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ATHEROGENESIS AND THE HUMORAL IMMUNE RESPONSE TO MODIFIED LIPOPROTEINS

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Abstract

Modified forms of LDL are immunogenic and activate both cell-mediated and humoral immune responses. Both types of responses are pro-inflammatory and are probably primary players in the perpetuation of the chronic inflammatory reaction characteristic of atherosclerosis. The immunologic response to modified LDL can be directed to MHC-II-associated peptides in the case of T helper cells, and to a variety of epitopes -modified lysine groups, modified phospholipids, proteins that become associated with oxidized LDL (such as β 2GP1) – in the case of B cell responses. T cell activation is likely to play a major role through cross-activation of macrophages. Humoral responses to modified LDL are pathogenic as a consequence of the formation of antigen-antibody complexes containing modified LDL and IgG antibodies. Those immune complexes induce cholesterol ester accumulation in macrophages and macrophage-like cells, and induce the release of proinflammatory cytokines, chemokines, oxygen active radicals, and matrix metalloproteinases from those cells. There is no conclusive evidence supporting a protective role for IgM antibodies in humans, possibly because autoantibodies to modified lipoproteins are predominantly of the IgG isotype.

Keywords

Atherosclerosis; autoimmunity; LDL antibodies; phospholipid antibodies; immune complexes; vascular inflammation

Introduction

Modified lipoproteins are likely to play a critical role in the induction and/or perpetuation of the chronic vascular inflammation. Besides their moderate pro-inflammatory effects[1],[2], modified lipoproteins induce cell-mediated[3] and humoral responses [4]. The precise nature of the epitopes that elicit autoimmune responses has yet to be defined, but *in vitro* modified human LDL, e.g. copper oxidized, MDA modified, and AGE modified, are immunogenic in laboratory animals and are also recognized by spontaneously formed human autoantibodies [4–7]. Human autoantibodies reactive with oxLDL were the first to be purified and characterized[8–10] and human AGE-LDL antibodies have also been isolated and

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characterized [11]. Peptides derived from oxidized LDL (oxLDL) also appear to be recognized by T cells [3]. At the vessel wall level, both types of response are likely to play a significant role. Immune complexes formed by modified LDL and corresponding antibodies (mLDL-IC) are potent macrophage activators[12,13] and macrophage activation leads to the overexpression of MHC-II molecules, cytokine and chemokine release, and expression of co-stimulatory molecules. This will create ideal conditions for cross-activation between macrophages and Th1 lymphocytes [14]. Activated macrophages also release oxygen active radicals, enhancing the opportunity for LDL modification[15], which will increase the immunogenic load, induce a more vigorous antibody response, and increase the formation of mLDL-IC. The immune system also appears to recognize other by-products of macrophage activation, such as heat shock proteins (Hsp) [16]. Hsp are also immunogenic[17], and are also able to activate macrophages through interactions with Toll-like receptors[18] and lipid rafts [19,20]. Therefore, a variety of circuits are activated by modified lipoproteins and mLDL-IC, setting the conditions for a chronic inflammatory reaction in the vessel walls.

Modified lipoprotein antibodies

Initially, the attention of clinical investigators concentrated on finding evidence supporting a pathogenic role for oxLDL and MDA-LDL antibodies, using them as a surrogate measurement of LDL with different degrees of modification. The results of these studies were rather disappointing, because the results were often conflicting and failed to produce a clear cut indication of the clinical value of modified lipoprotein antibody assays as biomarkers for the development and/or progression of atherosclerosis. While some groups reported a positive correlation between the levels of oxLDL antibodies and different endpoints considered as evidence of atherosclerotic vascular disease, such as progression of carotid atherosclerosis or risk for the future development of myocardial infarction [21–27], others failed to show such correlation or showed an inverse correlation [28–38].

These discrepancies are not surprising. Levels of antibody formed against a specific antigen are highly variable, depending on individual variations in the immune response. Furthermore the avidity of the antibody to the respective antigen also shows individual variations. Thus, the measurement of free circulating autoantibodies depends not only on the magnitude of the antibody response but also on antibody avidity and on the amount of antigen present in circulation. If the average avidity of circulating autoantibody is sufficiently high and antigen is present in circulation, soluble IC are formed and in their presence the assays for serum oxLDL antibody concentrations become inaccurate and underestimate the absolute concentration of circulating oxLDL antibody [38,39]. The characterization of purified oxLDL and AGE-LDL antibodies gave a further impetus to the study of their potential pathogenic role. Ox LDL antibodies, the first to be characterized, were found to be predominantly of the IgG isotype and, within this isotype, of subclasses 1 and 3 [9–11]. IgG1 and IgG3 antibodies have been defined as pro-inflammatory, based on their ability to activate the complement system by the classical pathway and to interact with Fcγ receptors in phagocytic cells [40]. The involvement of IgG1 and IgG3 antibodies in immune complex disease is also well recognized [41].

