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Atherosclerosis:
The Past, the Present and the Future

by

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Abbreviation List:

CuZnSOD	copper-zinc superoxide dismutase	LDL	low density lipoprotein
ECM	extracellular matrix	IL-1	interlecukin-1
EDRF	endothelium-derived releasing factor	MCP-1	monocyte chemoattractant protein
EGF	epidermal growth factor	MnSOD	manganese superoxide dismutase
FGF	fibroblast growth factor	PDGF	platelet-derived growth factor
ICAM-1	intracellular adhesion molecule-1	PUFA	polyunsaturaed fatty acid
ICAM-2	intracellular adhesion molecule-2	ROS	reactive oxygen species
IFN-γ	interferon-γ	SMC	smooth muscle cell
IGF	insulin-like growth factor	VCAM	vascular cell adhesion molecule

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1. Abstract

Atherosclerosis is a complex and common disease. It is the major cause of heart disease and stroke, which together remain the leading cause of death in the United States. The mechanisms of atherosclerogenesis remain unclear. The most attractive hypothesis proposed has been that atherosclerosis begins because the innermost layer of the artery, the endothelium, becomes damaged. The interactions between endothelium cells and oxidized low-density lipoprotein (LDL), and between smooth muscle cells (SMCs) and oxidized LDL are important parts of atherosclerogenesis. Many molecules are also involved, such as adhesion molecules and some mitogens including platelet-derived growth factor (PDGF). With the rising regards of the important roles of free radicals in the process of diseases, many scientists believe that atherosclerosis is a free radical disease. This paper will discuss the process of atherosclerosis followed by a proposed plan for future research.

2. Introduction

Atherosclerosis is a disease of the arteries characterized by fatty deposits on the intimal, or inner, lining. The presence of fatty deposits, called plaques, leads to an important loss of arterial elasticity with narrowing of the artery as shown in Figure 1. This constriction to smooth blood-flow ultimately deprives vital organs of their blood supply. Clots may lodge in arteries supplying the heart, causing myocardial infarction (heart attack), or the brain, causing stroke [1]. Atherosclerosis is the major cause of heart disease and stroke, which together remain the leading cause of death in the United States [2].

Atherosclerosis is a slow, progressive disease that may start in childhood. In some people this disease progresses rapidly in their third decade. In others it doesn't become threatening until they are in their fifties or sixties [3]. The major targets of Atherosclerosis are the aorta of the coronary and cerebral arteries. The three basic proceses leading to the formation of atherosclerotic lesions are: (i) invasión of the artery wall by leukocyes, particularly monocytes and T-lymphocytes; (ii) smooth muscle cell (SMC) phenotypic modulation, proliferation, and synthesisi of extracellular matrix; and (iii) intracellular lipoprotein uptake in macrophage and SMC, and lipid accumulation [4].

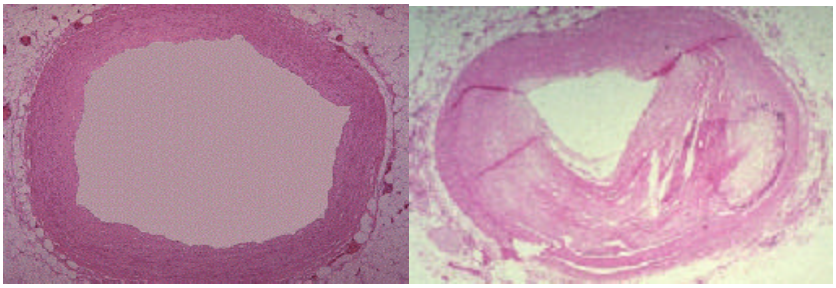

Figure 1. The comparison of a normal artery and atheroscletic artery. The figure on the left is showing a section of an artery (normal). The figure on the right has plaque buildup (http://adminweb.ucis.dal.ca/cprrc/atherosc.htm).

The most attractive hypothesis proposed has been that atherosclerosis begins because the innermost layer of the artery, the endothelium, becomes damaged [5]. Three possible causes of damage to the arterial wall are: elevated levels of cholesterol and triglyceride in the blood, high blood pressure and tobacco smoke. Because of the damage, over time lipids, cholesterol, fibrin, platelets, cellular debris, calcium and other substances are deposited in the artery wall. These substances may stimulate the cells of the artery wall to produce still other substances that result in further accumulation of cells in the innermost layer of the artery wall where the atherosclerotic

lesions form. These cells accumulate and many of them divide. At the same time, lipoprotein builds up within and around these cells. They also form connective tissue.

Since Goldstein and Brown's observation that oxidative modification of low-density lipoprotein (LDL) leads to foam cell formation, there has been intense interest in the relationship between oxidative stress and atherosclerosis [6]. With the understanding of the pathophysiological roles played by reactive oxygen species (ROS) and lipid peroxidation in the vasculature, there is also accumulating evidence that antioxidant compounds have beneficial effects in atherosclerosis [7]. In 1980, a factor released by the endothelium upon stimulation with acetylcholine was discovered, which is called endothelium-derived releasing factor (EDRF) [8]. Additional investigations have identified nitric oxide as EDRF and have associate abnormalities of EDRF action with atherosclerosis. This paper introduces the pathogenesis of atherosclerosis and discusses the role of reactive oxygen species (ROS) in atherosclerosis, followed by a proposal for future approaches.

