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High Levels of AGE-LDL, and of IgG Antibodies Reacting With MDA-lysine Epitopes Expressed by oxLDL and MDA-LDL in Circulating Immune Complexes Predict Macroalbuminuria in Patients With Type 2 Diabetes

Maria F. Lopes-Virella [a,*], Kelly J. Hunt [b], Nathaniel L. Baker [b], Gabriel Virella [c], The VADT Group of Investigators

[a] Department of Medicine and Laboratory Services, Medical University of South Carolina and Ralph H. Johnson VA Medical Center, Charleston, SC
[b] Department of Public Health Services, Medical University of South Carolina, Charleston, SC
[c] Department of Microbiology and Immunology, Medical University of South Carolina, Charleston, SC

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ABSTRACT

Background: Circulating immune complexes (IC) containing modified forms of LDL (mLDL) are strongly pro-inflammatory and when present in high levels are associated with the development of diabetic complications.
Objective: We investigated whether levels of oxidized LDL (oxLDL), malondialdehyde-LDL (MDA-LDL) and advanced glycation end products-LDL (AGE-LDL) as well as IgG and IgM antibodies reacting with MDA-lysine epitopes expressed by oxLDL and MDA-LDL isolated from circulating IC were associated with progression to macroalbuminuria in type 2 diabetes (VADT cohort).
Methods: Levels of mLDL in IC were measured in 905 patients, a median of two years after entry into the study. Participants were followed for an average of 3.7 years for renal outcomes. Generalized logistic regression models were used to quantify the association of increased levels of biomarkers and development of abnormal albuminuria. Normal, persistent micro- (ACR ≥ 30), incident micro- (ACR ≥ 30) and incident macroalbuminuria (ACR ≥ 300) were the outcomes of interest.
Results and conclusions: Patients with macro (n = 78) or non-persistent microalbuminuria (n = 81) at baseline were excluded. Odds ratios for endpoints in relation to high versus low (defined using a median split) biomarker levels are found in Fig. 1. Our study demonstrates that high levels of AGE-LDL as well as of IgG antibodies (but not IgM antibodies) reacting with MDA-LDL lysine epitopes in circulating IC predict the development of macroalbuminuria in patients with type 2 diabetes. These data support the pathogenic role of modified LDL IgG antibodies but not the protective role of modified LDL IgM antibodies.

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1. Introduction

Nephropathy is the second major cause of morbidity and mortality in diabetes mellitus (DM) (Locatelli et al., 2003). Overt nephropathy is usually preceded by increased levels of albumin in the urine (DCCT, 1993; K/DOQI, 2002). Microalbuminuria is associated not only with risk of developing renal insufficiency (Mogensen, 1987; Viberti, Jarrett, & Keen, 1982) but also cardiovascular disease (Gerstein et al., 2001) in patients with DM. The progression from microalbuminuria to macroalbuminuria is a reflection of deterioration of renal function that can be reduced or prevented by adequate treatment mainly by adequate glucose control. Consequently there is considerable interest in uncovering mechanisms responsible for the development and progression of albuminuria in diabetic patients and in identifying early biomarkers that may be predictive of this complication of diabetes.

The pathological mechanism(s) responsible for the early development of renal dysfunction such as microalbuminuria are not yet well defined. There is, however, strong evidence implicating endothelial dysfunction and inflammation in the early stages of renal disease (Araki et al., 2007; Dalla Vestra et al., 2005; Lopes-Virella et al., 2008; Schram, Chaturvedi, Schalkwijk, Fuller, & Stehouwer, 2005; Schram et al., 2003). The deposition in the kidney glomeruli of glycated proteins (Goh & Cooper, 2008; Tan, Forbes, & Cooper, 2007) and of advanced glycation end-product (AGE)-modified proteins (Goldin, Beckman, Schmidt, & Creager, 2006; Tan et al., 2007), both able to generate reactive oxygen species (ROS) (Forbes, Coughlan, & Cooper, 2008; Goldin et al., 2006; Tan et al., 2007) have been proposed as key factors intimately linked to renal inflammation.

