Meeting Summary

Lipoproteins and the Pathobiology of the Arterial Intima

Ninth Paavo Nurmi Symposium

Petri T. Kovanen and German Camejo

On September 7-9, 1989, a symposium on the interaction between lipoproteins and the arterial intima was held at Haikko Manor, near Helsinki, Finland. This Ninth Paavo Nurmi Symposium included 29 presentations selected to include both the pathology of the intima and the metabolism of lipoproteins in the intima. In particular, discussion focused on the relation between the accumulations of cholesterol in the intracellular and extracellular compartments of the arterial intima, that is, the transition from fatty streaks to atheromas.

Pathology of the Arterial Intima

Dr. Herbert Stary of New Orleans, La., opened the first session of the symposium with a review of his recent morphological observations on the temporal sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries during the first 40 years of life. His address set the stage for the meeting by defining the pathoanatomic changes underlying the process of atherosclerosis. Dr. Stary first defined the normal intima as a tissue composed of smooth muscle cells (of two phenotypes), isolated macrophages, and rare mast cells, all embedded in an extracellular matrix made up of several components. In Dr. Stary’s opinion, a substantial proportion of the extracellular lipid is derived from disintegrated lipid-laden foam cells. By increasing the intercellular space, the pooled extracellular lipid disrupts the structural coherence of smooth muscle cells. As Dr. Stary pointed out, the term “abnormal” should be reserved for intima that is associated with tissue damage, and so this term can be applied to this accumulation of extracellular lipid, which damages the arterial structure by displacing normal cells and the matrix of the intima. Thus, accumulation of extracellular lipid paves the way for pathological changes in the arterial wall, that is, it transforms the fatty streak into an atheroma.

Dr. John Guyton of Houston, Tex., next reported his morphological observations on extracellular lipid deposition in early atherosclerotic lesions of the human aorta. He argued in favor of direct deposition of extracellular lipid from tissue lipoproteins, rather than from dying intimal macrophages, the supporting evidence coming from the size of the lipid droplets.

Dr. Wolfgang Völker of Munster, F.R.G., then demonstrated that lipid deposits form lesions in extracellular areas rich in large chondroitin sulfate proteoglycans but not in areas rich in collagen bundles or in dermanian sulfate proteoglycans.

Dr. L. Maximilian Buja of Houston, Tex., compared the cellular pathology of human familial hypercholesterolemia (FH) with that of the related animal model, the Watanabe hereditary hyperlipidemic (WHHL) rabbit, and contrasted it with the classical hypercholesterolemia model of atherosclerosis, the cholesterol-fed rabbit. In human FH homozygotes and in the homozygotic WHHL rabbit, the cellular pathology of atherosclerosis shows similarities in ultrastructural features. In contrast, in normal rabbits fed a diet high in cholesterol and fat, the development of the lesion is arrested at the fatty streak stage. These significant differences in the pathobiology of atherogenesis appear to depend on the lipoprotein composition of the plasma rather than on differences in the responsiveness of the arterial intima to hypercholesterolemia per se.

The final contribution in this session was made by Dr. Erkki Pesonen of Helsinki, Finland. He reported, based on an autopsy study, that Finnish children who descend from grandparents from eastern Finland, an area with exceptionally high mortality due to coronary heart disease, have significant narrowing of the coronary arteries. He suggested that intimal thickening could be a morphological manifestation of hereditary disposition to coronary heart disease.

Influx of Low Density and High Density Lipoproteins Into the Arterial Intima

Dr. Thomas Carew of La Jolla, Calif., reported on findings in cholesterol-fed normal rabbits. In “lesion-prone” areas of the aorta, marked retention of intact low density lipoprotein (LDL) was observed even at a prelesion stage (16 days after the start of cholesterol feeding). There was no indication of increased entry or decreased degradation of LDL in the lesion-prone areas. Dr. Carew suggested that the reason for the local increase in the concentration of LDL could be oxidation of LDL, causing both increased binding of LDL to the extracellular matrix and increased uptake of LDL by the local monocyte/macrophages.
Dr. Steen Stender of Hellerup, Denmark, then gave an account of studies in humans in which lipoproteins were labeled in the cholesteryl ester moiety and then injected into normocholesterolemic patients undergoing vascular surgery. Accumulation of the radiolabel from injected lipoproteins was then measured in the arterial specimens obtained during surgery. A large variation in the rate of influx was observed. Inasmuch as the variation was independent of plasma cholesterol concentration, the rate of influx appeared to depend on local tissue factors.