The pathogenic role of immune complexes formed by oxLDL and oxLDL antibodies (oxLDL-IC)

The involvement of LDL-IC in the pathogenesis of atherosclerosis was suggested over a decade ago by the elegant studies of Yla-Herttuala and co-workers [8,42]. These authors purified oxLDL and the corresponding IgG antibodies from atheromatous lesions of humans and Watanabe hyperlipidemic rabbits, thus demonstrating that the ingredients necessary for the formation of LDL-IC are present in the damaged arterial wall. Several groups, including ours, have reported a significant correlation between soluble LDL-IC and the presence of CAD

[37,38,43]. Orekhov and coworkers reported that the level of cholesterol in isolated circulating IC, which reflects the amount of LDL-IC in circulation, correlated with the severity of coronary atherosclerosis [43,44]. Turk et al. reported that the concentrations of Apo-B in circulating IC was significantly higher in patients with coronary heart disease [45]. A prospective study involving 98 diabetic subjects recruited as part of the Pittsburgh EDIC study showed that LDL-IC and oxLDL antibodies, correlated with the development of coronary artery disease [CAD] over a period of seven years, but while the correlation with LDL-IC levels was direct, the correlation with oxLDL antibody levels was inverse [37,38]. To further evaluate the validity of the hypothesis that modified LDL (mLDL)-IC play a pathogenic role in diabetes we screened 1050 patients from the DCCT/EDIC cohort for mLDL-IC and evaluated the impact of these IC in progression of carotid intima-media thickness [IMT], a surrogate marker for CAD. We found that, after adjustment for age, gender, IMT at year 1, ultrasonography equipment used to measure IMT, DCCT randomization group, smoking, hypertension, HbA1c, logarithm of the albumin excretion rate [AER] and C-Reactive Protein [CRP] levels, mLDL-IC levels were good predictors of progression of internal carotid IMT [46]. Furthermore, in both cohorts the levels of modified LDL-IC were higher in patients with abnormal albumin excretion rate [AER] [47]. In the same study we found significant correlations between the levels of LDL-IC and those of soluble ICAM-1, CRP, and fibrinogen. More recently, in collaboration with Orchard and others, we obtained data confirming that mLDL-IC is a risk factor for incident atherosclerosis and nephropathy [48,49]. Therefore, we have accumulated strong epidemiological evidence supporting the pathogenic role of modified LDL-IC in atherosclerosis.

The atherogenic and pro-inflammatory properties of LDL-IC in humans have also been established by *in vitro* studies of the properties of LDL-IC, either isolated from patient's sera by polyethylene glycol [PEG] precipitation [39,50–53] or prepared *in vitro* with rabbit antibodies or with isolated human antibodies. The exposure of human monocyte-derived macrophages to mLDL-IC isolated from the sera of diabetic patients and from some healthy controls with high levels of circulating LDL-IC is followed by intracellular accumulation of cholesterol and cholesterol esters [39,52,54]. At the same time, the macrophages ingesting isolated LDL-IC become activated and release proinflammatory cytokines, and oxygen active radicals [55]. Insoluble IC prepared with human LDL and rabbit LDL antibody also promote the transformation of macrophages into foam cells [56] and deliver activating signals as a consequence of their interaction with FcγRI [57] resulting in the release of pro-inflammatory cytokines, such as IL-1 and TNF, oxygen active radicals, and metalloproteinases [55,56,58, 59]. LDL-IC prepared with human oxLDL and oxLDL antibodies isolated from patients with type 1 diabetes can also induce cholesterol and cholesterol ester accumulation in a variety of macrophage-like cell lines, including THP-1, U937, and MonoMac-6, which are also activated as reflected by the release of pro-inflammatory cytokines, including IL-1, IL-6, IL-12, and TNF [12,13], as well as of metalloproteinases [MMP-1] [59]. Indeed, human LDL-IC were shown to induce CE accumulation in those cell lines to a much higher degree than oxLDL, even when oxLDL concentrations were about ten times greater than the amount of oxLDL contained in the oxLDL-IC was used as control [13]. The accumulation of cholesteryl esters by macrophages incubated with LDL-IC is dependent on IC uptake through Fcγ receptors, primarily the high affinity FcγRI [60] and appears to be a consequence of the delayed degradation of ingested LDL [61]. Signaling through FcγR is also responsible for the activation of MonoMac cells which results in the release of pro-inflammatory cytokines [12]. A recent report that double KO mice, Apo-E^{-/-} and FcγR^{-/-}, seem protected against the development of atherosclerosis [62] can be considered as indirect evidence of the involvement of LDL-IC in this animal model, because modified lipoproteins or free antibodies to those modified lipoproteins will not interact with Fcγ receptors.

Can the immune response to modified lipoproteins be protective?

Some authors have raised questions concerning the pathogenic role of oxLDL antibodies and postulated a protective effect for the humoral response to oxLDL in humans[63] based on animal studies. In some of these studies Apo-E deficient mice and LDL-receptor-deficient [LDLr^{-/-}] mice deliberately immunized with homologous malondialdehyde-LDL [MDA-LDL] showed a reduction in the development of atheromatous lesions[64,65]. Similar observations were reported in hypercholesterolemic rabbits immunized with autologous oxLDL [66]. Still, it must be noted that the data obtained in active immunization experiments is not free of contradictions. For example, the studies carried out immunizing LDLr^{-/-} mice with MDA-LDL showed the same "protective" effect after immunization with unmodified LDL, and the reduction in atherosclerosis development was seen in the absence of antibodies to modified LDL [67]. In hypercholesterolemic rabbits immunized with autologous oxLDL [66] antibodies to oxLDL developed spontaneously in non-immunized hypercholesterolemic control rabbits, as well as in rabbits immunized with native LDL [which actually showed the greatest "protective" effect of immunization]. Also in contradiction with the proposed protective role of oxLDL and MDA-LDL antibodies was the observation of Palinski et al. who reported increased oxLDL autoantibody titers in LDLr^{-/-} mice that were significantly correlated with the extent of atherosclerosis [68].

In addition, it must be stressed that antibodies resulting from deliberate immunization have very different characteristics from those emerging spontaneously [4], and that there are significant species-related differences in lipoprotein metabolism and adaptive immune responses to modified lipoproteins. Therefore, the extrapolation of data obtained in animal models may be quite irrelevant for the understanding of human atherosclerosis [69].

