

Editorials

Antibodies against oxidized LDL for non-invasive diagnosis of atherosclerotic vascular disease

See page 1572 for the article to which this Editorial refers

Atherosclerosis is a chronic inflammatory disorder and oxidation of low density lipoproteins (LDL) is thought to play a central role in atherogenesis. Plasma levels of oxidized LDL and malondialdehyde-modified LDL (MDA-modified LDL) have been demonstrated to be significantly higher in patients with coronary artery disease^[1]. MDA-modified LDL was able to discriminate between patients with acute coronary syndromes and those with stable angina^[2]. Oxidation of LDL generates highly immunogenic oxidized phospholipids and proteins. The titre of antibodies against oxidized LDL (anti-oxLDL antibodies) or MDA-modified LDL has been evaluated as a marker or predictor of the extent of atherosclerosis in several studies^[3–9].

In this issue, Monaco *et al.*^[10] describe higher levels of anti-oxLDL antibodies in patients with peripheral artery disease without overt coronary artery disease than in patients with stable or unstable angina free from clinical peripheral vascular disease. The authors demonstrate a significant correlation between the titre of anti-oxLDL antibodies and the number of arterial stenoses above 70% in the overall population and suggest that the greater titre of antibodies in patients with peripheral vascular disease probably reflects the greater atherosclerotic burden in these patients. These data are in line with several^[3–6], but are inconsistent with other studies^[7–9]. To assess the value of antibodies against oxidized LDL as a potential new tool for non-invasive diagnosis of atherosclerotic vascular disease, it is important to analyse methodological and biological factors, which may underlie the different outcome of these studies.

A first explanation for the inconsistency of published results is the lack of standardization of the enzyme immunoassays for the measurement of antibodies against oxidized LDL by different groups. When copper oxidized LDL is used as antigen in enzyme immunoassays, batch-to-batch variability in reactivity should be avoided by monitoring fluorescence (360→430 nm) as oxidation progresses, to

determine when the reaction should be stopped^[11]. Even so, immunocompetition experiments have demonstrated that IgG recognizing LDL oxidized by horseradish peroxidase and H₂O₂ (HRP) did not cross-react with copper oxidized LDL and vice versa^[12]. This issue is carefully controlled for in the present investigation because both copper oxidized LDL and LDL oxidized by HRP was used as antigen.

Second, immune complex formation *in vivo* may lead to spurious low free anti-oxLDL levels. Anti-oxLDL levels were significantly lower in type 1 diabetes coronary artery disease patients than in controls^[9]. In contrast, immune complexes containing apo B were significantly higher in patients than in controls. Together, these findings might reflect a higher affinity of anti-oxLDL antibodies in diabetes patients^[9].

Third, whereas the majority of anti-oxLDL antibodies are IgG, a significant portion are IgM^[13], which are not measured in the assays. The relative amount of IgG and IgM antibodies may differ significantly between patients and longitudinally over time. Longitudinal follow-up data on the titre of the antibodies in individual patients are lacking. Several biological factors may modulate the titre of antibodies in patients over time. Atherogenesis progresses discontinuously and thus variations in oxidized LDL and anti-oxLDL antibody formation are likely. Although the present study did not find any difference in the anti-oxLDL titre between stable and unstable angina pectoris patients, determination of the anti-oxLDL titre at different time-points after the event and a quantification of IgM antibodies is necessary to resolve this issue.

It should be borne in mind that the presence of oxidative neoepitopes is not limited to oxidized LDL, e.g. these epitopes are present on apoptotic cells^[14]. Elevated levels of anti-oxLDL antibodies are found in patients with systemic diseases such as systemic lupus erythematosus, rheumatoid arthritis, chronic juvenile arthritis, periarteritis nodosa and scleroderma^[15]. Furthermore, Shaw *et al.*^[16] recently demonstrated that IgM autoantibodies against oxidized phospholipids in apo E^{-/-} mice, a murine model of

accelerated atherosclerosis, belong to a class of highly conserved natural antibodies known to participate in the immune response against phosphorylcholine and to confer protection against *Streptococcus pneumoniae*. Because phosphorylcholine is a component of many prokaryotic and eukaryotic organisms, this raises the possibility that certain infections may increase the titre of these natural antibodies and may influence atherogenesis^[16].

Notwithstanding some unresolved issues with regard to the use of anti-oxLDL titre as a new tool for the non-invasive diagnosis of atherosclerotic vascular disease, the potential value of these assays is underscored by the present investigation and a recent study of George *et al.*^[17], who demonstrated, using artificial neural networks, that the best predictor of the degree of coronary atherosclerosis was the anti-oxLDL titre. The ongoing evaluation of anti-oxLDL antibodies in the Physicians Health Study, the Framingham Offspring study and the PDAY (Pathobiological Determinants of Atherosclerosis in Youth) study may further highlight their diagnostic value. Whether these antibodies merely constitute an epiphenomenon or whether they have a direct effect on the progression of atherosclerosis, is an ongoing theme in basic research.

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