Conflicts of interest: None of the authors has conflicts of interest related to this article.

* Corresponding author at: 114 Doughty St, Charleston, SC 29425. Tel.: +1 843 789 6823; fax: +1 843 789 6854.
E-mail address: virellam@musc.edu (M.F. Lopes-Virella).

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Since patients with type 2 diabetes besides having hyperglycemia have also increased susceptibility to oxidation (Lopes-Virella, Klein, & Virella, 1996; Mironova, Klein, Virella, & Lopes-Virella, 2000) they usually have significantly increased levels of lipoprotein glyco-oxidation, specifically of glyco-oxidized LDL (Lopes-Virella et al., 1996).

In humans, modified forms of LDL are immunogenic, eliciting the production of autoantibodies, leading to the formation of circulating immune complexes (IC) which can be isolated from the peripheral blood of patients with type 1 (Virella et al., 2003) and type 2 DM (Lopes-Virella et al., 2012). The mLDL isolated from IC is heterogeneous, reacting mainly with rabbit antibodies to AGE-LDL, copper-oxidized LDL (oxLDL) and malondialdehyde-modified LDL (MDA-LDL). The main epitope of AGE-LDL is carboxymethyl-lysine (CML) while oxLDL and MDA-LDL share MDA-lysine as their major recognizable epitope (Virella et al., 2004). The antibodies recognizing MDA-lysine epitopes involved in IC formation are predominantly of the IgG isotype, subclasses 1 and 3 (Virella et al., 2000; Virella et al., 2003), with strong pro-inflammatory potential (Virella, 2007). IgM antibodies of the same specificity are also present in LDL IC isolated from patients with type 1 diabetes, but in considerably lower concentrations than IgG antibodies (Virella et al., 2008).

Earlier studies using the cholesterol content of isolated circulating IC as an indicator of the concentration of modified forms LDL in the IC (mLDL-IC) showed that the mLDL-IC concentration was higher in patients with type 1 diabetes when macroalbuminuria was present than in patients with microalbuminuria or in patients with normal albuminuria (Atchley, Lopes-Virella, Zheng, & Virella, 2002) and that higher concentrations of modified LDLs in IC could predict the development of nephropathy (Yishak et al., 2006). The development of specific capture assays for different LDL modifications allowed us to add specificity to our studies, and we demonstrated that higher levels of oxLDL and AGE-LDL in circulating IC were associated with increased odds to develop abnormal albuminuria (Lopes-Virella, Carter, Baker, Lachin, & Virella, 2012), likely due to the deposition or in situ formation of pro-inflammatory IC in the glomeruli.

The pro-inflammatory effects of modified LDL-IC depend primarily on the predominant isotype of the antibodies involved in IC formation. Ex vivo studies have shown that oxLDL-IC formed with human oxLDL and human IgG antibodies are clearly pro-inflammatory (Saad, Virella, Chassereau, Boackle, & Lopes-Virella, 2006). In contrast, complexes formed with IgM antibodies are less likely to be involved as causal agents of tissue inflammation because of their predominant intravascular distribution and inability to engage Fc receptors in phagocytic cells (Virella, 2007). Numerous reports have been published supporting the concept that IgM antibodies to modified LDL (Karvonen, Paivansalo, Kesaniemi, & Horkko, 2003; Tsimikas et al., 2007) and to oxidized phosphorylcholine (de Faire et al., 2010; Frostegard, 2010; Gigante et al., 2014; Su et al., 2008) do not cause inflammation and that actually may have athero-protective properties.

