 1 diabetes, hyperglycaemia, and the heart. Lancet 2008;371:1790–1799
6. Ahmed N, Thornalley PJ. Advanced glycation endproducts: what is their relevance to diabetic complications? Diabetes Obes Metab 2007;9:233–245
7. Fosmark DS, Berg JP, Jensen AB, et al. Increased retinopathy occurrence in type 1 diabetes patients with increased serum levels of the advanced glycation endproduct hydroimidazolone. Acta Ophthalmol 2009;87:498–500
8. Yao D, Taguchi T, Matsumura T, et al. High glucose increases angiopoietin-2 transcription in microvascular endothelial cells through methylglyoxal modification of mSin3A. J Biol Chem 2007;282:31038–31045
9. Rabbani N, Godfrey L, Xue M, et al. Glycation of LDL by methylglyoxal increases arterial atherogenicity: a possible contributor to increased risk of cardiovascular disease in diabetes. Diabetes 2011;60:1973–1980
10. Brown BE, Dean RT, Davies MJ. Glycation of low-density lipoproteins by methylglyoxal and glycolaldehyde gives rise to the in vitro formation of lipid-laden cells. Diabetologia 2005;48:361–369
11. Brown BE, Rashid I, van Reyk DM, Davies MJ. Glycation of low-density lipoprotein results in the time-dependent accumulation of cholesteryl esters and apolipoprotein β-100 protein in primary human monocyte-derived macrophages. FEBS J 2007;274:1530–1541
12. Virella G, Thorpe SR, Alderson NL, et al.; DCCT/EDIC Research Group. Autoimmune response to advanced glycosylation end-products of human LDL. J Lipid Res 2003;44:487–493
13. Tsimikas S, Miyanohara A, Hartvigsen K, et al. Human oxidation-specific antibodies reduce foam cell formation and atherosclerosis progression. J Am Coll Cardiol 2011;58:1715–1727
14. Engelbertsen D, Anand DV, Fredrikson GN, et al. High levels of IgM against methylglyoxal-modified apolipoprotein B100 are associated with less coronary artery calcification in patients with type 2 diabetes. J Intern Med 2012;271:82–89
15. Engelbertsen D, Vallejo J, Quach TD, Fredrikson GN, Alm R, Hedblad B, et al. Low levels of IgM antibodies against an advanced glycation endproduct-modified apolipoprotein B100 peptide predict cardiovascular events in nondiabetic subjects. J Immunology 2015;195:3020–3025
16. Wu M, Chen Y, Wilson K, et al. Intraretinal leakage and oxidation of LDL in diabetic retinopathy. Invest Ophthalmol Vis Sci 2008;49:2679–2685
17. Fredrikson GN, Anand DV, Hopkins D, et al. Associations between autoantibodies against apolipoprotein β-100 peptides and vascular complications in patients with type 2 diabetes. Diabetologia 2009;52:1426–1433
18. Fredrikson GN, Hedblad B, Berglund G, et al. Identification of immune responses against aldehyde-modified peptide sequences in apoB associated with cardiovascular disease. Arterioscler Thromb Vasc Biol 2003;23:872–878
19. Lopes-Virella MF, Bebu I, Hunt KJ, et al.; DCCT/EDIC Research Group. Immune complexes and the risk of CVD in type 1 diabetes. Diabetes 2019;68:1853–1860
20. Lopes-Virella MF, McHenry MB, Lipsitz S, et al.; DCCT/EDIC Research Group. Immune complexes containing modified lipoproteins are related to the progression of internal carotid intima-media thickness in patients with type 1 diabetes. Atherosclerosis 2007;190:359–369
21. Sousa GR, Pober D, Galderisi A, et al. Glycemic control, cardiac autoimmunity, and long-term risk of cardiovascular disease in type 1 diabetes mellitus. Circulation 2019;139:730–743
