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Innate Immune Proteins C1q and Mannan-Binding Lectin Enhance Clearance of Atherogenic Lipoproteins by Human Monocytes and Macrophages

Deborah A. Fraser and Andrea J. Tenner

Atherosclerosis is now widely accepted to be a chronic inflammatory disorder that is characterized by the accumulation of modified lipoproteins in the arterial intima. C1q and mannann-binding lectin (MBL) are not only recognition components in the activation of inflammation via the complement cascade, but they are also able to directly modulate phagocyte activation. Studies in C1q−/− and MBL−/− mice suggest that these molecules play a protective role in the early atherosclerotic lesion in the absence of, or prior to, expression of other complement components. However, in later stages, complement activation becomes an inappropriate inflammatory response, contributing to disease pathology. Therefore, to investigate possible molecular interactions of C1q and MBL in atherosclerotic lesions, we examined the influence of C1q and MBL in the clearance of native and modified lipoproteins by human monocytes and monocyte-derived macrophages. Both C1q and MBL are shown to bind and enhance the monocyte/monocyte-derived macrophage clearance of modified forms of low-density lipoprotein (LDL), including oxidized LDL and acetylated LDL, but not native LDL. Modified forms of LDL activate the classical complement pathway, but no lectin pathway activation was detected. Interestingly, monocytes that ingested modified LDL in the presence of C1q or MBL upregulated surface CD80 and CD31, as well as CCL2 chemokine gene expression. However, C1q and MBL also significantly reduced levels of free cholesterol accumulation in monocytes and human monocyte-derived macrophages that ingested oxidized LDL, while enhancing high-density lipoprotein–specific cholesterol efflux from these cells. These results suggest a novel pathway in which C1q and MBL influence removal and metabolism of atherogenic forms of LDL in the early stages of atherosclerosis. 


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The online version of this article contains supplemental material.

Abbreviations used in this paper: ABC, ATP-binding cassette; AcLDL, acetylated low-density lipoprotein; C1qD, serum depleted of C1q; C1qD-MBLD, human serum depleted of C1q and mannan-binding lectin; Dil, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; E-LDL, enzyme-modified low-density lipoprotein; HDL, high-density lipoprotein; HMDM, human monocyte-derived macrophage; LDL, low-density lipoprotein; MBL, mannan-binding lectin; MFI, mean fluorescence intensity; NHS, normal human serum; OPD, o-phenylenediamine; OxLDL, oxidized low-density lipoprotein.
and homogeneous, as assessed by SDS-PAGE. Protein concentration was
M-CSF was purchased from PeproTech (Rocky Hill, NJ). C1q was isolated
Calbiochem (EMD Chemicals, Darmstadt, Germany). Recombinant human
ration dates. Human high-density lipoprotein (HDL) was purchased from
was obtained from Molecular Probes (Invitrogen, Eugene, OR). Human
MBLs were purified as described (22).

Materials and Methods

Materials

1,1’-Diocadecyl-3,3’,3’-tetramethylindocarbocyanine perchlorate (DiI) was purchased from Molecular Probes (Invitrogen, Eugene, OR). Human LDL and their matched-lot modified forms, AcLDL and OxLDL, were purchased from Intracel (Frederick, MD) and used within specified expiration dates. Human high-density lipoprotein (HDL) was purchased from Calbiochem (EMD Chemicals, Darmstadt, Germany). Recombinant human M-CSF was purchased from PeproTech (Rocky Hill, NJ). C1q was isolated from plasma-derived normal human serum (NHS) by ion-exchange chromatography followed by size-exclusion chromatography according to the method of Tenner et al. (19) and modified as described (20). The C1q preparations used were fully active, as determined by hemolytic titration, and homogeneous, as assessed by SDS-PAGE. Protein concentration was determined using an extinction coefficient (E) of 280 nm of 6.82 for C1q (21). During the purification of C1q, serum depleted of C1q (C1qD) was collected after passage of plasma-derived serum over the ion-exchange resin and stored at −70°C until use. Serum depleted of both C1q and MBL (C1qD-MBLD) was prepared by passing C1qD over a column of mannose-agarose (Sigma-Aldrich, St. Louis, MO) that had been equilibrated into veronal buffer containing magnesium and calcium. Recombinant or human plasma MBLs were purified as described (22).