3. Pathogenesis of Atherosclerosis

The vascular walls are relatively rich in extracellular matrix (ECM). In the capillaries, ECM forms the basal lamina, and in the aorta, ECM is the major portion of the media and the adventitia [9]. The extracellular matrix is the result of the biosynthetic activity of most cells of the organisms. The selection of the quality and expression of the quantity of these ECM macromoleculs requires a precise "programming" to be able to determine the differentiation of the vascular wall cells. And any deviation from this precise "programming" can cause problems. The development of atherosclerosis is a good example of such deviations from the normal programming of the biosynthesis of ECM macromolecules. This section concerns two major cell

types of the arterial wall: endothelial cells and smooth muscle cells as well as the extracellular matrix macromolecules they synthesize. There are also other cell types that are involved in the process of atherosclerosis such as monocytes, macrophages and platelets and other molecules such as chemotactic factors. They are beyond the scope of this paper.

3.1 Structure of Blood Vessels

In general, the blood vessel wall is composed of three layers (as shown in Figure 2 [10]): the intima, media and adventitia. The intima consists of a single layer of endothelial cells lying on a continuous basement membrane composed of type IV collagen and structural glycoproteins. The media is composed of smooth muscle cells surrounded by a connective tissue matrix made up of collagen fibres (type I and type III), elastic fibres, glycosaminoglycans, and structural glycoproteins. The adventitia is the outer layer containing fibroblasts, adipocytes, mast cells, and an extracellular matrix of collagen fibres, lipids, and structural glycoproteins.

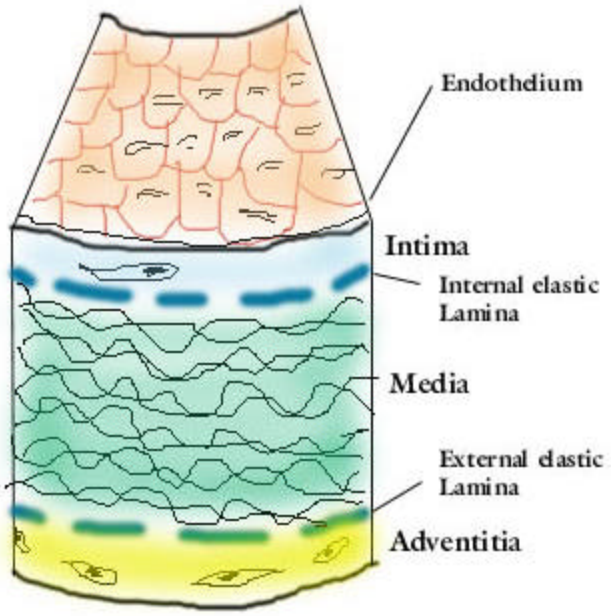

Figure 2: Diagram of the crosssection of blood vessel walls [10].

3.2 Cells Involved in Atherosclerosis

a. Endothelial cells

Although the size of the blood vessels varies extensively from the capillaries to the veins and arteries, they all have lining the luminal surface a single layer of endothelial cells surported by an underlying ECM [10]. Morphology of the endothelium varies between large and small vessels. In large veins and arteries, the endothelium exists as a tightly packed sheet of polygonal cells. In small venules and capillaries, individual endothelial cells form the vessel through which the blood cells pass in single file.

The integrity of endothelial cells is related to atherogenesis [11], and this relationship is still being investigated in many laboratories. It is beyond the shadow of doubt that macromolecules of the configuration of LDL can gain entrance to the artery wall via the transport vesicles which traverse the endothelial cells [12]. Therefore, endothelium works as the gate keeper and regulator of lipoprotein transport. The endothelium cells also have potential to express leukocyte adhesion molecules, attractant protein, and majoy histocompatibility antigens [13]. Many factors can induce endothelial cell injury and cause it dysfunction. The results of endothelial cell injury are increased endothelial permeability, increased leukocyte adhesion, accumulation of macrophages in subendothelial intima and SMC activation and proliferation [14]. Lipoproteins (mainly LDL and also VLDL), oxidized lipoprotein, and circulating cells (particularly monocytes and T-lymphocytes) enter the subendothelial space and form the fatty streaks, then monocytes transform to macrophages and foam cells in the vessel wall, and eventually results in complex advanced lesion of atherosclerosis as shown in Figure 3 [15].

b. Smooth muscle cells

Smooth muscle cells are the predominant cells in the raised fibrous plaque [16]. In a normal artery, SMC are the major component of the medial layer, although they may also be present in the intima in some arterial segments. When SMC is stimulated to migrate and proliferate as it is in some pathological conditions, it becomes much more active synthetically and more responsive to stimuli causing cell proliferation. Furthermore, it changes its morphological appearance. Rough endoplasmic reticulum, free ribosomes and the Golgi apparatus become more prominent [17].