There is no human data, either resulting from clinical studies or from *in vitro* investigations, that unambiguously supports a protective role of IgG oxLDL antibodies relative to the development of atherosclerosis. A report that a human-derived IgG oxLDL monoclonal antibody inhibited the uptake of oxLDL by macrophages[70] suggested such a protective role, but the recombinant monoclonal antibody was synthesized as Fab fragments[70] and the inhibition of oxLDL uptake was just a consequence of the fact that Fab fragments cannot activate complement and do not interact with Fcγ receptors [FcγR] [40,71]. Not surprisingly, Fab-LDL immune complexes were unable to activate macrophages and did not trigger an inflammatory reaction.

Are IgM antibodies to modified LDL protective?

More recently, the emphasis of research into the protective role of the humoral immune response in relation to atherosclerosis shifted into a proposed protective role of IgM antibodies. Such protective role for IgM antibodies is logical, given their low affinity, predominant intravascular distribution, and lack of interaction with Fc receptors of phagocytic cells [40, 72,73]. Data supporting this protective role of IgM antibodies has been obtained with different mouse models, in which oxLDL antibodies are predominantly IgM. This observation was the basis in support of a theory postulating that it is possible to induce a protective Th2-dependent antibody response to oxLDL [74]. It has also been proposed that IgM antibodies to modified LDL may exist as "natural" antibodies, part of a protective innate immune response [75]. The postulated existence of antibodies found in normal individuals in complete absence of antigenic stimulation has never been proven, and probably will never be, because of unpredictable cross-reactivities that may be responsible for the apparent emergence of "natural" antibodies. On the other hand, there are well-known examples of "natural" antibodies that emerge as a result of cross-reactions, such as the case of isohemagglutinins of the ABO system and some anti-bacterial antibodies [76]. Cross-reactivity explains some experimental findings that some

consider as examples of innate immunity to lipoproteins. For example, the immunization of hypercholesterolemic mice with *Streptococcus pneumoniae* result in the synthesis of protective IgM antibodies directed against phosphorylcholine epitopes shared with oxLDL [77]. Recently it was also shown that immunization of both pregnant NZW rabbits and LDL receptor-deficient mice with oxLDL resulted in cross-immunization of the progeny, which responded with the synthesis of IgM antibodies and formation of IgM-oxLDL immune complexes, which in turn appeared to have a protective effect against the development of atherosclerosis [78]. Irrespective of semantic and philosophical issues about the origin of “natural” antibodies, these observations raised considerable interest among those that would like to develop a preventive strategy against atherosclerosis based on immunization protocols. However, a recent report on the effects of pneumococcal vaccination in humans failed to demonstrate the induction of circulating IgM antibodies to oxLDL [79]. Nevertheless, a protective role of IgM antibodies to modified forms of LDL has also been proposed in humans, based on a negative correlation between IgM MDA-LDL antibody levels and carotid intima-media thickness [80,81]. The sum of published data, however, is contradictory. Mayr et al. reported that IgM oxLDL antibodies were inversely associated with incident and progressive carotid atherosclerosis in univariate analyses but were not independent predictors in multivariate analyses [15]. More recently, Fredrikson et al. reported that IgM antibodies to oxLDL correlate with a more rapid progression of carotid disease, as judged by IMT measurements [82].

The protective role of IgM antibodies to oxLDL in humans may not be as clear-cut as it is in some animal models for several reasons. First, in humans, modified lipoproteins elicit an spontaneous adaptive immune response directed against Apo-B epitopes unique of modified lipoproteins [4]. Several studies have shown that IgG is always the predominant isotype in affinity chromatography-purified oxLDL antibodies [13] and the same was true for AGE-LDL antibodies isolated from patients with type 1 diabetes [11]. A recent study conducted by us in a group of 36 patients with type 1 diabetes using stringent experimental conditions supports a pathogenic role for IgG oxLDL antibodies than a protective role for IgM oxLDL antibodies when nephropathy is used as the end-point. For this study we developed a protocol to assay IgG and IgM oxLDL antibodies contained in circulating immune complexes isolated from patient's sera. The circulating IC were precipitated from serum samples with 4% polyethylene glycol, resuspended, and fractionated in immobilized protein G columns. IgG and IgM oxLDL antibodies were assayed in the eluate and washout fractions of the Protein G column, respectively [83]. This approach has the advantage of measuring the specific antibodies of true significance, i.e., those able to form stable IC. Our preliminary data has shown that oxLDL antibodies isolated from IC are predominantly of the IgG isotype [$24 \pm 16 \mu\text{g/mL}$ for IgG antibodies vs. $3.7 \pm 2.8 \mu\text{g/mL}$ for IgM antibodies]. Statistical analysis of the data using conditional logistic regression analysis showed that for a 1 standard deviation increase in oxLDL IgG antibodies in IC, the odds of nephropathy being present increased by a factor of 2.3 (OR=2.34; 95% CI [0.78, 7.03]; $p=0.13$). On the other hand, increases in IgM antibody concentration were not found to show a negative correlation with the development of nephropathy (OR=0.927 for 1 SD change; 95% CI [0.44, 1.96]; $p=0.84$) [83].

The role of phospholipid antibodies in atherosclerosis

Phospholipid antibodies are classically associated with systemic lupus erythematosus (SLE) and with the phospholipid antibody syndrome, whose major clinical presentation is hypercoagulability and venous thrombosis [84]. It has also been proposed that these antibodies may play a pathogenic role in the accelerated atherosclerosis that afflicts patients with SLE and with phospholipid antibodies in general [85,86].

To better understand the pathogenic role of phospholipid antibodies (PLA) it must be realized that this is an “umbrella” designation for a highly heterogeneous group of antibodies [87]. PLA

have not been as extensively characterized as oxLDL antibodies, but it has been established that most PLA that exist in patients with SLE and phospholipid antibody syndrome react with beta-2-glycoprotein 1 (β 2GP1) and are predominantly of the IgG isotype [84,87]. The atherogenic mechanism of these antibodies seems to involve the association of β 2GP1 with oxLDL, followed by the formation of pro-inflammatory IgG- β 2GP1-oxLDL complexes [84, 88].

