Abstract—Oxidized phospholipids are found in the vasculature of animal models of atherosclerosis, in human atherosclerotic lesions, and in other inflammatory diseases. Oxidized phospholipids cause vascular and nonvascular cells to initiate an inflammatory reaction. Metabolites of arachidonic acid, such as 12-hydroxyeicosatetraenoic acid, can mimic some of the inflammatory properties of oxidized phospholipids. In vitro and in vivo normal high-density lipoprotein (HDL), normal apolipoprotein A-I, and apolipoprotein A-I mimetic peptides, each likely acting in a different manner, prevent the inflammatory reaction characteristic of atherosclerosis, and this is associated with decreased levels of oxidized lipids in tissues and cells. HDL from animal models of atherosclerosis or from humans with atherosclerosis or from humans or animals with other chronic inflammatory diseases does not prevent the inflammatory reaction characteristic of atherosclerosis and may even enhance the inflammatory reaction. In mice and perhaps humans, ≈30% of the steady-state plasma HDL-cholesterol pool is derived from the small intestine. The metabolism of phospholipids by gut bacteria has been recently implicated in atherosclerosis in both mice and humans. Studies with apolipoprotein A-I mimetic peptides suggest that the small intestine is a major tissue regulating systemic inflammation in mouse models of atherosclerosis and may be important for determining the functionality of HDL. (Arterioscler Thromb Vasc Biol. 2012;32:2553-2560.)

Key Words: apolipoprotein A-I ■ apolipoprotein A-I mimetic peptides ■ high-density lipoprotein ■ hydroxyeicosatetraenoic acid ■ hydroxyoctadecadienoic acid ■ lipoproteins ■ oxidized lipids ■ small intestine

High-Density Lipoprotein and 4F Peptide Reduce Systemic Inflammation by Modulating Intestinal Oxidized Lipid Metabolism

Mohamad Navab, Srinivasa T. Reddy, Brian J. Van Lenten, Georgette M. Buga, Greg Hough, Alan C. Wagner, Alan M. Fogelman

M uc h work in the field has suggested that oxidation of low-density lipoprotein (LDL) was a key event in atherogenesis as it led to enhanced uptake and foam cell formation. However, it was seen almost at the same time that the many oxidized lipids in oxidized-LDL could be responsible for the proinflammatory effects of oxidized-LDL. Studies to define these oxidized lipids led to the discovery of oxidized phospholipids (Ox-PL) as a major class of proinflammatory lipids.1 During this period, studies to explain how high-density lipoprotein (HDL) influences atherogenesis led to the realization that it had a major role in providing antioxidant properties to dampen the proinflammatory properties of oxidized-LDL. 2 In turn, this led to the development of apolipoprotein (apo)A-I mimic peptides such as 4F and others.3 Subsequent studies showed that not only was 4F peptide able to inhibit atherosclerosis, but it potently inhibited inflammation in a surprisingly wide variety of animal models of disease.3

In preliminary studies in humans,4 4F peptide was administered orally at doses from 0.43 to 7.14 mg/kg. The plasma levels achieved were very low (Cmax 15.9±6.5 ng/mL). However, peptide administration at doses of 4.3 and 7.14 mg/kg significantly improved the HDL inflammatory index, whereas doses of 0.43 and 1.43 mg/kg did not.4 In a second clinical trial5 it was decided to achieve high plasma levels of peptide by using low doses (0.042–1.43 mg/kg) of peptide administered intravenously or subcutaneously. Despite achieving very high plasma levels of peptide (Cmax 3255±640 ng/mL), there was no improvement in HDL inflammatory index.5 This led to a reappraisal of why the peptide was so effective in mice and led to the surprising discovery that a major site of action for the 4F peptide may be in the intestine, even when it is administered subcutaneously.6

This pathway of discovery has led us to the following 5 hypotheses, which are depicted in the Figure. Hypothesis 1 is that the oxidation of normal lipids by metabolic pathways or by...
In this article, we will review the many lines of evidence garnered from many laboratories that have led us to construct these 5 novel hypotheses of how HDL and apoA-I mimetic peptides, such as 4F, which bind these lipids act on endothelial cells or on enterocytes in the small intestine. The cells respond by producing cytokines that act on liver cells or macrophages, leading to the production of acute phase proteins. Normal high-density lipoprotein (N-HDL) or apoA-I mimetic mimetic peptides (apoA-I mimetic) block this sequence of events. However, if the acute phase reaction is established the normal HDL is converted to acute phase HDL (AP-HDL), which loses its ability to inhibit this process or may even interact with the cells to amplify the acute phase reaction. The numbers shown in the figure relate to each of the 5 hypotheses discussed in the text of this review.