The interaction of SMCs with lipoproteins, especially the LDLs, has also become a very improtant part of the formation of atherosclerosis [18]. The arterial SMCs can take up relatively large quantities of LDLs and store some of these in vacuoles, perhaps by an

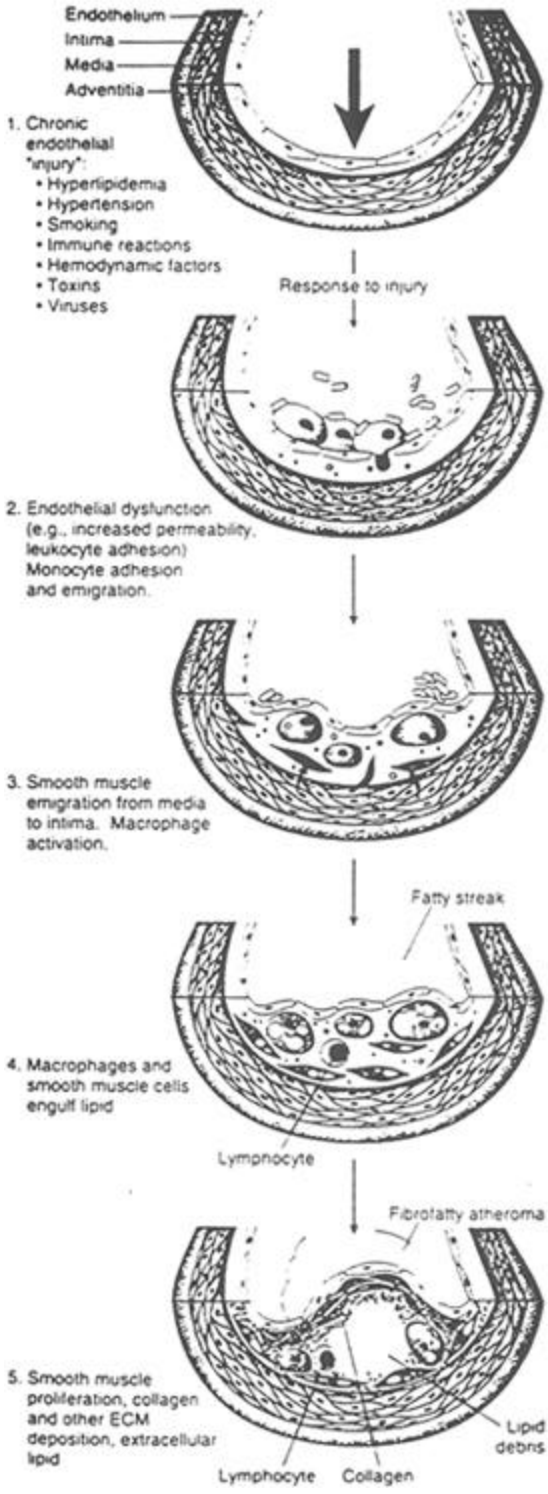

Figure 3. The hypothetical process of atherosclerosis [15].

unregulated, non-receptor scavenger mechanism, whereas the quantity of LDL taken up by SMCs under usual circumstances is regulated by the LDL receptor system and its "feedback" mechanisms. Features and functions of the arterial SMC are shown in Figure 4.