22. Holte KB, Juel NG, Brox JI, et al. Hand, shoulder and back stiffness in long-term type 1 diabetes; cross-sectional association with skin collagen advanced glycation end-products. The Dialong study. J Diabetes Complications 2017;31:1408–1414
23. Larsen JR, Tsunoda T, Tuzcu EM, et al. Intracoronary ultrasound examinations reveal significantly more advanced coronary atherosclerosis in people with type 1 diabetes than in age- and sex-matched non-diabetic controls. Diab Vasc Dis Res 2007;4:62–65
24. Holte KB, Svanteson M, Hanssen KF, Haig Y, Solheim S, Berg TJ. Undiagnosed coronary artery disease in long-term type 1 diabetes. The Dialong study. J Diabetes Complications 2019;33:383–389
25. Svanteson M, Holte KB, Haig Y, Kløw NE, Berg TJ. Coronary plaque characteristics and epicardial fat tissue in long term survivors of type 1 diabetes identified by coronary computed tomography angiography. Cardiovasc Diabetol 2019;18:58
26. Sveen KA, Karimé B, Jørum E, et al. Small- and large-fiber neuropathy after 40 years of type 1 diabetes: associations with glycemic control and advanced protein glycation: the Oslo Study. Diabetes Care 2013;36:3712–3717
27. Tall AR, Yvan-Charvet L. Cholesterol, inflammation and innate immunity. Nat Rev Immunol 2015;15:104–116
28. Son S, Hwang I, Han SH, Shin JS, Shin OS, Yu JW. Advanced glycation end products impair NLRP3 inflammasome-mediated innate immune responses in macrophages. J Biol Chem 2017;292:20437–20448
29. Ott C, Jacobs K, Haucke E, Navarrete Santos A, Grune T, Simm A. Role of advanced glycation end products in cellular signaling. Redox Biol 2014;2:411–429
30. Hörkkö S, Bird DA, Miller E, et al. Monoclonal autoantibodies specific for oxidized phospholipids or oxidized phospholipid-protein adducts inhibit macrophage uptake of oxidized low-density lipoproteins. J Clin Invest 1999;103:117–128
31. Gordin D, Harjutsalo V, Tinsley L, et al. Differential association of microvascular attributions with cardiovascular disease in patients with long

duration of type 1 diabetes. Diabetes Care 2018;41:815–822
32. Gerstein HC, Ambrosius WT, Danis R, et al.; ACCORD Study Group. Diabetic retinopathy, its progression, and incident cardiovascular events in the ACCORD trial. Diabetes Care 2013;36:1266–1271
33. Hägg S, Thorn LM, Putaala J, et al.; FinnDiane Study Group. Incidence of stroke according to presence of diabetic nephropathy and severe diabetic retinopathy in patients with type 1 diabetes. Diabetes Care 2013;36:4140–4146
34. Fairweather D. Sex differences in inflammation during atherosclerosis. Clin Med Insights Cardiol 2015;8(Suppl. 3):49–59
35. Tames FJ, Mackness MI, Arrol S, Laing I, Durrington PN. Non-enzymatic glycation of apolipoprotein B in the sera of diabetic and non-diabetic subjects. Atherosclerosis 1992;93:237–244
36. Karvonen J, Päivänsalo M, Kesäniemi YA, Hörkkö S. Immunoglobulin M type of autoantibodies to oxidized low-density lipoprotein has an inverse relation to carotid artery atherosclerosis. Circulation 2003;108:2107–2112
37. Tsimikas S, Brilakis ES, Lennon RJ, et al. Relationship of IgG and IgM autoantibodies to oxidized low density lipoprotein with coronary artery disease and cardiovascular events. J Lipid Res 2007;48:425–433
38. Lewis MJ, Malik TH, Ehrenstein MR, Boyle JJ, Botto M, Haskard DO. Immunoglobulin M is required for protection against atherosclerosis in low-density lipoprotein receptor-deficient mice. Circulation 2009;120:417–426
39. Kyaw T, Tipping P, Bobik A, Toh BH. Opposing roles of B lymphocyte subsets in atherosclerosis. Autoimmunity 2017;50:52–56