Oxidized lipids act on endothelial cells or on enterocytes in the small intestine. The cells respond by producing cytokines that act on liver cells or macrophages, leading to the production of acute phase proteins. Normal high-density lipoprotein (N-HDL) or apoA-I mimetic mimetic peptides (apoA-I mimetic) block this sequence of events. However, if the acute phase reaction is established the normal HDL is converted to acute phase HDL (AP-HDL), which loses its ability to inhibit this process or may even interact with the cells to amplify the acute phase reaction. The numbers shown in the figure relate to each of the 5 hypotheses discussed in the text of this review.

**Figure.** Free arachidonic acid (AA) is metabolized to an oxidized fatty acid (Ox-AA), such as 12-hydroxyeicosatetraenoic acid. Phospholipids (PL) are also oxidized (Ox-PL). These oxidized lipids act on endothelial cells or on enterocytes in the small intestine. The cells respond by producing cytokines that act on liver cells or macrophages, leading to the production of acute phase proteins. Normal high-density lipoprotein (N-HDL) or apoA-I mimetic mimetic peptides (apoA-I mimetic) block this sequence of events. However, if the acute phase reaction is established the normal HDL is converted to acute phase HDL (AP-HDL), which loses its ability to inhibit this process or may even interact with the cells to amplify the acute phase reaction. The numbers shown in the figure relate to each of the 5 hypotheses discussed in the text of this review.

Oxidized lipids can initiate an inflammatory response and are also formed in an inflammatory reaction. To understand the sequence of events, Napoli et al11 followed the time course of the appearance of Ox-PL and monocytes in aortas of human fetuses. They found that the presence of Ox-PL preceded the appearance of the monocytes,11 The findings of Nishi et al12 highlight the importance of Ox-PL to human disease. They found that the vulnerability of plaques was related to the amount of LDL containing oxidized phosphatidylcholine in the lesion. Tsimikas et al13 reported that after percutaneous angioplasty there was a dramatic increase in plasma levels of Ox-PL confirming the presence of Ox-PL that were associated with both lipid and protein moieties of the lipoprotein.9 Podrez et al10 demonstrated that a variety of Ox-PL beyond those described by Watson et al10 and Subbagounder et al14 are present in lesions and that they interact with CD36 in the mouse as first suggested by Boullier et al.9 A simple phospholipid, such as 1-palmitoyl-2- arachidonoyl-sn-glycero-3-phosphorylcholine, when air oxidized produces hundreds of compounds,7 and hence it is not surprising that there are a myriad of Ox-PL found in nature.

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In monkey and rabbit models of atherosclerosis, Tsimikas et al15 demonstrated that during regression of lesions Ox-PL increased in plasma and decreased in lesions, consistent with the findings of this group in humans.13 Interestingly, Tsimikas et al16 found that Ox-PL in human plasma is largely associated with Lp(a) lipoprotein and is strongly associated with angiographically documented coronary artery disease (CAD), particularly in patients 60 years of age or younger. Leibundgut et al17 found that Ox-PL are also present in plasminogen, which is homologous to Lp(a), and affects fibrinolysis.

Although it is not known precisely how diet-induced inflammation produces Ox-PL, the process seems to be widespread in nature as Fang et al18,19 demonstrated that Ox-PL pathways. The role of phospholipid oxidation products in atherosclerosis has recently been reviewed.1 Watson et al10 and Subbagounder et al demonstrated by liquid chromatography-electrospray ionization/multi-stage mass spectrometry that Ox-PL were present in fatty streaks from cholesterol-fed rabbits7,8 and in lesions of apolipoprotein E–null mice.7 Subbagounder et al also demonstrated that the group at the sn-2 position of Ox-PL determines the specific bioactivity and that the substitution of stearoyl for palmitoyl at the sn-1 position or ethanolamine for choline at the sn-3 position of the phospholipid did not alter bioactivity. Subbagounder et al further showed that all parts of the phospholipid molecules are required for these bioactivities.