Cells

Human peripheral blood monocytes were isolated by counterflow elutriation using a modification of the technique of Lionetti et al. (23) as described (24). Greater than 90% of the cells in each preparation were monocytes (CD11b/CD14 positive, CD3 negative) according to flow cytometry analysis. All blood samples were collected in accordance with the guidelines and approval of the University of California Irvine Institutional Review Board. Freshly isolated monocytes were used immediately, or cultured for 7 d in RPMI 1640 media supplemented with 10% FCS (ThermoFisher-Hyclone, Milford, MA), 25ng/ml M-CSF, 1% L-glutamine, 1% penicillin/streptomycin to generate human monocyte-derived macrophages (HMDM), with 50% volume exchange with fresh media every 3-5 d.

Binding assay

Immuno 2HB plates (ThermoFisher-Hyclone) were coated with lipoproteins or BSA control protein at 50 μg protein/ml in PBS, overnight at room temperature. Remaining sites were blocked with PBS containing 5% milk for 1 h at 37°C. Dilutions of purified C1q or MBL in PBS/1% milk were incubated for 2 h at room temperature. Wells were washed with PBS/ 0.05% Tween 20, prior to addition of JH11 (25) monoclonal anti-C1q (0.5 μg/ml) or 1173 polyclonal anti-MBL (26) (2 μg/ml) in PBS/1% milk and incubation for 90 min at room temperature. Wells were washed and HRP-conjugated secondary Ab (1/2000 dilution; Jackson ImmunoResearch Laboratories, West Grove, PA) was added for 30 min at room temperature. The binding assay was developed by the addition of substrate o-phenylenediamine (OPD) (Sigma-Aldrich). Binding of C1q or MBL was assessed by measurement of the average absorbance of duplicate sample wells at 405 nm.

Complement activation assay

Immuno 2HB plates were coated with lipoproteins, BSA, ormann (50 μg/ml; Sigma-Aldrich) as described above. Remaining sites were blocked with PBS/1% BSA for 1 h at 37°C. Dilutions of C1q (0.01–10 μg/ml) or MBL (10 μg/ml) were made into serum depleted of C1q and MBL as a source of other complement components (1/10 in gelatin veronal buffer containing magnesium and calcium), and incubated for 30 min at 37°C to allow for complement activation to occur. Wells were washed with PBS/0.05% Tween 20 prior to incubation with monoclonal anti-C3d (1/1000 dilution; BD Biosciences, San Diego, CA) for 1 h at room temperature. Wells were washed and HRP-conjugated secondary Ab (1/10000 dilution; Jackson ImmunoResearch Laboratories) was added for 45 min at room temperature. The assay was developed by the addition of substrate OPD, and C3b deposition was assessed by measurement of the average absorbance at 405 nm of duplicate sample wells.

Lipoprotein uptake assay

LDL, AcLDL, and OxLDL, were labeled with DiI as follows: 0.5 mg (protein) LDL or modified LDL were incubated in 1 ml PBS with 150 μg DiI for 16 h, 37°C under sterile conditions. Unconjugated dye was removed by buffer exchange of DiI-labeled lipoproteins into sterile PBS using a PD-10 desalting column (GE Healthcare, Piscataway, NJ). Protein concentration of each preparation was determined by BCA assay (ThermoFisher-Pierce) according to the manufacturer’s instructions. Dil-labeled lipoproteins were stored at 4°C in the dark and used within 3 wk of preparation. Freshly elutriated monocytes, or macrophages harvested using Versene/EDTA (Invitrogen), were resuspended at 1 × 10⁵ cells/ml in phagocytosis buffer (RPMI 1640, 25 mM HEPES, 5 mM MgCl₂) and incubated for 30 min at 37°C to allow for complement activation to occur. Wells were washed with PBS/0.05% Tween 20 prior to incubation with monoclonal anti-C3d (1/1000 dilution; BD Biosciences, San Diego, CA), and approval of the University of California Irvine Institutional Review Board. Freshly isolated monocytes were used immediately, or cultured for 7 d in RPMI 1640 media supplemented with 10% FCS (ThermoFisher-Hyclone, Milford, MA), 25ng/ml M-CSF, 1% L-glutamine, 1% penicillin/streptomycin. Plates were centrifuged at 70 × g for 3 min. OxLDL at 10 μg protein/ml was added to wells in the
presence or absence of 75 μg/ml C1q for 2–24 h. Cells were harvested with 0.25% trypsin, extracted with chlorofom/isopropanol/Triton X-100 (7:11:0.1) using a microhomogenizer, and levels of free cholesterol were then measured by enzymatic colorimetric assay using a cholesterol quantitation kit (BioVision, Mountain View, CA) as described in detail in the provided manufacturer’s protocol.