Other types of phospholipid antibodies react with phosphorylcholine (PC)[81] and lysophosphatidylcholine (LPC) [86], formed during LDL oxidation. These antibodies are detected using cardiolipin[89] or commercial preparations of PC complexed with bovine serum albumin[81], and the reaction of patient's sera with this substrate can be inhibited by oxLDL. The phospholipid antibodies detected by these methods seem to be predominantly of the IgM isotype[81,89] and it has been postulated that they recognize PC epitopes shared by *Streptococcus pneumoniae*. Mice immunized with *Streptococcus pneumoniae* produce antibodies (predominantly of the IgM isotype) that appear to cross-react with PC in oxLDL [75,77]. The proposed protective role of IgM antibodies in relation to the development of atherosclerosis in humans has been previously discussed in this review, and remains hypothetical, given the contradictory results reported by different groups [15,80–82,90].

Most studies comparing cardiolipin antibodies and oxLDL antibodies suggest that the β 2GP1 and PC/PLC antibodies are independent populations, with limited crossreactivity with oxidized LDL [9,10,90–92]. There is no reason why patients cannot recognize modified lysine and phospholipids epitopes independently, and absorption studies cannot truly determine whether a single cross-reactive antibody or multiple antibodies are involved. Our studies with affinity chromatography-purified oxLDL antibodies have the same limitations, but certainly indicate that anticardiolipin reactivity is not uniformly present in all purified antibodies,[9] it is of very low level,[10] and the fact that preincubation with oxLDL does not affect the reactivity of purified oxLDL antibodies with cardiolipin[10] raises issues about whether those are true antibodies of very low affinity or just non-specifically absorbed immunoglobulins. Thus, it seems essential to define the specificity of PLAs when investigating their role in atherosclerosis, and focus on those antibodies directed against phospholipid epitopes of oxLDL, equally represented in SLE and in the general population.

Conclusions

The sum of available evidence suggests that, in humans, the humoral immune response to modified LDL is pathogenic. The predominance of IgG over IgM antibodies favors the formation of IgG-containing IC with proinflammatory properties. *In vitro* studies have demonstrated that immune complexes formed with modified LDL and IgG antibodies have significantly stronger pro-atherogenic (as judged by leading to foam cell formation) and pro-inflammatory (as judged by the release of proinflammatory cytokines) properties than modified LDL by itself [12,13,56,61]. Clinical studies have shown that high levels of mLDL-IC are related to intima-media thickening and diabetic nephropathy. While most studies on the pro-inflammatory and pro-atherogenic properties of LDL-containing immune complexes have been performed with oxLDL and corresponding antibodies, there is no reason to believe that IC involving IgG antibodies reacting with any other LDL modifications, including modified LDL phospholipids or oxLDL- β 2GP1 complexes, cannot be equally pathogenic. The theoretically appealing possibility that IgM antibodies may have anti-inflammatory effects has neither been satisfactorily proven nor disproved and needs further investigation. Preliminary data suggesting that dietary modification may reduce the levels of oxLDL and increase the levels of IgM PC antibodies[93] certainly increases the interest in determining whether high levels of IgM mLDL antibodies may have a beneficial effect on the evolution of atherosclerosis. Alternative interventions could involve controlled upregulation of T regulatory cells (Tregs),

which has a strong theoretical appeal[94,95], and has been reported as an effective form of manipulation of transplant rejections responses[96], but is a rather distant target at this time. The only practical approach at this time is to reduce the antigenic load, either by dietary modification, or by pharmacological means. Statins have been shown to reduce the levels of circulating modified LDL-IC in two studies[97,98], and have also anti-inflammatory and anti-oxidant effects [99–101]. Their effects on modified LDL-IC levels should be investigated in a well designed trial with sufficient numbers of patients and methodological rigor, designed to determine how much of the benefits attributed to statins can be correlated with the reduction of the major pro-inflammatory insult that mLDL-IC appear to represent. On the meantime the search for alternative and more specific ways to reduce the modification of LDL, which would consequently reduce the immune response to modified lipoproteins and the formation of LDL-IC, should receive continued attention from the atherosclerosis research community.