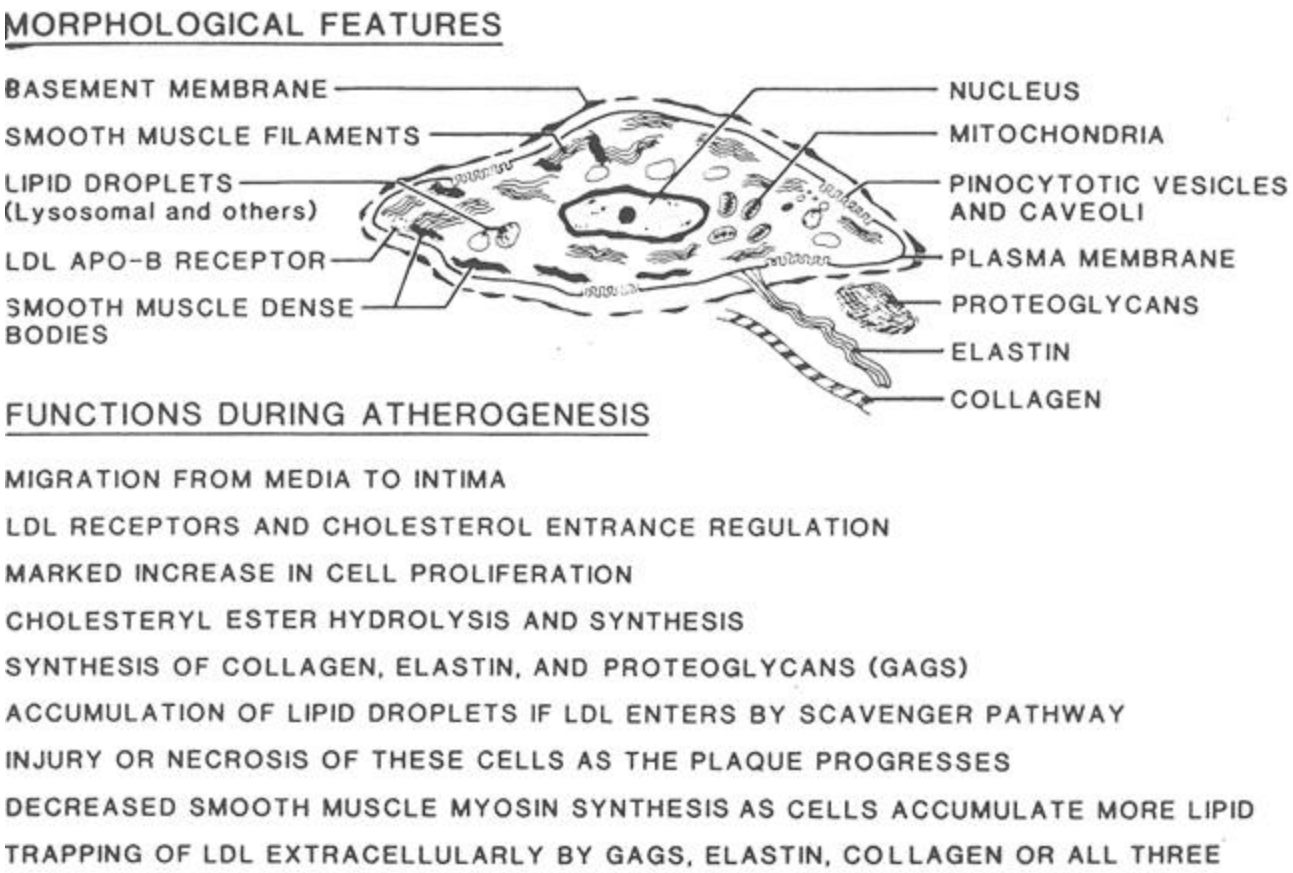

Figure 4. Features and functions of the arterial smooth muscle cell [18].

3.3 Molecules Involved in Atherosclerosis

There are a lot of molucules involved in the development of atherosclerosis, as shown in Figure 5 [18]. This section discusses several important molecular components: the adhesion molecules and some growth factors, especially the one that is called platelet-derived growth factor (PDGF).

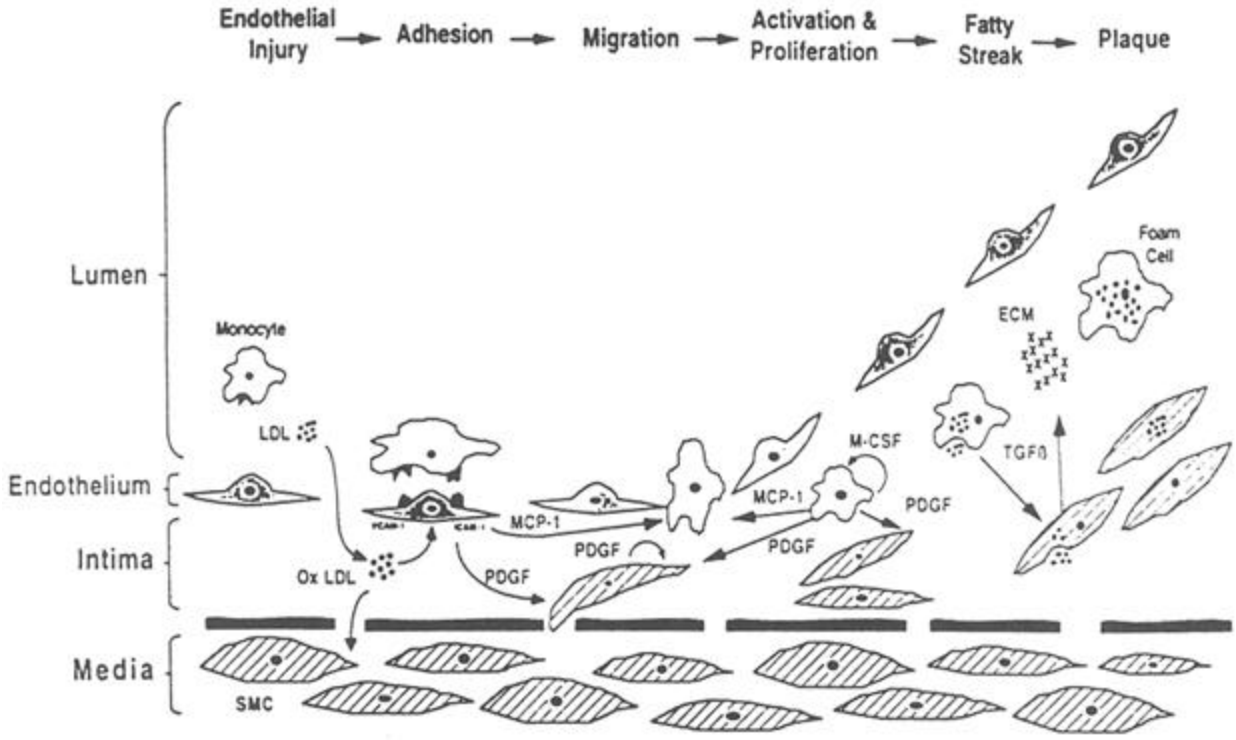

Figure 5. The athrosclerotic process involves many molecules [18].

a. Adhesion molecules

There are evidence showing that the expression of adhesion molecules is correlated with the extent of the atherosclerotic lesion [19, 20]. There are several different structual groups of adhesion factors which have been identified on endothelial cells and which interact with receptors on leukocytes and platelets. Intracellular adhesion molecule-1 (ICAM-1) and intracellular adhesion molecule-2 (ICAM-2) are cell surface glycoproteins found on many cell types. ICAM-1 is inducible on cultured endothelial cells by the inflammatory mediatiors interleukin-1 (IL-1), tumor necrosis factor (TNF), interferon-γ (IFN-γ) and endotoxin. ICAM-1 can bind lymphocytes, monocytes and neutrophils to endothelium. ICAM-2 is constitutively expressed on endothelial cells and appears to be a truncated form of ICAM-1. Vascular cell

adhesion molecule (VCAM) is also induced by cytokines and binds selectively to lymphocytes and to some degree to monocytes, but not to neutrophils [21].