Dr. Mahamad Navab of Los Angeles, Calif., described influx studies using a highly sophisticated in vitro model of the human aorta. In this system lacking the internal elastic lamina, the endothelial cells formed the major barrier to the passage of LDL particles. The barrier could be effectively disrupted by lipopoly saccharide (LPS) from Escherichia coli. However, if LPS was first complexed with LDL, then the complexes would be transported intact across the endothelium without affecting the barrier function of the endothelium. Yet, the complexes initiated an inflammatory response as indicated by their ability to stimulate the endothelial and smooth muscle cells to produce attractants to monocytes.

Taken together, the results presented in this section favored the notion that, of the apolipoprotein (apo) B-containing lipoproteins with densities of less than 1.063 g/ml, only the LDL particles are atherogenic (in terms of producing extracellular deposition of lipids). High density lipoprotein (HDL) particles also were found to flow into the arterial intima, thus suggesting an interplay of these two classes of lipoprotein particles in the arterial intima.

Metabolism of Low Density Lipoprotein–Derived Cholesterol in the Arterial Intima

The first part of this session was devoted to the oxidative modification of LDL and its effects on the cellular metabolism of LDL. Dr. Sampath Parthasarathy of La Jolla, Calif., compared cell-mediated and copper-mediated oxidative modifications of LDL. His clearcut conclusion was that the conditions for the two differ dramatically, with oxidative modification of LDL requiring micromolar concentrations of copper in the absence of cells but only nanomolar concentration when cells are involved.

Dr. Toru Kita of Kyoto, Japan, next reported on the antioxidant potential of the drug probucol. He emphasized that probucol prevents the progression of atherosclerosis in homozygous WHHL rabbits without inducing any changes in plasma LDL cholesterol levels. The results in vivo strongly suggest that probucol could slow down the progression of atherosclerosis by preventing oxidative modification of LDL. Moreover, the ability of probucol to prevent the development of atherosclerosis strongly suggested that oxidation of LDL does actually occur in vivo in the WHHL rabbit and that this oxidation is causally related to the development of atherosclerosis.

This fascinating experiment in rabbits now awaits its human counterpart.

Dr. Margaret Haberland of Los Angeles, Calif., then reviewed her experience on modified lipoproteins and atherosclerosis, placing emphasis on LDL modified by malondialdehyde (MDA). Using a monoclonal antibody technique, she provided direct evidence for the existence in vivo of MDA-modified protein within the arterial lesions of WHHL rabbits. The distribution of apo B-100 closely paralleled the extracellular deposition of MDA-modified protein. The absence of detectable amounts of MDA-modified LDL in the plasma strongly suggested that the modification reaction is restricted to the immediate environment of the arterial lesion.

Dr. Henry Hoff of Cleveland, Ohio, then suggested that the oxidatively modified LDL particles are key elements in the production of macrophage foam cells in human atherosclerotic lesions. LDL-like lipoprotein particles isolated from human atherosclerotic lesions were found to be internalized by the LDL receptor or the scavenger receptor on macrophages in culture, the latter uptake mechanism leading to lipid loading of the cells. An interesting hypothesis was put forward by Dr. Hoff. He suggested that aldehydes may form bridges between LDL particles, provided these are present at high concentrations. Then, the macrophages but not the smooth muscle cells may phagocytize these aggregates independently of the LDL or scavenger receptor.

Dr. M.J. Mitchinson of Cambridge, England, described observations on the presence of ceroid in vivo and its formation in vitro. Ceroid, an oxidative product of lipids, could be formed by macrophages from LDL in vitro when these cells were exposed to emulsions of cholesterol linolate or cholesteryl arachidonate. The intracellularly accumulated ceroid granules closely resemble those seen in foam cells of human atherosclerotic lesions. Most interestingly, various antioxidants such as probucol and α-tocopherol inhibited ceroid formation.