The binding of oxidized-LDL to the scavenger receptor CD36 in mice was demonstrated to be attributable to Ox-PL that were associated with both lipid and protein moieties of the lipoprotein.9 Podrez et al10 demonstrated that a variety of Ox-PL beyond those described by Watson et al10 and Subbagounder et al14 are present in lesions and that they interact with CD36 in the mouse as first suggested by Boullier et al.9 A simple phospholipid, such as 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine, when air oxidized produces hundreds of compounds,7 and hence it is not surprising that there are a myriad of Ox-PL found in nature.

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accumulated in lesions induced by cholesterol feeding zebra fish larvae.

The presence of Ox-PL at sites of inflammation is not restricted to atherosclerosis. For example, Imai et al\textsuperscript{20} found Ox-PL in lungs of human and animals infected with severe acute respiratory syndrome (SAARS), anthrax, or H5N1. Pulmonary challenge with inactivated H5N1 avian influenza virus rapidly induced acute lung injury and Ox-PL formation in mice.\textsuperscript{20} Consistent with these findings, Crowe et al\textsuperscript{21} found that interleukin-17 RA-null mice had markedly better survival and less Ox-PL formation in the lungs after influenza infection. Ox-PL has also been found in the mucosa of the small intestine of mice that are genetically prone to polyp formation and colon cancer.\textsuperscript{22} In humans, Ox-PL has been found in brain lesions of patients with multiple sclerosis\textsuperscript{23} and in skin lesions of patients with leprosy.\textsuperscript{24} Ox-PL have also been found in nonalcoholic fatty liver disease, and Ox-PL levels correlated with disease severity in humans.\textsuperscript{25} In a mouse model of this disease, administration of an apoA-I mimetic peptide known to bind Ox-PL with extraordinary high affinity\textsuperscript{26} (the 4F peptide) significantly reduced hepatic fibrosis.\textsuperscript{27} However, the authors did not measure Ox-PL levels in their study.\textsuperscript{27} The presence of Ox-PL in human eyes was seen to increase with age and was increased in eyes from patients with age-related macular degeneration.\textsuperscript{28} In a mouse model of sclerodermia, the hearts contained higher levels of antibody to Ox-PL than controls and the tissue levels of these antibodies decreased\textsuperscript{29} with administration of the 4F peptide.

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The presence of Ox-PL in a wide range of inflammatory conditions in species ranging from zebra fish to humans is consistent with hypothesis 1 proposed in this review. Some of the studies cited in this section are also consistent with hypothesis 4 proposed in this review.

### Oxidized Lipids Cause Vascular and Nonvascular Cells to Initiate an Inflammatory Reaction

In vitro, a mixture of Ox-PL made by air oxidation of 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine causes human aortic endothelial cells (ECs) to bind monocytes (but not neutrophils) and causes the cells to secrete cytokines, including the potent monocyte chemoattractant factor monocyte chemoattractant protein 1.\textsuperscript{1} Individual components of air oxidized 1-palmitoyl-2-arachidonoyl-sn-glycero-3-phosphorylcholine have different effects in vitro. For example, 1-palmitoyl-2-glutaroyl-sn-glycero-3-phosphorylcholine stimulates ECs to bind both neutrophils and monocytes, whereas 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine only stimulates monocyte binding and strongly inhibits lipo-polysaccharide-mediated induction of neutrophil binding and expression of E-selectin protein and mRNA.\textsuperscript{10} In rabbits, vascular inflammation was seen to tightly track with the content of Ox-PL, oxidized fatty acids, and malondialdehyde in lesions.\textsuperscript{31} Interestingly, the relative levels of oxidized fatty acids derived from arachidonic acid, linoleic acid, and oleic acid in plasma also tracked well with levels in lesions.\textsuperscript{31}