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REFERENCES

1. Witztum JL. The role of oxidized LDL in the atherogenic process. *J Atheroscler Thromb* 1994;1:71–75. [PubMed: 9222872]
2. Lopes-Virella MF, Virella G. The role of immune and inflammatory processes in the development of macrovascular disease in diabetes. *Front Biosci* 2003;8:s750–s768. [PubMed: 12957881]
3. Stemme S, Faber B, Holm J, et al. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. *Proc Natl Acad Sci USA* 1995;92:3893–3897. [PubMed: 7732003]
4. Virella G, Thorpe S, Alderson NL, et al. Definition of the immunogenic forms of modified human LDL recognized by human autoantibodies and by rabbit hyperimmune antibodies. *J Lipid Res* 2004;45:1859–1867. [PubMed: 15258197]
5. Steinbrecher UP, Fisher M, Witztum JL, et al. Immunogenicity of homologous low density lipoprotein after methylation, ethylation, acetylation, or carbamylation: generation of antibodies specific for derivatized lysine. *Journal of Lipid Res* 1984;25:1109–1116. [PubMed: 6439810]
6. Palinski W, Yla-Herttuala S, Rosenfeld ME, et al. Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* 1990;10:325–335. [PubMed: 1693068]
7. Steinbrecher UP. Oxidation of human low density lipoprotein results in derivatization of lysine residues of apolipoprotein B by lipid peroxide decomposition products. *J Biol Chem* 1987;262:3603–3608. [PubMed: 3102491]
8. Yla-Herttuala S, Palinski W, Butler S, et al. Rabbit and human atherosclerotic lesions contain IgG that recognizes epitopes of oxidized LDL. *Arterioscler Thromb* 1994;14:32–40. [PubMed: 7506053]
9. Mironova M, Virella G, Lopes-Virella MF. Isolation and characterization of human anti-oxidized LDL autoantibodies. *Arterioscler Thromb Vasc Biol* 1996;16:222–229. [PubMed: 8620336]
10. Virella G, Koskinen S, Krings G, et al. Immunochemical characterization of purified human oxidized low-density lipoprotein antibodies. *Clinical Immunol* 2000;95:135–144. [PubMed: 10779407]
11. Virella G, Thorpe SR, Alderson NL, et al. Autoimmune response to advanced glycosylation end-products of human Low Density Lipoprotein. *J Lipid Res* 2003;44:487–493. [PubMed: 12562876]
12. Saad AF, Virella G, Chassereau C, et al. OxLDL immune complexes activate complement and induce cytokine production by MonoMac 6 cells and human macrophages. *J Lipid Res* 2006;47:1975–1983. [PubMed: 16804192] Epub 2006 Jun 19.
13. Virella G, Atchley DH, Koskinen S, et al. Pro-atherogenic and pro-inflammatory properties of immune complexes prepared with purified human oxLDL antibodies and human oxLDL. *Clinical Immunol* 2002;105:81–92. [PubMed: 12483997]

14. Hansson GK. Regulation of immune mechanisms in atherosclerosis. *Ann N Y Acad Sci* 2001;947:157–165. [PubMed: 11795263]discussion 165-156.
15. Mayr M, Kiechl S, Tsimikas S, et al. Oxidized low-density lipoprotein autoantibodies, chronic infections, and carotid atherosclerosis in a population-based study. *J Am Coll Cardiol* 2006;47:2436–2443. [PubMed: 16781371]
16. Mayr M, Kiechl S, Willeit J, et al. Infections, immunity, and atherosclerosis: associations of antibodies to *Chlamydia pneumoniae*, *Helicobacter pylori*, and cytomegalovirus with immune reactions to heat-shock protein 60 and carotid or femoral atherosclerosis. *Circulation* 2000;102:833–839. [PubMed: 10952949]
17. Wick G, Kleindienst R, Schett G, et al. Role of heat shock protein 65/60 in the pathogenesis of atherosclerosis. *International Archives of Allergy & Immunology* 1995;107:130–131. [PubMed: 7542057]
18. Qazi KR, Oehlmann W, Singh M, et al. Microbial heat shock protein 70 stimulatory properties have different TLR requirements. *Vaccine* 2007;25:1096–1103. [PubMed: 17049413]
19. Wang R, Kovalchin JT, Muhlenkamp P, et al. Exogenous heat shock protein 70 binds macrophage lipid raft microdomain and stimulates phagocytosis, processing, and MHC-II presentation of antigens. *Blood* 2006;107:1636–1642. [PubMed: 16263790]
20. Svensson PA, Asea A, Englund MC, et al. Major role of HSP70 as a paracrine inducer of cytokine production in human oxidized LDL treated macrophages. *Atherosclerosis* 2006;185:32–38. [PubMed: 15993884]
21. Bellomo G, Maggi E, Poli M, et al. Autoantibodies against oxidatively modified low-density lipoproteins in NIDDM. *Diabetes* 1995;44:60–66. [PubMed: 7813815]
22. Erkkilä AT, Närönen O, Lehto S, et al. Autoantibodies against oxidized low-density lipoprotein and cardiolipin in patients with coronary heart disease. *Arterioscl Thromb Vasc Biol* 2000;20:204–209. [PubMed: 10634819]
23. Lehtimäki T, Lehtinen S, Solakivi T, et al. Autoantibodies against oxidized low density lipoprotein in patients with angiographically verified coronary artery disease. *Arterioscler Thromb Vasc Biol* 1999;19:23–27. [PubMed: 9888862]
24. Maggi E, Chiesa R, Melissano G, et al. LDL oxidation in patients with severe carotid atherosclerosis. A study of in vitro and in vivo oxidation markers. *Arterioscler Thromb* 1994;14:1892–1899. [PubMed: 7981176]
25. Palinski W, Yla-Herttuala S, Rosenfeld ME, et al. Antisera and monoclonal antibodies specific for epitopes generated during oxidative modification of low density lipoprotein. *Arteriosclerosis* 1990;10:325–335. [PubMed: 1693068]
26. Salonen JT, Yla-Herttuala S, Yamamoto R, et al. Autoantibody against oxidised LDL and progression of carotid atherosclerosis. *Lancet* 1992;339:883–887. [PubMed: 1348295]
27. Takeuchi M, Makita Z, Yanagisawa K, et al. Detection of noncarboxymethyllysine and carboxymethyllysine advanced glycation end products (AGE) in serum of diabetic patients. *Mol Med* 1999;5:393–405. [PubMed: 10415164]
28. Boullier A, Hamon M, Walters-Laporte E, et al. Detection of autoantibodies against oxidized low-density lipoproteins and of IgG-bound low density lipoproteins in patients with coronary artery disease. *Clin Chim Acta* 1995;238:1–10. [PubMed: 7554289]
29. Festa A, Kopp HP, Scherthaner G, et al. Autoantibodies to oxidised low density lipoproteins in IDDM are inversely related to metabolic control and microvascular complications. *Diabetologia* 1998;41:350–356. [PubMed: 9541177]
30. Hulthe J, Wiklund O, Hurt-Camejo E, et al. Antibodies to oxidized LDL in relation to carotid atherosclerosis, cell adhesion molecules, and phospholipase A(2). *Arterioscler Thromb Vasc Biol* 2001;21:269–274. [PubMed: 11156864]
31. Hulthe J, Bokemark L, Fagerberg B. Antibodies to oxidized LDL in relation to intima-media thickness in carotid and femoral arteries in 58-year-old subjectively clinically healthy men. *Arterioscler Thromb Vasc Biol* 2001;21:101–107. [PubMed: 11145940]
32. Virella G, Virella I, Leman RB, et al. Anti-oxidized low-density lipoprotein antibodies in patients with coronary heart disease and normal healthy volunteers. *Internat J Clin Lab Res* 1993;23:95–101.