Dr. Seppo Ylä-Herttuala of Tampere, Finland, (currently in La Jolla, Calif.) showed that LDL-like particles isolated from human atherosclerotic lesions have physical, immunologic, and biologic properties resembling those of oxidized LDL. He also showed that, in contrast to the atherosclerotic intima, the normal human intima lacks these characteristics. The LDL-like particles isolated from atherosclerotic lesions were found to contain MDA- and 4-hydroxynonenal-lysine adducts in the apo B portion, to have a chemoattractive effect on monocytes, and to be degraded in macrophages in vitro at an increased rate.

Finally, Dr. Olli Jaakkola of Tampere, Finland, reported results on lipoprotein uptake by macrophages and smooth muscle cells derived from human aortic fatty streaks. In these primary cultures, the macrophages were capable of accumulating cholesteryl esters by uptake of acetyl-LDL and β-migrating very low density lipoprotein. The smooth muscle cells were also capable of accumulating cholesterol.
from the two types of lipoproteins but to a lesser extent than the macrophages.

Taken together, the data presented in this session gave strong evidence for the hypothesis that LDL particles become oxidized in the human arterial wall and that such modified LDL particles are taken up by the local macrophages, thereby producing foam cells.

Finally, Dr. Jorma Kokkonen of Helsinki, Finland, reported on results obtained with isolated rat serosal mast cells and LDL. It was found that stimulation of mast cells, leading to exocytosis of their secretory granules, results in binding of LDL to the heparin proteoglycan component of the granules. Such granules coated with LDL then became phagocytosed by cocultured macrophages, resulting in "piggy-bag" uptake of LDL, with a resultant accumulation of LDL-derived cholesterol as cholesteryl ester droplets in the cytoplasm of the macrophages.

**Interaction of Low Density Lipoproteins and Lp(a) With the Extracellular Matrix of the Arterial Intima**

Dr. Gerald Berenson of New Orleans, La., then discussed the relations between the structures of arterial proteoglycans and their capacity to serve as a mechanism of retention of apo B–containing lipoproteins in the arterial intima. Data were presented showing that arterial proteoglycans and glycosaminoglycans in association with LDL are taken up in a nonregulable way by mouse macrophages, suggesting that the proteoglycans play a role in both the intracellular and extracellular accumulations of LDL-derived cholesterol.

Dr. Göran Bondjers of Gothenburg, Sweden, then discussed the evidence that accumulation of apo B–containing lipoproteins is due to their retention rather than to their increased influx. He presented data showing that human arterial proteoglycans can select subpopulations of LDL with higher affinity for the proteoglycan. These subfractions appeared smaller in size, richer in polar surface lipids, and more positive than those with lower affinity.

Dr. German Camejo of Gothenburg, Sweden, using human arterial chondroitin sulfate proteoglycan fractions, presented data indicating that the association induced substantial changes in the surface structure of LDL. The alterations, detected by changes in proteolytic sensitivity, reactivity with a monoclonal antibody, and association with glycosaminoglycans, appeared to be most marked in the lysine and arginine regions of the apo B-100, which are responsible for the association with the proteoglycans and glycosaminoglycans.

In the report by Dr. Ulrike Beisiegel of Hamburg, F.R.G., the mechanisms by which apo B–containing lipoproteins may contribute to the progress of atherosclerotic lesions were extended to include lipoprotein(a) (Lp[a]). The antigen apo(a) was used in these immunohistochemical studies on the localization of Lp(a) in fresh arterial biopsies. Apo(a) and apo B showed essentially the same extracellular distribution and appeared to be frequently accompanied by fibrin. Both the apo B-100 and apo(a) portions of Lp(a) appear to contribute to the association of Lp(a) with arterial proteoglycans and glycosaminoglycans, as reported by Dr. Gert Kostner of Graz, Austria. These insoluble complexes of Lp(a) were shown to be taken up more avidly by peritoneal macrophages than the native Lp(a).

Dr. Christian Ehnholm of Helsinki, Finland, communicated his latest results obtained together with Dr. Antti Vaheerä, also of Helsinki. They have observed that incubation of Lp(a) or isolated apo(a) with fibronectin results in proteolytic cleavage of the latter, distinct from that caused by plasmin or kallikrein. The proteolytic activity of apo(a) is of the serine proteinase type and displays specificity for arginine rather than for lysine bonds. This fascinating new observation could add to our understanding of the molecular mechanisms underlying the association between Lp(a) and atherosclerosis.