In human samples from autopsies and hearts of atherosclerotic patients, ICAM-1 was detected in endothelial cells over plaques, intimal vascular smooth muscle cells, and macrophages. VCAM-1 was detected in luminal endothelial cells in advanced coronary artery plaques and neovascular endothelial cells at base of plaques, It was also found in focal endothelial cells of uninvolved vessels with diffused intimal thickening and in macrophages. These CAMs are likely to mediate leukocyte infiltration [22].

b. Mitogens and growth factors

A number of mitogens and growth factors secreted by endothelial cells and other cells are decisive factors in the growth and propagation of the atherosclerotic plaque. These mitogens include platelet-derived growth factor (PDGF), insulin-like growth factor (IGF), epidermal growth factor (EGF), fibroblast growth factor (FGF) and so on [23]. PDGF will be discussed in this paper.

PDGF is not only a major mitogen of SMCs, but also is a chemoattractant for SMCs and monocytes. It is present in three isoforms, which interact with several different cell receptors. PDGF gene expression is low in the normal vascular wall tissue and high in sites prone to SMC proliferation such as the intima of atherosclerotic plaques. PDGF is produced by endothelial cells, smooth-muscle cells and macrophages. It is among a dozen of different growth factors that stimulate smooth-muscle cells to proliferate and produce large amounts of ECM. PDGF can bind to the cell-surface PDGF receptor on contractile smooth-muscle cells and switch these cells to the synthetic smooth-muscle cell type, as shown in Figure 6. As these cells proliferate and synthesize ECM, they form a fatty streak, which eventually lead to atherosclerosis [24].

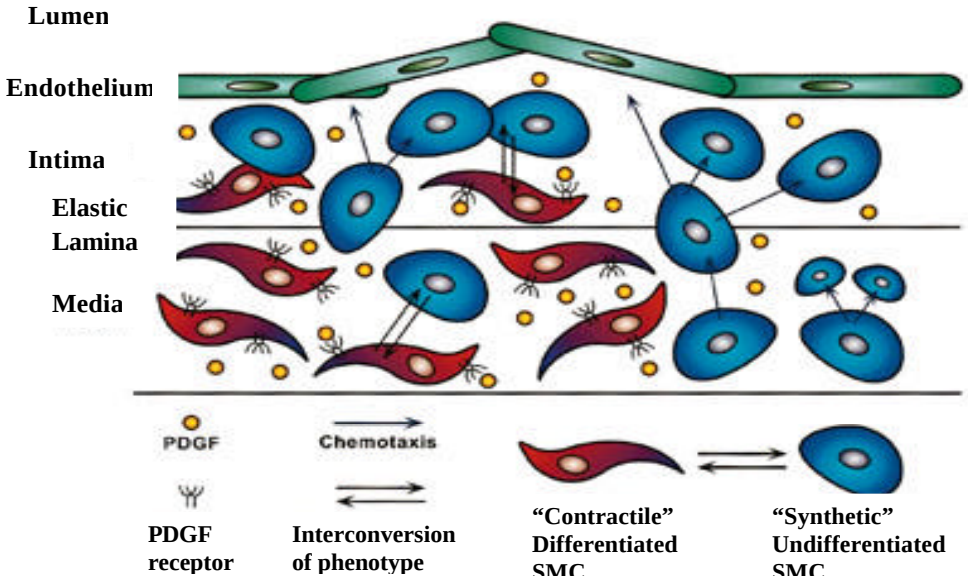

Figure 6. Smooth muscle cell proliferation in a growing atherosclerotic plaque [24].

4. ROS in Atherosclerosis

The study of free radical biology has so grown in recent years that it has generated dedicated journals such as *Free Radicals in Biology and Medicine*. The increase in the scientific literature reflects the opinion that oxidative stress may be involved in the pathogenesis of many diseases. The three major types of cellular damage resulting from ROS involve lipids, protein oxidaiton and oxidation of DNA. The oxidative modificaiton of low-density lipoprotein (LDL) cholesterol was postulated to be a pivatol step in atherogenesis. The most common evidence in athrosclerotic leision is the fatty streak in which lipids accumulated by SMC, monocytes and macrophages. The amount of low-density lipoprotein (LDL) oxidation occurs in areas where the antioxidant concentration is low, such as the arterial wall. This section discusses the possible roles of lipoprotein in the process of atherosclerosis. We first start out with LDL oxidation.

4.1 LDL Oxidation

The chemical events of LDL oxidation are very complex. Although the exact séquense of events leading to LDL oxidation *in vivo* is unknown, initiation begins with either abstraction of hydrogen atoms from polyunsaturaed fatty acid (PUFA) within LDL by various ROS or by direct enrichment of the LDL with lipoperoxides from cells. Either mechanism leads to a loading of LDL with lipoperoxides which change into more reactive intermediates that can initiate oxidation in neighboring PUFAs or PUFAs in nearby LDL particles [25]. *In vitro*, the decomposition of lipoperoxides to more reacive peroxyl radicalsl is dependent on the presence of transition metals (eg. copper or iron) and can be inhibited by metal chelators.

Importantly, the oxidation process is autocatalytic such that, a single hydrogen abstraction could lead to oxidation of the entire LDL particle as well as neighboring LDL particles. This process may be facilitated by LDL's intrinsic phospholipase A_2 activity., which leaves off oxidized fatty acids from lecithin, generating isolecithin. The oxidized fatty acids released by phospholipase A_2 are more mobile and presumably may help spread the oxidation process to other areas of the LDL particle [26]. Initially, the oxidation process procedes slowly, but eventually the antioxidant content withiin LDL is depleted and the number of fatty acid lipoperoxides amplify such that the oxidation process rapidly accelerates (the propagation phase). Eventually, PUFAs are cleaved into a variety of reactive aldehydes, ketones and other short chanin fragments (the termination phase). These in turn may bind to apopretein B-100 in LDL which leads to decreased recognition and binding by the LDL receptor. In addition, new epitopes are formed that lead to recognition and enhanced uptake of modified LDL by the scavenger receptor of macrophages. There may in fact be a family of "scavenger" receptors whose function is to remove modified or altered proteins, including LDL [27].