**Modulation of gene expression by C1q**

Human monocytes or HMDM were added to 8-well Labtek chamber slides, 1.25 × 10⁵ cells/well, in X-VIVO 15 media, which was supplemented with 1% l-glutamine and 1% penicillin/streptomycin. Slides were centrifuged at 70 × g for 3 min. OxLDL at 10 μg protein/ml was added to wells in the presence or absence of 75 μg/ml C1q or 10 μg/ml MBL. After 2 h, RNA was harvested from cells remaining in the wells using an Illustra RNA extraction kit (GE Healthcare), and cDNA was synthesized by RT-PCR using the Moloney murine leukemia virus reverse transcriptase kit (Invitrogen) according to the manufacturer’s instructions. Levels of mRNA for GAPDH, ATP-binding cassette (ABC)-A1, ABC-G1, and MCP-1/CCL2 were analyzed by real-time quantitative RT-PCR (iCycler; Bio-Rad, Hercules, CA) using probes designed in-house (Supplemental Table 1) and synthesized by Integrated DNA Technologies (San Diego, CA). The mRNA expression levels were normalized to GAPDH as an internal standard, and they were expressed as fold difference from monocytes/HMDM in the absence of lipoprotein.

**HDL-specific cholesterol efflux assays**

Freshly elutriated human monocytes or HMDM (day 7) were labeled with 1 μCi/ml [³H]cholesterol (PerkinElmer, Boston, MA) for 24 h in RPMI 1640 containing 10% FCS. Cells were washed twice with PBS and equilibrated for 2 h in RPMI 1640 containing 0.2% fatty acid-free BSA (Sigma-Aldrich) at 37°C. Cells were washed into fresh RPMI 1640 containing 2% fatty acid-free BSA and plated at 1 × 10⁶ cells/ml in 24-well plates, and OxLDL was then added to specific wells at 10 μg protein/ml in the presence or absence of 75 μg/ml C1q for 2 h before HDL (50 μg protein/ml) was added to wells for a further 2–24 h incubation. Cholesterol efflux was determined by measuring radioactivity in the culture medium of protein/ml) was added to wells for a further 2–24 h incubation. Cholesterol efflux was determined by measuring radioactivity in the culture medium of

**Results**

**C1q and MBL bind modified forms of LDL**

C1q binding to modified forms of LDL has previously been reported (10). To confirm this in our system, and to investigate binding of MBL, a plate binding assay was developed. LDL-, OxLDL-, AcLDL-, or BSA (control)-coated wells were incubated with dilutions of purified C1q (0–150 μg/ml) or MBL (0–80 μg/ml), and binding was assessed by Ab detection. To confirm that equivalent levels of unmodified/modified LDL were coated on the plate, levels of lipoprotein binding were assessed in parallel wells by the addition of anti–apolipoprotein B-100 (data not shown). As was previously reported, C1q bound to OxLDL, and in addition we observed binding to AcLDL, but not to unmodified LDL or BSA control, as measured by A405 nm (Fig. 1). Binding was dose-dependent on the concentration of C1q (Fig. 1A). Similar to C1q, MBL also bound to modified forms of LDL, but not to LDL alone (Fig. 1B).