Furnkranz et al\textsuperscript{22} directly applied Ox-PL to carotid arteries in mice and found that the arteries responded by inducing a set of atherosclerosis-related genes including monocyte chemoattractive protein 1, keratinocyte-derived chemokine, tissue factor, interleukin 6, heme oxygenase 1, and early growth response 1. In isolated perfused carotid arteries, Ox-PL triggered rolling and firm adhesion of monocytes in a P-selectin and keratinocyte-derived chemokine-dependent manner.\textsuperscript{32} Monocyte adhesion to ECs induced by Ox-PL seems to involve activation of cytosolic phospholipase A2 and 12-lipoxygenase; indeed, 12-HETE mimicked the effects of Ox-PL.\textsuperscript{33} Honda et al\textsuperscript{34} found that the 12-lipoxygenase pathway was critical for mildly oxidized-LDL (which contains Ox-PL) to induce monocyte binding to human aortic EC. Similar to Ox-PL, 12-HETE induces monocyte but not neutrophil binding to human aortic EC.\textsuperscript{35} The number of compounds that can be generated by enzymatic pathways, such as the lipoxygenase pathways, is dramatically increased by the crossover of multiple pathways and the ability of the lipids that are formed to undergo nonenzymatic rearrangements.\textsuperscript{36} The complexity is further increased by the ability of some enzyme systems to oxidize fatty acids to biologically active molecules while they are still esterified to cholesterol or phospholipids.\textsuperscript{37} Thus, the biologic activity of 12-HETE used in the examples mentioned earlier is just that, an example. The number of biologically active oxidized lipids present in nature is very large.

The mechanism(s) by which Ox-PL induce cells to initiate an inflammatory response is complex.\textsuperscript{38-47} Ox-PL seems to have oxidation-specific epitopes, which are recognized as danger-associated molecular patterns by pattern recognition receptors involved in innate immunity.\textsuperscript{48} Antioxidant enzymes can modulate the stress response induced by Ox-PL.\textsuperscript{49} Ox-PL have also been shown to significantly alter the phenotype of macrophages accounting for some of the characteristics of macrophages that have been noted in atherosclerotic lesions.\textsuperscript{50}

Inflammatory response of vascular cells in vitro and in vivo to a variety of oxidized lipids and the striking similarity between the response to Ox-PL and to a metabolite of arachidonic acid, such as 12-HETE, is consistent with both hypothesis 1 and hypothesis 2 of this review.

### In Vitro and In Vivo Normal HDL, Normal ApoA-I, and ApoA-I Mimetic Peptides Prevent Inflammatory Reaction Characteristic of Atherosclerosis and this Is Associated With Decreased Levels of Oxidized Lipids In Vitro and In Vivo

The role of normal HDL and mimetics of HDL in preventing inflammatory reaction initiated by oxidized lipids involves multiple components of HDL.\textsuperscript{51,52} In vitro studies demonstrated that an apoA-I mimetic peptide (4F) prevented the formation and secretion of Ox-PL in response to influenza A infection.\textsuperscript{53} In vivo, this peptide prevented the trafficking of macrophages into the aorta in response to influenza A infection in mice.\textsuperscript{54} In other in vitro studies, normal human HDL modulated the proinflammatory response of human aortic EC to Ox-PL to a signaling cascade that was anti-inflammatory.\textsuperscript{55}

An HDL-associated enzyme, paraoxonase 1 (PON1), which inhibited the response of ECs to Ox-PL in vitro when
delivered into arteries in vivo inhibited the response to balloon injury. Another component of normal HDL, apoM, has been reported to bind Ox-PL and increase the antioxidant effect of HDL. Feeding LDL receptor–null mice (LDLR−/−) a Western diet (WD) induced the formation of Ox-PL in their kidneys, which was accompanied by inflammatory changes similar to those in the arteries of these mice. Treating the mice with the 4F peptide significantly reduced tissue levels of Ox-PL and significantly reduced the inflammation in the tissues without changing plasma lipid levels. As noted earlier, the plasma and lesion content of oxidized fatty acids seem to parallel one another. Administration of the 4F peptide to mouse models of atherosclerosis reduced the plasma levels of oxidized fatty acids and resulted in more anti-inflammatory HDL. Another line of evidence suggesting that the mechanism by which apoA-I mimetic peptides work involves binding and removing Ox-PL comes from the finding that their use induces natural antibodies that recognize Ox-PL, which would be consistent with the plasma increase in Ox-PL that has been seen in animal models of regression.