33. van de Vijver LP, Steyger R, van Poppel G, et al. Autoantibodies against MDA-LDL in subjects with severe and minor atherosclerosis and healthy population controls. *Atherosclerosis* 1996;122:245–253. [PubMed: 8769687]
34. Wu R, de Faire U, Lemne C, et al. Autoantibodies to OxLDL are decreased in individuals with borderline hypertension. *Hypertension* 1999;33:53–59. [PubMed: 9931081]
35. Leinonen JS, Rantalaiho V, Laippala P, et al. The level of autoantibodies against oxidized LDL is not associated with the presence of coronary heart disease or diabetic kidney disease in patients with non-insulin-dependent diabetes mellitus. *Free Radic Res* 1998;29:137–141. [PubMed: 9790515]
36. Uusitupa MIJ, Niskanen L, Luoma J, et al. Autoantibodies against oxidized LDL do not predict atherosclerosis vascular disease in non-insulin-dependent diabetes mellitus. *Arterioscler Thromb Vasc Biol* 1996;16:1236–1242. [PubMed: 8857919]
37. Orchard TJ, Virella G, Forrest KY, et al. Antibodies to oxidized LDL predict coronary artery disease in type 1 diabetes: a nested case-control study from the Pittsburgh Epidemiology of Diabetes Complications Study. *Diabetes* 1999;48:1454–1458. [PubMed: 10389853]
38. Lopes-Virella MF, Virella G, Orchard TJ, et al. Antibodies to oxidized LDL and LDL-containing immune complexes as risk factors for coronary artery disease in diabetes mellitus. *Clinical Immunol* 1999;90:165–172. [PubMed: 10080827]
39. Mironova M, Virella G, Virella-Lowell I, et al. Anti-modified LDL antibodies and LDL-containing immune complexes in IDDM patients and healthy controls. *Clin Immunol Immunopath* 1997;85:73–82.
40. Virella, G. Biosynthesis, metabolism and biological properties of immunoglobulins. In: Virella, G., editor. *Medical Immunology*. 6th Ed. N.Y. and London: Informa; 2007. p. 65–72.
41. Virella, G.; Tsokos, G. Immune complex diseases. In: Virella, G., editor. *Medical Immunology*. N.Y. and London: Informa; 2007. p. 323–334.
42. Yla-Herttuala S, Palinski W, Rosenfeld ME, et al. Evidence for the presence of oxidatively modified low density lipoprotein in atherosclerotic lesions of rabbit and man. *J Clin Invest* 1989;84:1086–1095. [PubMed: 2794046]
43. Orekhov AN, Kalenich OS, Tertov VV, et al. Lipoprotein immune complexes as markers of atherosclerosis. *Internat J Tissue React* 1991;13:233–236.
44. Orekhov AN, Kalenich OS, Tertov VV, et al. Diagnostic value of immune cholesterol as a marker for atherosclerosis. *J Cardiovasc Risk* 1995;2:459–466. [PubMed: 8749274]
45. Turk Z, Sesto M, Skodlar J, et al. Soluble LDL-Immune Complexes in Type 2 Diabetes and Vascular Disease. *Horm Metab Res* 2002;34:196–201. [PubMed: 11987029]
46. Lopes-Virella MF, McHenry MB, Lipsitz S, et al. 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. [PubMed: 16530770]
47. Atchley DH, Lopes-Virella MF, Zheng D, et al. Oxidized LDL-Anti-Oxidized LDL immune complexes and diabetic nephropathy. *Diabetologia* 2002;45:1562–1571. [PubMed: 12436340]
48. Costacou T, Lopes-Virella MF, Zgibor JC, et al. Markers of endothelial dysfunction in the prediction of coronary artery disease in type 1 diabetes. The Pittsburgh Epidemiology of Diabetes Complications Study. *J Diabetes Complications* 2005;19:183–193. [PubMed: 15993351]
49. Yishak AA, Costacou T, Virella G, et al. Novel predictors of overt nephropathy in subjects with type 1 diabetes. A nested case control study from the Pittsburgh Epidemiology of Diabetes Complications cohort. *Nephrol Dial Transplant* 2006;21:93–100. [PubMed: 16144851] Epub 2005 Sep 2006.
50. Klimov AN, Denisenko AD, Popov AV, et al. Lipoprotein-antibody immune complexes: Their catabolism and role in foam cell formation. *Atherosclerosis* 1985;58:1–15. [PubMed: 4091875]
51. Klimov AN, Denisenko AD, Vinogradov AG, et al. Accumulation of cholesteryl esters in macrophages incubated with human lipoprotein-antibody autoimmune complex. *Atherosclerosis* 1988;74:41–46. [PubMed: 3214480]
52. Mironova MA, Klein RL, Virella GT, et al. Anti-modified LDL antibodies, LDL-containing immune complexes, and susceptibility of LDL to in vitro oxidation in patients with type 2 diabetes. *Diabetes* 2000;49:1033–1041. [PubMed: 10866057]