**Modified forms of LDL activate the classical, but not lectin, complement pathway**

C1q binding to modified forms of LDL has previously been shown to activate the classical complement pathway (10). Because we have shown that MBL also binds modified forms of LDL, we investigated the ability of modified forms to activate the lectin complement pathway, using an ELISA-based plate assay to measure deposition of complement activation product C3b. Similar to previous reports, we show that OxLDL and AcLDL but not unmodified LDL are able to activate the classical pathway of complement, leading to deposition of C3b (Fig. 2A). Interestingly, although MBL also binds modified forms of LDL (Fig. 1B), this recognition step did not lead to activation of the lectin complement pathway (Fig. 2B).

In this assay, MBL was shown to be able to activate the lectin pathway via recognition of mannan, a known lectin pathway activator.

**FIGURE 1.** C1q and MBL bind modified forms of LDL. LDL, OxLDL, AcLDL, or BSA control protein were immobilized on a plate and incubated with 0.5–150 μg/ml C1q (A) or 0.5–80 μg/ml MBL (B). Binding was assessed by ELISA using the OPD substrate with signal absorbance measured at 405 nm. Data are from a single experiment, performed in duplicate wells, representative of three individual experiments.

**FIGURE 2.** Modified forms of LDL activate complement via the classical pathway but not the lectin pathway. LDL, OxLDL, AcLDL, BSA (control protein), or mannan (control lectin pathway activator) were immobilized on a plate and incubated with 0.01–10 μg/ml C1q (A) or 20 μg/ml C1q or MBL (B) in C1q-D-MBLD. Complement activation of the classical (A, B) or lectin pathway (B) was assessed by measuring deposition of C3b by ELISA using the OPD substrate, with signal absorbance measured at 405 nm. Data are from single experiments, performed in duplicate wells, representative of three individual experiments.
C1q and MBL enhance ingestion of modified LDL by monocytes and macrophages

Defense collagens have been shown to enhance the phagocytosis of various particles, including targets coated with IgG/complement and apoptotic cells (17), mediated by their collagen-like domain. To assess their role in clearance of atherogenic lipoproteins, DiI-labeled LDL, OxLDL, and AcLDL were incubated with human monocytes and HMDM in the presence or absence of purified C1q or MBL and lipoprotein uptake was assessed by flow cytometry. C1q enhanced monocyte clearance of modified LDL (OxLDL and AcLDL), but not unmodified LDL (Fig. 3A). This enhancement of modified LDL uptake was seen at both 1 and 10 μg of protein/ml (Fig. 3B) and was dose-responsive to C1q concentration (data not shown), with maximal C1q-mediated clearance at physiological levels of C1q (75 μg/ml). Similar C1q-mediated enhancements were seen in HMDM, in cells from a range of donors and multiple batches of lipoprotein (Fig. 3D). Purified MBL also enhanced monocyte and HMDM clearance of OxLDL and AcLDL (Fig. 3C and data not shown). Enhanced clearance of OxLDL was dose-responsive to MBL concentration (data not shown). Differences in mean fluorescence intensity (MFI) between modified forms of lipoproteins are due to variation in DiI-labeling efficiency rather than reflective of relative ingested amounts. Addition of NHS also enhanced clearance of OxLDL, but this enhancement was not seen with C1qD-MBLD (Fig. 3E). Reconstitution of C1qD-MBLD serum with 75 μg/ml C1q (approximate normal plasma level (27)) restored the ability of C1qD-MBLD serum to enhance clearance of OxLDL. However, no enhancement of uptake was observed with the reconstitution of 10 μg/ml MBL to C1qD-MBLD serum (approximate high-normal plasma level (28)). Ingestion of DiI-labeled modified LDL into vesicular compartments was confirmed by confocal microscopy (Supplemental Fig. 1).