Oxidation of LDL may be initiated by a number of different mechanisms. However, cell-mediated oxidation of LDL may be the most relavent to our understanding of LDL oxidation *in vivo.* In tissure culture, all the cells normally present in the artery wall, including endothelial cells, smooth muscle cells, macrophages and lymphocytes can oxidize LDL [28]. The mechanisms by which cells initiate oxidation of LDL are poorly defined.

Table 1. Mechanisms By Which Oxidized LDL May Be Atherogenic [25].

1	It has enhanced uptake by macrophages leading to cholesteryl ester enrichment and foam cell formation
2	It is chemotactic for circulating monocytes and T-lymphocytes.
3	It inhibits the motility of tissue macrophages
4	It is cytotoxic.
5	It renders LDL more susceptible to aggregation, which leads to enhanced macrophage uptake
6	It can alter gene expression of neighboring arterial cells such as induction of MCP-1, CSF, IL-1 and endothelial expression of adhesion molecules.
7	It can adversely alter coagulation pathways, such as by induction of tissue facotor.
8	It can adversely alter vasomotor properties of coronary arteries
9	It is immunogenic and can elicit autoantibody formation and reactive T-cells.

4.2 Atherogenic Properties of Oxidized LDL

Oxidized LDL takes on a variety of properties that make it more atherogenic than unmodified LDL. These are outlined in Table 1 [25]. Every early step in the initial stages of atherosclerosis is the focal adherence of monocytes to the artery wall. This may result from

expression of specific leukocyte adherence molecules such as VCAM-1on endothelial cells. These adherence proteins are expressed prior to monocyte binding to the endothelium and accumulate over areas of the artery wall which later become sites of foam cell formation. LDL that has been minimally oxidized stimulateds the expression and secretion of many different cytokines. *In vitro*, adding minimally modified LDL to endothelial and smooth muscle cells stimulates their secretion of monocyte chemoattractant protein (MCP-1) [29]. This is shown in figure 7 [30]. Presumably, this cytokine will attract monocytes to the endothelium where they bind to specific adherence proteins. During LDL oxidation, phosphatidylcholine is formed and can stimulated expression of the adherence molecule VCAM-1, and is one example of the many products of modified lipoproteins that likely influence monocyte recruitment.

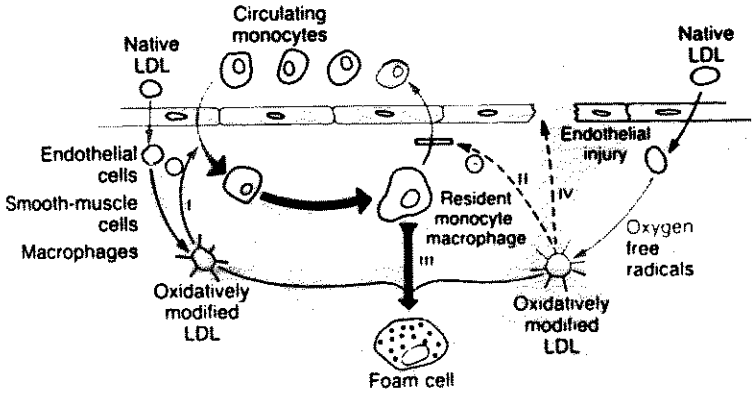

Figure 7. Proposed mechanisms by which oxidized LDL contributes to atherosclerosis [30].

4.3 Effects of Antioxidants on Atherosclerosis

a. a **-tocopherol**

Because the above hypothesis implicates oxidatively modified LDL in the pathogenesis of atherosclerosis, treatment with antioxidant, such as α-tocopherol, could conceivably retard the progression or actually regress the atherosclerotic lesion [31]. α-tocopherol is a chain-breaking

antioxidant that prevent oxidation by trapping peroxyl free radicals (shown in Figure 8 [32]), thereby providing first-line protection against lipid peroxidation. In models involving normal animals fed a high-cholesterol diet, significant inhibition of atherosclerosis with vitamin E treatment has been shown in monkeys [31]. Moreover, several studies have linked the atherosclerosis-inducing effect of cigarette smoke with low antioxidant status [33, 34]. Cigarette smokers can be considered to be under oxidative stree, even if antioxidant concentrations are normal, as cigarette smoke is extremely high in free radicals. Although the red blood cells and lipoprotein particles of smokers have similar concentrations of vitamin E compared to nonsmokers, the lipids of these cells and particles are more susceptible to oxidation. Supplementation with vitamin E prevents lipid peroxidation in smokers.