The studies in this section are consistent with hypothesis 3 and hypothesis 4 as proposed in this review.

### HDL From Animal Models of Atherosclerosis or From Humans With Atherosclerosis or From Mammals With Other Chronic Inflammatory Diseases or HDL with ApoA-I Modified by Products Found in Inflammatory Reactions Does Not Prevent Inflammatory Reaction Characteristic of Atherosclerosis and May Even Enhance Inflammatory Reaction

Van Lenten et al were the first to report that during an acute phase response anti-inflammatory HDL becomes proinflammatory in rabbits and humans. Subsequently, Navab et al reported that injection of Ox-PL into mice genetically susceptible to diet-induced atherosclerosis (C57BL/6J mice), but not in mice resistant to diet-induced atherosclerosis (C3H/HeJ mice), induced an acute phase response with elevations of apoJ and decreased PON1 activity.

Hedrick et al reported that feeding an atherogenic diet to LDLR−/− mice for 3 days did not decrease hepatic PON1 mRNA but caused a dramatic decrease in plasma PON1 activity and mass. The decreased activity and mass were temporally related to an increase in the lipid hydroperoxide content of HDL with a decrease in HDL-cholesterol, native apoA-I and apoA-II levels. As the native apoA-I disappeared from the circulation, higher molecular weight forms of apoA-I appeared, some of which contained epitopes recognized by an antibody that recognizes Ox-PL (EO6). At present there are no studies that present specific evidence of the levels of various components of Ox-PL in the vessel wall and as function of HDL mass and functionality.

The ability of HDL to prevent the formation of Ox-PL was found to be reduced in mice after exposure of the mice to second-hand cigarette smoke. The role of Ox-PL in modulating HDL function has been previously reviewed. The genetic control of the anti-inflammatory properties of HDL in mice and the inverse relationship of these anti-inflammatory properties in humans with the ability of HDL to promote cholesterol efflux from cholesterol loaded macrophages was reported by Navab et al.

Vaisar et al reported that HDL from patients with coronary heart disease (CAD) is associated with many acute phase proteins, supporting the proposal that HDL is changed in the presence of chronic inflammation. The effects of chronic inflammation on HDL were favorably modified in humans with a regimen of combined statin and niacin treatment. Abnormalities in HDL have been identified in a variety of inflammatory states including patients with systemic lupus erythematosus, rheumatoid arthritis, and diabetes mellitus. Kontush and Chapman have emphasized the importance of HDL subpopulations. Bhattacharyya et al reported that in humans there is a strong relationship between the activity of the HDL-associated enzyme PON1, systemic oxidative stress, and cardiovascular risk. Moreover, the levels of metabolites of arachidonic acid in these patients strongly tracked with PON1 activity and risk for cardiovascular events.

The work of Undurti et al showed that modification of HDL by an enzyme released from macrophages and neutrophils during inflammation (myeloperoxidase) generates a proinflammatory HDL particle. The levels of hemoglobin (a potent oxidant when freed from red blood cells) and haptoglobin (an acute phase reactant) that were associated with HDL in CAD patients significantly predicted the inflammatory properties and function of their HDL. HDL from patients with another chronic inflammatory condition (rheumatoid arthritis) was associated with acute phase proteins and complement factors similar to that reported in patients with CAD.

Modifying apoA-I with malondialdehyde (but not other reactive carbonyls) blocked cholesterol efflux by the ATP binding cassette A1 pathway and HDL from atherosclerotic lesions contained more malondialdehyde than normal HDL. Besler et al reported that HDL from patients with stable CAD or an acute coronary syndrome did not have anti-inflammatory properties when presented to ECs in vitro and did not stimulate EC repair because it failed to induce EC NO production. They found that HDL from these subjects (1) activated endothelial lectin-like oxidized-LDL receptor, (2) this triggered endothelial protein kinase C βII activation, which (3) inhibited EC NO-activating pathways and NO production. They identified reduced PON1 activity as a molecular mechanism leading to generation of HDL with protein kinase C βII-activating properties, which was in part attributable to increased formation of malondialdehyde in HDL.