C1q and MBL modulate monocyte activation and chemokine responses during the clearance of OxLDL

Given that C1q has also been shown to modulate gene expression and function in phagocytic cells (29), we examined the effects of C1q on monocyte responses during lipoprotein clearance. Surface

![FIGURE 3](http://www.jimmunol.org/)
expression of cellular activation markers was measured by flow cytometry in monocytes that had ingested OxLDL in the presence or absence of C1q and cultured a further 24 h (Fig. 4A). Although uptake of OxLDL alone had no effect on levels of costimulatory molecule CD80, HLA-DR/MHC class II, or adhesion molecule CD31/PECAM-1, the presence of C1q enhanced surface expression levels of these molecules in monocytes from three individual donors. These molecules also remained elevated after 48 h of culture (data not shown). Because chemokines play an important role in the recruitment of monocytes to the vascular lesion in atherosclerosis, we also investigated the effect of C1q and MBL on MCP-1/CCL2 gene expression by quantitative RT-PCR (Fig. 4B). Ingestion of 10 μg of protein/ml OxLDL reduced gene expression levels of MCP-1/CCL2 in HMDM after 2 h. However, the presence of C1q or MBL on OxLDL actually significantly enhanced gene expression levels above levels in OxLDL-treated HMDM and also levels in HMDM alone. Similar enhancements by C1q of MCP-1/CCL2 gene expression in response to OxLDL were also seen in human monocytes at 2 h (data not shown).

**C1q and MBL modulate cholesterol accumulation in monocytes and HMDM during ingestion of OxLDL**

Because we have shown that C1q enhances the uptake of modified lipoproteins (Fig. 3), we measured cholesterol accumulation in HMDM incubated with OxLDL in the presence or absence of C1q or MBL (Fig. 5). As expected, HMDM that had ingested OxLDL had enhanced levels of free cholesterol compared with those cultured in the absence of lipoprotein. At 2 h, no difference was seen in free cholesterol levels in HMDM treated with OxLDL alone or in the presence of C1q or MBL. However, after 24 h, coincubation of HMDM with OxLDL and either C1q or MBL significantly reduced free cholesterol levels in HMDM that had ingested OxLDL. Similar results were obtained with human monocytes at 2 and 24 h (data not shown).

**C1q and MBL enhance cholesterol efflux in OxLDL-loaded monocytes and macrophages**

To investigate the mechanism of the C1q-mediated reduction in cholesterol accumulation in HMDM and monocytes that ingested OxLDL, we investigated the effect of C1q on genes associated with cholesterol efflux, ABC transporter molecules ABC-A1 and ABC-G1, by quantitative RT-PCR. Incubation of C1q or MBL bound to OxLDL with human monocytes for 2 h significantly enhanced mRNA levels of cholesterol transporter genes ABC-A1 and ABC-G1 in both human monocytes and HMDM above levels seen with OxLDL only (Fig. 6A, 6B and data not shown). To identify whether the increased gene expression of cholesterol transporter molecules was associated with an effect on cholesterol efflux, we assessed the effect of C1q (75 μg/ml) or MBL (10 μg/ml) on cholesterol efflux in [3H]cholesterol-loaded human monocytes and HMDM. Our results show that, similar to the literature (30), OxLDL suppressed HDL-specific cholesterol efflux from HMDM and monocytes (Fig. 6C, 6D). Soluble C1q alone did not significantly enhance levels of HDL-specific efflux from HMDM or monocytes (data not shown). However, the presence of either C1q or MBL on OxLDL restored/sustained HDL-specific efflux levels similar to those of control [3H]cholesterol-loaded macrophages in the absence of OxLDL (Fig. 6C, 6D). C1q was also seen to enhance apolipoprotein A1-specific efflux in human monocytes and HMDM (data not shown).