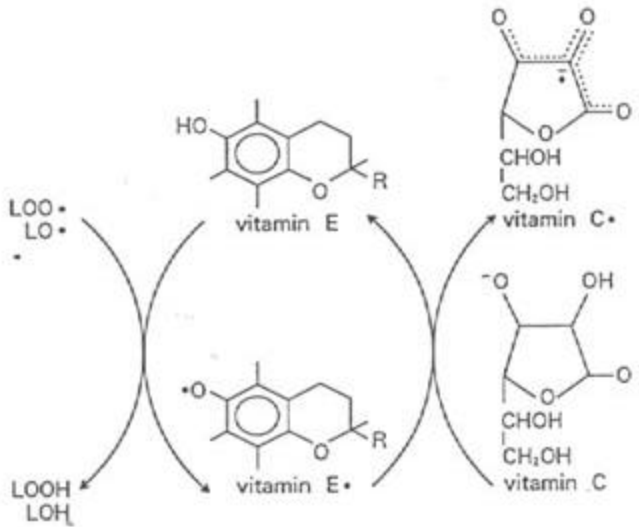

Figure 8. Chain reaction of vitamin E (a **-tocopherol) with lipid radicals and vitamin C** [32].

b. Antioxidant enzyme system

Cells in our body have balanced antioxidant enzyme system to protect from oxidative damage, as shown in Figure 9 [35].

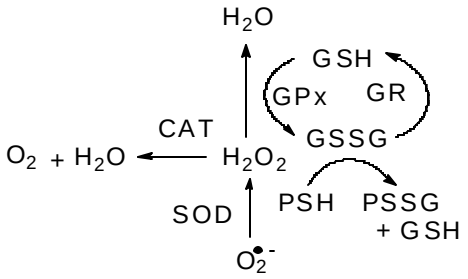

Figure 9. The antioxidant enzyme system [35].

Manganese superoxide dismutase (MnSOD) and Copper-Zinc superoxide dismutase (CuZnSOD) both convert superoxide ($O_2^{\bullet -}$) to produce hydrogen peroxide (H_2O_2) during normal oxygen metabolism and contribute to the redox state of the cell. MnSOD is located in the mitochondrial matrix whereas CuZnSOD is in the cytosole. It has been shown that MnSOD can be induced in atherosclerotic arteries, may be as a reply to increased mitochondrial oxidation [36]. Although *in vitro* studies showed that CuZnSOD inhibited cell-mediated oxidation of LDL, CuZnSOD transgenic mice did not reduce the extent of atherosclerotic lesion development [37]. However, their role in preventing atherosclerosis still needs investigation.

5. Future plans for research

Although there are a huge bunch of papers and books about atherosclerosis, from the above information, we can see that there are still two important points remaining unclear. First, the mechanisms of atherogenesis are unclear. We know that LDL oxidation contributes to the process of atherogenesis, but we don't know the siganal transduction pathways that are involved in which LDL is oxidized and accumulated upon the artery walls. Second, the role of antioxidant

enzymes in preventing atherogenesis remains undefined. The following hypothesis aims at answering these questions.

5.1 Hypothesis

The hypothesis is that using proteomics technique, we will be able to identify proteins that change expression during atherosclerosis and those proteins most probably are involved in the signal transduction pathways leading to atherosclerosis; With this technique, we are also be able to identify proteins that are influenced by MnSOD overexpression and those proteins are candidates that are disturbed by MnSOD on the atherosclerogenetic signal transduction pathways. Following steps can be done to prove this hypothesis.

5.2 Identify proteins that may be involved in the signal transduction pathways for Atherosclerosis.

Specific Aim 1: Set up an animal model suitable for atherosclerosis analysis.

In this study, we will choose mice as the animal model. Mice are fed with normal chow supplemented with high cholesterol and butter fat for 14 weeks prior to base-line analysis (supplemented diet group). Blood will be drawn 1 week prior to the end of the experimental diet, plasma cholesterol is then quantified. Mice are divied into three groups with mean cholesterol levels not significantly different. One group of mice are killed for analysis of atherosclerosis (base-line group). Remaining mice are injected intravenously with either AdMnSOD (AdMnSOD atherosclerotic group) or control Adnull (atherosclerotic control group) virus. The supplemented diet are continued after vector administration. Blood samples are collected and centrifuged to obtain plasma, which is stored at $-20^{o}C$ for lipid analyses and at $4^{o}C$ for FPLC. Mice are killed 6 weeks after vector administration for analysis of atherosclerosis. Control group uses age and sex matched mice and fed with normal diet. *Rational:* high

cholesterol and butter fat diet can lead to atherosclerosis. And mouse is a classic model for atherosclerosis analysis [38].

Specific Aim 2: Analysis of atherosclerotic lesions

a. Evaluation of whole aortas

The entire aorta, from junction with the heart to the iliac bifurication, is removed from mice, cut open and staind with the Oil red O staining solutions as described by Paigen [39], and examined under a dissecting micorscope. The spots stained with Oil red O solutions are atherosclerotic lesions. Visual estimation of the area covered by Oil red O staining lesions will be made.

b. Evaluation of aortic cross sections

The heart and the upper section of the aorta are removed. Extraneous tissue are trimmed. The heart and the aortas are sectioned from the apex to the base of the heart and beyond into the ascending aorta up to the aortic arch. Sectioning continues along the ascending aorta away from the heart until the valve cusps are no longer visible. Every cross-section slide is stained with Oil red O staining solution and evaluated for atherosclerotic lesions [39].