The importance of HDL in promoting cholesterol efflux independent of HDL-cholesterol levels was demonstrated by the studies of Khera et al, and it was suggested by Heinecke to be a possible therapeutic target. Cavigiolio et al reported that oxidative damage to apoA-I inhibited the disassociation of apoA-I from HDL to the lipid poor form of apoA-I that is critical for promoting cholesterol efflux via ATP binding cassette A1.

Thus, (1) HDL and its associated proteins are highly susceptible to modification by lipid oxidation products and...
enzymes produced or released at sites of inflammation; (2) the proteins and enzymes associated with HDL are significantly changed to a proinflammatory phenotype during an acute phase response; (3) the ability of HDL to promote cholesterol efflux and to be anti-inflammatory is dramatically reduced during a chronic acute phase reaction such as seen in CAD patients.

The studies in this section are consistent with hypothesis 3 proposed in this review.

**Small Intestine Is Important in Modulating Systemic Inflammation**

In mice, ≈30% of the steady-state plasma HDL-cholesterol pool is derived from the small intestine. Studies in chylomicrons suggest that a similar fraction of HDL in humans comes from the small intestine. The metabolism of phospholipids by gut bacteria has been recently implicated in atherosclerosis in both mice and humans. The level of oxidized lipids, phospholipids, and fatty acids in germ-free animals or animals treated with broad spectrum antibiotics are currently unknown.

Studies with the 4F peptide suggest that the small intestine is a major tissue-regulating systemic inflammation in mouse models of atherosclerosis and may be an important site for determining the functionality of HDL. To test this hypothesis Navab et al administered the 4F peptide at equal doses orally or by subcutaneous injection (subcutaneous) to LDLR−/− mice on a WD. Plasma and liver peptide levels were 298-fold and 96-fold higher, respectively, after subcutaneous administration, whereas peptide levels in the intestine only varied by 1.66±0.33-fold. Levels of metabolites of arachidonic and linoleic acids known to bind with high affinity to the peptide were significantly reduced in intestine, liver, and hepatic bile to a similar degree whether the peptide was administered subcutaneously or orally. However, levels of 20-HETE, which is known to bind to the 4F peptide with low affinity, were unchanged. Peptide treatment reduced serum amyloid A and triglyceride levels and increased HDL-cholesterol levels similarly after subcutaneous or oral administration. Plasma levels of metabolites of arachidonic and linoleic acids significantly correlated with serum amyloid A levels. Feeding metabolites of arachidonic acid (eg, 12- or 15-HETE) in mouse chow consistent with a reduction in phospholipase activity. The studies cited in this review are consistent with hypothesis 4 and hypothesis 5 proposed in this review.

**Summary**

The studies cited in this review are consistent with hypothesis 1 that the oxidation of normal lipids by metabolic pathways or by nonenzymatic means produces oxidized lipids which trigger an inflammatory response in many tissues including the vasculature. The cited studies are also consistent with hypothesis 2 that metabolites of arachidonic acid, such as 12-HETE (and likely many other oxidized fatty acids including those esterified to cholesterol or phospholipids), can act similar to Ox-PL to induce inflammation. Although some of the biologic activities of metabolites of arachidonic acid, such as 12-HETE and Ox-PL, are similar (eg, both induce binding of monocytes but not neutrophils to ECs), the pathways by which they exert their biologic activity may not be similar and remain to be defined by future research. The studies described in this review are consistent with hypothesis 3 that HDL contains proteins and enzymes that can inactivate or remove these proinflammatory lipids, but in some circumstances, such as a systemic acute phase response, the proteins and enzymes associated with HDL are altered so that the inflammatory response is either not inhibited or is enhanced. The studies reviewed here are also consistent with hypothesis 4 that apoA-I mimetic peptides, such as 4F, reduce inflammation by binding and removing oxidized lipids.
from tissues. And finally, the studies cited in this review are consistent with hypothesis 5 that oxidized lipids in the small intestine are important in modulating systemic inflammation, and the intestine is a major site for the action of apoA-I mimetic peptides, such as 4F, which bind these oxidized lipids. Although these studies are consistent with each of these 5 hypotheses, definitive proof of each of these hypotheses is lacking and will require extensive future research by many laboratories.

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References


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