**Discussion**

These studies present data supporting a novel role for innate immune proteins C1q and MBL during the clearance of modified lipoproteins and cholesterol metabolism by human monocytes and HMDM. In this study, we show that both C1q and MBL can directly bind modified lipoproteins, independent of Ab (Fig. 1). Studies have already shown that the collagenous domains of the macrophage scavenger receptor mediate binding specificities for polyanionic ligands, including AcLDL (31), although the isolated collagenous domain of C1q, which shares similar specificity for polyanions, did not bind AcLDL. We were also unable to detect binding to modified LDL using isolated fragments of C1q collagenous domain or the C1q globular head domain (data not shown), suggesting that the intact molecule is required for recognition or that multivalent binding by hexameric heads is required for high-avidity binding. Similarly, in a recent report, C1q was shown to bind another form of modified LDL, enzyme-modified LDL (E-LDL), via its globular head domains, which recognize unesterified fatty acids formed by cholesterol esterase in these particles (32). Consistent with a recognition step via its globular heads, we see activation of the classical complement pathway by C1q recognition of modified, but not native, LDL as measured by C3b deposition in a modified ELISA assay (Fig. 2). Activation of the classical pathway by modified forms of LDL may therefore contribute to the inflammatory environment of the atherosclerotic region. Studies by Biró et al. (10) using purified complement components in a C1 assay showed that other forms of modified LDL, such as E-LDL, bind and activate C1
under physiological conditions. These studies also suggest that C-reactive protein is required for activation of the classical pathway by OxLDL. Because our studies used MBL- and C1q-depleted serum as a source of other complement components, C-reactive protein would also be present, and therefore the relative contributions of E-LDL versus other modified LDL particles to complement activation during the early stages of atherogenic disease remain to be established. Interestingly, in our assay, although MBL was able to bind modified lipoproteins, they did not activate complement via the lectin pathway. MBL has long been associated with atherosclerosis. In humans, MBL deficiency has been correlated with severity of atherosclerotic disease (15). Human population studies have also shown that high levels of MBL (>1 mg/l) were associated with a greatly decreased risk of myocardial infarction in hypercholesterolemic individuals (33). More recent studies have shown that MBL is abundantly present in early lesions in a mouse model of atherosclerosis. This MBL was shown to be produced locally by myeloid cells during early atherogenesis and provided direct evidence for the critical role of MBL in controlling the development of atherosclerotic lesions (16). Studies have also shown that cells of the monocyte/macrophage lineage are also a major site of C1q biosynthesis (34) and that C1q is often upregulated in response to injury (35) and can be produced in the absence of other complement components (36). C1q has also been shown to be produced locally by dendritic cells, macrophages, and macrophage foam cells in atherosclerotic lesions (37). Direct evidence for the role of C1q in atherogenesis was provided by Bhatia et al. (14), who showed that C1q reduces early development of lesions in a murine model of atherosclerosis, and suggested that this may be due to the role of C1q in the clearance of apoptotic cells (14). Collectively, these studies identify critical protective functions of these innate immune molecules beyond the activation of complement, particularly in early stages of disease.

It has been shown for other disease states that C1q may have protective effects early in disease, prior to initiation of complement activation (38). C1q and MBL are known activation ligands for phagocytosis, rapidly triggering enhanced phagocytosis of a variety of targets, including sheep erythrocytes suboptimally opsonized by IgG or complement, and apoptotic cells (17). In this study, purified C1q and MBL also enhanced ingestion of modified forms of LDL (OxLDL and AcLDL), but not unmodified LDL, similar to their binding specificities, suggesting that binding of C1q to the lipoprotein is required for the enhancement of clearance (Fig. 3). Although the mechanism of enhanced clearance is presently not known, C1q has been shown to act as a bridging molecule to enhance the uptake of other particles via its collagen-like domain, including apoptotic cells (39, 40). This domain is shared by MBL, and directed mutagenesis in that domain has disrupted the enhancement of phagocytic activity (26), suggesting that the enhanced clearance is triggered via
interaction with a common phagocytic receptor. However, although several candidate receptors have been proposed, none has been definitively identified (8). Uptake assays performed using normal human serum and CLqD-MBLD as a source of complement demonstrated a significant contribution of CLq and/or MBL to lipoprotein clearance in the presence of other serum components, including downstream complement components. Addition of NHS significantly enhanced the ingestion of OxLDL by human monocytes (Fig. 3E). This enhancement of ingestion was not seen with the addition of CLqD-MBLD, suggesting that CLq and/or MBL play a role in the serum-mediated enhancement of lipoprotein clearance by these phagocytes. However, reconstitution of CLqD-MBLD with serum levels of CLq (CLqD-MBLD plus CLq), but not of MBL (CLqD-MBLD plus MBL), restored serum-mediated uptake of OxLDL. This suggests that CLq cannot only enhance OxLDL clearance via direct interaction with phagocytic cells, but also via classical pathway activation (in the presence of other complement components, such as in NHS) and C3b opsonization of modified LDL. However, the association of MBL with MBL-associated serine proteases or other complement components found in serum not only do not result in complement activation, but it also may mask the ability of MBL to enhance OxLDL clearance via direct phagocyte interactions.