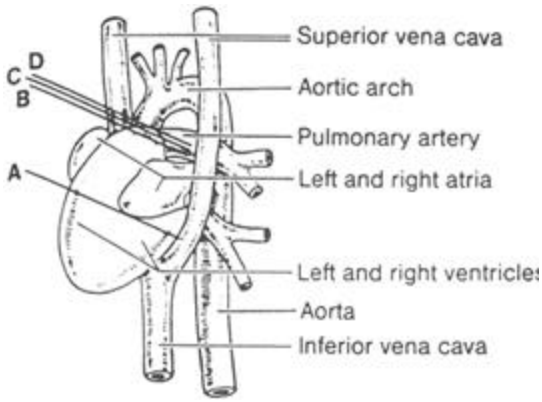

Figure 10. Anatomy of a mouse heart and aorta [39]. Line A represents a line between the tips of the atria; Line B represents the beginning of the aortic sinus; Line C represents the beginning of one cross section of the aorta and Line D represents the end of this cross section. The average distance between Line C and Line D is about 280 μm.

Rational: Pathologic properties of atherosclerotic lesions can be measured by evaluating the extend of lesions along whole aorta and aorta cross-sections.

Specific Aim 3. Analysis of LDL and LDL oxidation

a. LDL purification and analysis of cholesterol levels. LDL are purified from plasma by sequential untracentrifugations. Protein concentrations in plasma and LDL are determined according to the method of Lowry [40]. Plasma cholesterol and triglyceride levels are measured by FPLC gel filtration on two superose 6 columns. The cholesterol concentrations in the FPLC fractions are determined using an enzymatic assay kit.

Lipoprotein are isolated from plasma samples by sequential untracentrifuagtion. The plasma samples obtained at various time points during experiments are pooled from mice each of the AdMnSOD atherosclerotic and Adnull atherosclerotic group. The VLDL, LDL and HDL are isolated by tube slicing. The lipoprotein frations are analyzed for the lipid and protein contents.

b. Detection of oxidized LDL by ELISA. The oxidized LDL in blood plasma were detected by ELISA using anti-oxLDL monoclonal antibodies (mAb). Ninety-six well plates are coated with plasma samples. Primary and secondary antibodies are added consequently. Sulfuric acid and the optical density (OD) are measured with a microplate reader to determine the concentration of oxidized LDL.

Rational: LDL oxidation are highly correlated with atherosclerosis.

Specific Aim 4: identify proteins that may involved in the atherosclerosis process.

Among the techniques of functional genomics, both DNA micorarrays and proteomics hold great promise for the study of complex biological systems with applications in molecular medicine. These novel and powerful gene expression profiling techniques permit the analysis of the expression levels of thousands genes simultaneously both in health and disease. DNA microarray focuses on the mRNA level whereas proteomics is used to analyze global patterns of gene expression at the protein level. Proteins are frequently the functional molecules and,

therefore, the most likely to reflect differences in gene expression. Some messengers are transcribed but not translated, and the number of mRNA copies does not necessarily reflect the number of functional protein molecules. Therefore, we are going to use the proteomics techniques to approach our hypothesis, to identify proteins involved in atherosclerogenic signal transduction pathways.

Step 1. Protein seperation with 2D PAGE.

After analyzing the LDL contents and atherosclerotic lesions of the supplemented diet group of mice, total protein will be isolated from artery walls of the base-line group mice and control group mice, separated on a high resolution two-dimensional polyacrylamide gel electrophoresis (2D PAGE). Proteins will be separated both in terms of their isoelectric point (p*I*) and molecular weight. This technique was originally described by O'Farrell [41].

Step 2. Protein detection and quantification

The proteins on the gels will be detected by silver nitrate staining and Coomasie Blue staining. Quantitative fluorescence measurements can be performed with CCD-camera based systems as well as with laser scanner systems.

Step 3. Protein identification and database construction

Proteins that have detectable expression difference between control group and base-line group will be identified with Edman peptide sequencing. The peptide sequences will be blasted against NCBI protein database to identify the corresponding proteins isolated from 2D PAGE (http://www.ncbi.nlm.nih.gov/blast/html/blastcgihelp.html#protein_databases).

A comprehensive 2D PAGE database for atherosclerosis will be generated based on the protein expression profile obtained from the above steps. This database will be exceedingly important for the atherosclerosis research in that it provides a global overview of the proteins

that may be involved in the process of Atherosclerosis. And future research can be directed based on the information provided by this database.

5.3 Identify proteins that may be disturbed by MnSOD in the atherosclerogenic signal transduction pathways.

Same steps will be used as described in 5.2. To identify proteins that may be disturbed by MnSOD in atherosclerogenic signal transduction pathways, AdMnSOD atherosclerotic group, atherosclerotic control group and control group will be used for 2D PAGE analysis. Again, a comprehensive 2D PAGE database for effect of MnSOD on atherosclerosis will be generated based on the protein expression profile obtained from the above steps. Again, this database will be extremely important in that it provides great information about the effects that MnSOD may have on atherosclerosis.

6. Summary

Atherosclerosis is a major cause of heart disease and stroke. The mechanisms of atherosclerogenesis remain unclear. Evidences show that oxidized LDL plays a very important role in atherosclerogenesis. But the signal transduction pathways that lead to LDL oxidation are far beyond clear. With the rising regarding of the important roles of free radicals in the process of diseases, many scientists believe that atherosclerosis is a free radical disease. Antioxidants may play an important role in preventing or reversing atherosclerosis. But the exact roles of antioxidant in the process of atherosclerosis are still undefined. Since prometics techniques provide us a way to profile protein expression both in health and disease, it is a very promising technique to answer all the above questions. With the generation of a comprehensive atherosclerosis 2D PAGE database and effects of MnSOD on atherosclerosis database, future research can be directed based on the important information provided by these databases.

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