53. Orekhov, AN.; Tertov, VV. Antibody-like immunoglobulins G against low density lipoprotein that stimulate lipid accumulation in cultured cells. In: Malmadier, CL., editor. Hypercholesterolemia, Hypocholesterolemia, Hypertriglyceridemia. N.Y., N.Y: Plenum Press; 1990. p. 399-404.
54. Klimov AN, Denisenko AD, Vinogradov AG, et al. Accumulation of CE in macrophages incubated with human lipoprotein-antibody autoimmune complex. *Atherosclerosis* 1988;74:41–46. [PubMed: 3214480]
55. Virella G, Munoz JF, Galbraith GM, et al. Activation of human monocyte-derived macrophages by immune complexes containing low-density lipoprotein. *Clin Immunol Immunopath* 1995;75:179–189.
56. Griffith RL, Virella GT, Stevenson HC, et al. Low density lipoprotein metabolism by human macrophages activated with low density lipoprotein immune complexes. A possible mechanism of foam cell formation. *J Exp Med* 1988;168:1041–1059. [PubMed: 3171477]
57. Huang Y, Jaffa A, Koskinen S, et al. Oxidized LDL-containing immune complexes induce Fc gamma receptor I-mediated mitogen-activated protein kinase activation in THP-1 macrophages. *Arterioscler Thromb Vasc Biol* 1999;19:1600–1607. [PubMed: 10397676]
58. Kiener PA, Rankin BM, Davis PM, et al. Immune complexes of LDL induce atherogenic responses in human monocytic cells. *Arterioscler Thromb Vasc Biol* 1995;15:990–999. [PubMed: 7600130]
59. Huang Y, Fleming AJ, Wu S, et al. Fc-gamma receptor cross-linking by immune complexes induces matrix metalloproteinase-1 in U937 cells via mitogen-activated protein kinase. *Arterioscler Thromb Vasc Biol* 2000;20:2533–2538. [PubMed: 11116049]
60. Lopes-Virella MF, Binzafar N, Rackley S, et al. The uptake of LDL-IC by human macrophages: predominant involvement of the Fc gamma RI receptor. *Atherosclerosis* 1997;135:161–170. [PubMed: 9430365]
61. Lopes-Virella MF, Griffith RL, Shunk KA, et al. Enhanced uptake and impaired intracellular metabolism of low density lipoprotein complexed with anti-low density lipoprotein antibodies. *Arterioscler Thromb* 1991;11:1356–1367. [PubMed: 1911721]
62. Hernandez-Vargas P, Ortiz-Munoz G, Lopez-Franco O, et al. Fc gamma receptor deficiency confers protection against atherosclerosis in apolipoprotein E knockout mice. *Circ Res* 2006;99:1188–1196. [PubMed: 17053192]
63. Wick GXu Q. Atherosclerosis-an autoimmune disease. *Exp Gerontol* 1999;34:559–566. [PubMed: 10817811]
64. Palinski W, Miller E, Witztum JL. Immunization of low density lipoprotein (LDL) receptor deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. *Proc Natl Acad Sci USA* 1995;92:821–825. [PubMed: 7846059]
65. George J, Afek A, Gilburd B, et al. Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. *Atherosclerosis* 1998;138:147–152. [PubMed: 9678780]
66. Ameli S, Hultgardh-Nilsson A, Regnstrom J, et al. Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. *Arterioscler Thromb Vasc Biol* 1996;16:1074–1079. [PubMed: 8696949]
67. Freigang S, Horkko S, Miller E, et al. Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neoepitopes. *Arterioscler Thromb Vasc Biol* 1998;18:1972–1982. [PubMed: 9848892]
68. Palinski W, Tangirala RK, Miller E, et al. Increased autoantibody titers against epitopes of oxidized LDL in LDL receptor-deficient mice with increased atherosclerosis. *Arterioscler Thromb Vasc Biol* 1995;15:1569–1576. [PubMed: 7583529]
69. Virella G, Lopes-Virella MF. Humoral immunity and atherosclerosis. *Nature Medicine* 2003;9:243–244.
70. Shaw PX, Horkko S, Tsimikas S, et al. Human-derived anti-oxidized LDL autoantibody blocks uptake of oxidized LDL by macrophages and localizes to atherosclerotic lesions in vivo. *Arterioscler Thromb Vasc Biol* 2001;21:1333–1339. [PubMed: 11498462]
71. Virella, G. Immunoglobulin structure. In: Virella, G., editor. *Medical Immunology*. 6th Ed. NY and London: Informa; 2007. p. 55-63.