The presence of CLq during modified lipoprotein clearance also modulated monocyte differentiation and cellular responses (Fig. 4). Levels of costimulatory molecule CD80 and HLA-DR/MHC class II were elevated in cells that ingested OxLDL in the presence of CLq compared with OxLDL alone, suggesting that CLq is enhancing monocyte activation and maturation toward a macrophage-like differentiation state. CLq also upregulated surface expression of the adhesion molecule CD31/PECAM-1. CD31 is expressed at varying levels on all cells of the vascular region, and it is involved in leukocyte transmigration through arterial walls (41). Similar to data from cells exposed to immobilized CLq or CLq bound to apoptotic cells (39), CLq also enhanced gene expression of chemokine CCL2/MCP-1, a molecule responsible for the recruitment of leukocytes to sites of injury. These data suggest that CLq expressed locally in atherosclerotic lesions may be providing “find-me” and activation signals, enhancing chemotaxis and migration of monocytes to the area.

Infiltrating monocytes, or macrophages in the arterial wall, can function to export cholesterol out of lesions or to process cholesterol to be excreted (42). However, excessive lipoprotein levels in the vessel wall found in atherosclerosis can overwhelm these protective mechanisms. Macrophages that ingest more cholesterol than they are able to secrete store the excess cholesterol in lipid droplets within the cell, leading to formation of macrophage foam cells, which can induce inflammatory responses and undergo apoptosis/necrosis, and thus play a key role in regulating the pathology of plaques (43). Levels of free cholesterol in monocytes were enhanced early after ingestion of OxLDL and were unaffected by the presence of CLq or MBL (2 h; Fig. 5). However, interestingly, after 24 h, cholesterol levels were significantly reduced in cells that had ingested OxLDL in the presence of CLq or MBL compared with those that had ingested OxLDL alone (Fig. 5). This is similar to a report in which adiponectin, a CLq-like protein that is secreted by adipocytes, reduced lipid accumulation in macrophages (44). mRNA levels of ATP-binding cassette transporters ABC-A1 and ABC-G1, which remove cholesterol from macrophages via efflux to HDL and its principal apolipoprotein, apolipoprotein A-I, were increased in response to CLq and MBL (Fig. 6A, 6B and data not shown) and correlated with enhanced HDL-specific efflux of [3H]cholesterol in monocytes and HMDM that had ingested OxLDL (Fig. 6C, 6D). Although the release of excess cholesterol from monocytes and macrophages is important for the prevention of foam cell formation and efficient clearance of cholesterol from the atherogenic region, efflux activities of ABC-A1 and ABC-G1 may also dampen inflammatory responses in macrophages (45).

In summary, these data describe a novel role for CLq and MBL, present in early atherosclerotic regions, in enhancing lipoprotein uptake and modulating gene expression that leads to beneficial metabolism and excretion of excess cholesterol. Because mechanisms governing the removal of modified lipoproteins and processing of cholesterol are critical to the suppression of atherosclerosis, further investigation of the molecular components of these CLq- and MBL-mediated processes should lead to novel strategies or areas for therapeutic intervention in this disease.

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Disclosures

The authors have no financial conflicts of interest.

References


Supplemental Table 1. Primers used for Quantitative RT-PCR

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<th>Gene</th>
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<th>REV Primer (5’→3’)</th>
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Supplemental Figure 1.
Ingestion of Dil-labeled modified LDL into vesicular compartments was confirmed by confocal microscopy

Supplemental Figure 1. Confocal analysis of 10 mg protein/ml Dil-OxLDL (red) uptake by HMDM in the absence or presence of 75 mg/ml C1q, using the Zeiss LSM 710 confocal. HMDM were stained with FITC-labeled phalloidin (green) which recognizes actin, and nuclear stain DAPI (blue). Data are from a single experiment, representative of results obtained from different donors in at least 3 individual experiments.