We had two main objectives in the present study. The first objective was to investigate whether the correlations between high concentrations of mLDL in IC and albuminuria observed in patients with type 1 diabetes (Lopes-Virella, Carter, et al., 2012) were also observed in patients with type 2 diabetes; the second objective was to expand our previous observation in a small group of patients with type 1 diabetes in which significant positive associations of IgG oxLDL antibody concentration and serum creatinine and albumin excretion rate were observed while no significant associations were found between these parameters and IgM oxLDL antibody levels (Virella et al., 2008), to a much larger cohort of patients with type 2 diabetes.

2. Materials and Methods

2.1. The VADT design and population

The study design of the VADT study has been previously reported (Abraira et al., 2003). Briefly, 1791 veterans with type 2 diabetes and suboptimal glucose control were randomized in 20 participating sites to receive either intensive or standard glucose control. The goal for HbA1c levels was an absolute reduction of 1.5% in the intensive-therapy group, as compared with the standard-therapy group. A unique feature of the study was that other modifiable cardiovascular risk factors were treated aggressively and uniformly in both arms of the study. All patients were treated to guidelines according to the American Diabetes Association for blood pressure, hypertension, diet, exercise and diabetes education (ADA, 2000). All patients were prescribed aspirin and all patients with elevated lipid levels were prescribed statins, unless contraindicated. The study was approved by the IRB at each of the participating sites. All patients provided written informed consent.

Of the 1791 VADT study participants, 995 patients from 17 of the participating sites, approximately half from the standard arm and half from the intensive treatment arm, agreed to participate in a sub-study focused on determining the association between specific biomarkers and macrovascular disease. The biochemical, physical, and demographic profiles of the 995 patients in the substudy do not differ significantly from the 796 not included in the substudy with the exception of slightly lower age and LDL-cholesterol and slightly higher triglyceride levels as well as a higher prevalence of aspirin use at baseline in the substudy participants when compared to non substudy participants (Lopes-Virella, Baker, et al., 2012). The study population for the current report consists of 912 of the 995 participants enrolled in the substudy whose serum was available to measure modified forms of LDL and oxLDL antibodies in circulating IC. In 83 patients not enough serum was available to perform the measurements.

Enrollment for the VADT study occurred from December 2000 to May 2003. The baseline VADT cohort examination was standardized and included interviews, blood pressure measurements, anthropometric measurements, fasting venipuncture and urine collection for measurement of albumin creatinine ratio (ACR) (Abraira et al., 2003). VADT follow-up visits occurred every three months with ACR, creatinine and lipid levels being measured yearly. Measurement of MDA-LDL, oxLDL and AGE-LDL was performed on IC isolated from serum samples collected during a routine follow-up between August 2002 and March 2006, a median of 2 years (range: 0 to 5 years) after participants' baseline examination. Measurement of IgG in oxLDL-IC and IgM in oxLDL-IC was also performed at the same time. Serum samples were obtained after an overnight fast and stored at −80 °C until assayed. Patients were followed until lost to follow-up, death or May 2008.

2.2. Renal Outcome Measures

The current study was conducted to determine whether levels of mLDL in circulating IC were associated with the development of micro or macro albuminuria. Of the 912 participants with measured mLDL in circulating IC, 234 were excluded from the current analysis [i.e., 7 missing information on urine albumin creatinine ratio (ACR), 78 had macro-albuminuria (ACR > 300 mg/g) at the time of the biomarker measurements, 81 had micro-albuminuria (ACR of 30 to 300 mg/g) at the time of the biomarker measurements that regressed to normal levels (ACR <30 mg/g) during follow-up] and 68 who did not have ACR measured at least twice after biomarker measurements were made. The remaining 678 participants were classified into those with normal albuminuria throughout the study (n = 451), those with persistent micro-albuminuria throughout the study (n = 119), those with incident micro-albuminuria (n = 77) and those with incident macro-albuminuria (n = 31). Persistent micro-albuminuria was defined as those with an ACR ≥30 mg/g of creatinine at the time of the biomarker measurements who also met the definition of having micro-albuminuria during the subsequent study period. Incident micro-albuminuria was defined as having ACR < 30 mg/g of

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