**Efflux of Cholesterol From the Arterial Intima**

This last session was devoted to discussion of the early stages of reverse cholesterol transport occurring in the interstitial spaces of tissues. The speakers described experiments both in vitro and in vivo.

Dr. Gerd Schmitz of Munster, F.R.G., discussed the role of HDL in reverse cholesterol transport and its disturbances in Tangier disease and in HDL deficiency with xanthomas. He also described the clinical and biochemical defects occurring in Tangier disease and stated that an interesting future line of research will be to relate cytoskeletal mechanisms to the biochemical defects underlying Tangier disease.

Dr. Peter Slotte of Turku, Finland, reviewed the evidence for the role of HDL binding sites as functional receptors mediating the efflux of cholesterol from parenchymal cells, such as skin fibroblasts, aortic endothelial cells, and adipose cells. He concluded that the efflux of intracellular cholesterol to HDL particles occurs only if these acceptor particles are bound to specific HDL receptors.

Dr. Christopher Fielding of San Francisco, Calif., described his most recent findings concerning the initial steps in reverse cholesterol transport. The discovery of short-lived cholesterol acceptors, a new subclass of HDL particles, the "preβ-HDL," has enabled a more precise definition of the role of HDL in reverse chole-
terol transport. In this novel concept, short-lived acceptors lacking lecithin:cholesterol acyltransferase (LCAT) activity form a chain of intermediates in the formation of mature, spherical HDLs. Rapid movement of these intermediate particles from the interstitial fluid to the blood plasma with other HDL subclasses that show LCAT activity could provide the driving force for the initial steps in reverse cholesterol transport.

Dr. Paul Roheim of New Orleans, La., presented some recent data from his laboratory, where prenodal lymph has been collected from the paw of the dog to study the relation of HDL metabolism with reverse cholesterol transport. Plasma HDL, while interacting with cells, obtains newly synthesized apo E along with free cholesterol and phospholipids from them and becomes “modified” HDL. From this modified HDL, apo A-I is then released and thereby forms a preβ-apo A-I particle, which is not associated with any other lipoprotein particle. This unassociated preβ-apo A-I particle acts as a reserve for cholesterol efflux. If associated with apo A-IV, the particle can pick up some additional cholesterol.

Dr. Drago Reichl of London, England, (currently of Winston-Salem, N.C.) stated that 95% of apo B-containing lipoproteins of human lymph have the density characteristics of plasma LDL. The apo A-I-containing particles in the extravascular fluid differ dramatically from their counterparts in the plasma, the presence of large HDL particles being the most conspicuous difference between the lipoproteins in the plasma and those in the extravascular space.

In this session concerning the efflux of cholesterol from the arterial intima, there was full agreement on the role of HDL particles as the major, if not the sole, physiological carriers of cholesterol from the intracellular sites of tissues back to the plasma compartment.

Dr. Elspeth Smith of Aberdeen, Scotland, presented her data on lipoproteins directly derived from the arterial intima. In her presentation, all the facets of the main theme of this Ninth Paavo Nurmi Symposium were covered in an elegant fashion. In her address, Dr. Smith emphasized that not only the LDL particles but also the HDL particles may be modified. This is in full agreement with other reports in this symposium, whether dealing with peripheral lymph or with studies conducted with HDL particles in vitro. The preβ-HDL particles were given a central role in the initial steps of cholesterol removal from the arterial intima by all speakers dealing with the early events of reverse cholesterol transport occurring in various tissues.

**Concluding Remarks**

Dr. Petri Kovanen of Helsinki, Finland, summarized the presentations given at the symposium and presented a schematic model based on them, in which the basic science data presented at the symposium were used to explain how lowering the plasma concentration of LDL cholesterol and elevating the concentration of plasma HDL cholesterol should result in retardation of atheroma growth. According to this model, the intimal balance of cholesterol is directly related to the cholesterol balance in the bloodstream, reflected in the relative concentrations of LDL and HDL particles. Thus, lowering the concentration of LDL particles and increasing the concentration of HDL particles in the bloodstream should retard cholesterol accumulation in the arterial intima. Indeed, recent clinical studies favor this hypothesis, thus providing additional evidence in support of the pathological and biochemical findings presented at this Ninth Paavo Nurmi Symposium.
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Arterioscler Thromb Vasc Biol. 1991;11:452-455
doi: 10.1161/01.ATV.11.2.452
Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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