72. Virella, G. Humoral immune response and its induction by active immunization. In: Virella, G., editor. *Medical Immunology*. 6th Ed. N.Y. and London: Informa; 2007. p. 159-172.
73. Mariani, G.; Strober, W. Immunoglobulin Metabolism. In: Metzger, H., editor. *Fc Receptors and the Action of Antibodies*. Washington, DC: American Society for Microbiology Press; 1990. p. 94-180.
74. Binder CJ, Chang MK, Shaw PX, et al. Innate and Acquired Immunity in Atherogenesis. *Nature Medicine* 2002;8:1218-1226.
75. Binder CJ, Shaw PX, Chang MK, et al. The role of natural antibodies in atherogenesis. *J Lipid Res* 2005;46:1353-1363. [PubMed: 15897601]Epub 2005 May 1316.
76. Virella, G.; Tomlinson, S. Infections and Immunity. In: Virella, G., editor. *Medical Immunology*. 6th Ed. New York and London: Informa; 2007. p. 183-200.
77. Binder CJ, Horkko S, Dewan A, et al. Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between *Streptococcus pneumoniae* and oxidized LDL. *Nature Medicine* 2003;9:736-743.Epub 2003 May 2012.
78. Yamashita T, Freigang S, Eberle C, et al. Maternal immunization programs postnatal immune responses and reduces atherosclerosis in offspring. *Circ Res* 2006;99:e51-e64. [PubMed: 16946133]
79. Damoiseaux J, Rijkers G, Tervaert JW. Pneumococcal vaccination does not increase circulating levels of IgM antibodies to oxidized LDL in humans and therefore precludes an anti-atherogenic effect. *Atherosclerosis* 2007;190:10-11. [PubMed: 16774756]
80. Karvonen J, Paivansalo M, Kesaniemi YA, et al. Immunoglobulin M type of autoantibodies to oxidized low-density lipoprotein has an inverse relation to carotid artery atherosclerosis. *Circulation* 2003;108:2107-2112. [PubMed: 14530200]Epub 2003 Oct 2106.
81. Su J, Georgiades A, Wu R, et al. Antibodies of IgM subclass to phosphorylcholine and oxidized LDL are protective factors for atherosclerosis in patients with hypertension. *Atherosclerosis* 2006;188:160-166. [PubMed: 16307748]
82. Fredrikson GN, Hedblad B, Berglund G, et al. Association between IgM against an aldehyde-modified peptide in apolipoprotein B-100 and progression of carotid disease. *Stroke* 2007;38:1495-1500. [PubMed: 17363723]
83. Virella G, Carter RE, Saad A, et al. Distribution of IgM and IgG antibodies to oxidized LDL in immune complexes isolated from patients with type 1 diabetes and its relationship with nephropathy. *Clin Immunol*. 2008in print.
84. Matsuura E, Kobayashi K, Tabuchi M, et al. Oxidative modification of low-density lipoprotein and immune regulation of atherosclerosis. *Prog Lipid Res* 2006;45:466-486. [PubMed: 16790279]
85. Matsuura E, Kobayashi K, Tabuchi M, et al. Accelerated atheroma in the antiphospholipid syndrome. *Rheum Dis Clin North Am* 2006;32:537-551. [PubMed: 16880083]
86. Frostegard J. Atherosclerosis in patients with autoimmune disorders. *Arterioscler Thromb Vasc Biol* 2005;25:1776-1785. [PubMed: 15976324]
87. Lopez D, Garcia-Valladares I, Palafox-Sanchez CA, et al. Oxidized low-density lipoprotein/beta2-glycoprotein I complexes and autoantibodies to oxLig-1/beta2-glycoprotein I in patients with systemic lupus erythematosus and antiphospholipid syndrome. *Am J Clin Pathol* 2004;121:426-436. [PubMed: 15023048]
88. Kobayashi K, Lopez LR, Shoenfeld Y, et al. The role of innate and adaptive immunity to oxidized low-density lipoprotein in the development of atherosclerosis. *Ann N Y Acad Sci* 2005;1051:442-454. [PubMed: 16126986]
89. Wu R, Huang YH, Elinder LS, et al. Lysophosphatidylcholine is involved in the antigenicity of oxidized LDL. *Arterioscler Thromb Vasc Biol* 1998;18:626-630. [PubMed: 9555869]
90. Damoiseaux J, Jeyasekharan AD, Theunissen R, et al. Cross-reactivity of IgM and IgG anticardiolipin antibodies with oxidized-low density lipoproteins. *Ann N Y Acad Sci* 2005;1050:163-169. [PubMed: 16014530]
91. Loch H, Wiik A. Prevalence of antibodies against oxidised LDL in a cohort of 163 patients with positive anti-phospholipid antibodies and recent thrombosis. *Rheumatol Int* 2006;26:416-421. [PubMed: 16189653]
92. Sherer Y, Gerli R, Vaudo G, et al. Prevalence of antiphospholipid and antioxidantized low-density lipoprotein antibodies in rheumatoid arthritis. *Ann N Y Acad Sci* 2005;1051:299-303. [PubMed: 16126971]

93. Elkan AC, Sjoberg B, Kolsrud B, et al. Gluten-free vegan diet induces decreased LDL and oxidized LDL levels and raised atheroprotective natural antibodies against phosphorylcholine in patients with rheumatoid arthritis: a randomized study. *Arthritis Res Ther* 2008;10:R34. [PubMed: 18348715]
94. Raimondi G, Turner MS, Thomson AW, et al. Naturally occurring regulatory T cells: recent insights in health and disease. *Crit Rev Immunol* 2007;27:61–95. [PubMed: 17430097]
95. Yang XF. Factors regulating apoptosis and homeostasis of CD4+ CD25(high) FOXP3+ regulatory T cells are new therapeutic targets. *Front Biosci* 2008;13:1472–1499. [PubMed: 17981643]
96. Tao R, Hancock WW. Regulating regulatory T cells to achieve transplant tolerance. *Hepatobiliary Pancreat Dis Int* 2007;6:348–357. [PubMed: 17690028]
97. Lopes-Virella MF, Mironova M, Stephan E, et al. Role of simvastatin as an immunomodulator in type 2 diabetes. *Diabetes Care* 2004;27:908–913. [PubMed: 15047647]
98. Tsimikas S, Witztum JL, Miller ER, et al. High-dose atorvastatin reduces total plasma levels of oxidized phospholipids and immune complexes present on apolipoprotein B-100 in patients with acute coronary syndromes in the MIRACL trial. *Circulation* 2004;110:1406–1412. [PubMed: 15353498]Epub 2004 Sep 1407.
99. Bocan TM. Pleiotropic effects of HMG-CoA reductase inhibitors. *Curr Opin Investig Drugs* 2002;3:1312–1317.
100. Endres M. Statins: potential new indications in inflammatory conditions. *Atheroscler Suppl* 2006;7:31–35. [PubMed: 16503422]
101. Patel TN, Shishehbor MH, Bhatt DL. A review of high-dose statin therapy: targeting cholesterol and inflammation in atherosclerosis. *Eur Heart J* 2007;28:664–672. [PubMed: 17242008]