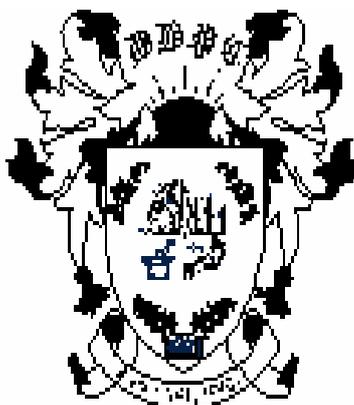


**CAUSES, SYMPTOMS, PATHOPHYSIOLOGY
AND DIAGNOSIS OF ATHEROSCLEROSIS- A REVIEW**

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Atherosclerosis (also known as Arteriosclerotic Vascular Disease or ASVD) is the condition in which an artery wall thickens as the result of a build-up of fatty materials such as cholesterol. It is a syndrome affecting arterial blood vessels, a chronic inflammatory response in the walls of arteries, in large part due to the accumulation of macrophage white blood cells and promoted by low density (especially small particle) lipoproteins (plasma proteins that carry cholesterol and triglycerides) without adequate removal of fats and cholesterol from the macrophages by functional high density lipoproteins (HDL), is commonly referred to as a hardening or furring of the arteries. It is caused by the formation of multiple plaques within the arteries (1).

The **atheromatous plaque** is divided into three distinct components:

1. The atheroma ("lump of wax", from *Athera*, wax in Greek.), which is the nodular accumulation of a soft, flaky, yellowish material at the center of large plaques, composed of macrophages nearest the lumen of the artery
2. Underlying areas of cholesterol crystals
3. Calcification at the outer base of older/more advanced lesions.

The following terms are similar, yet distinct, in both spelling and meaning, and can be easily confused: arteriosclerosis, arteriolosclerosis, and atherosclerosis. Arteriosclerosis is a general term describing any hardening (and loss of elasticity) of medium or large arteries (from the Greek *Arterio*, meaning *artery*, and *sclerosis*, meaning *hardening*); arteriolosclerosis is any hardening (and loss of elasticity) of arterioles (small arteries); atherosclerosis is a hardening of an artery specifically due to an atheromatous plaque. Therefore, atherosclerosis is a form of arteriosclerosis.

Atherosclerosis, though typically asymptomatic for decades, eventually produces two main problems: First, the atheromatous plaques, though long compensated for by artery enlargement, eventually lead to plaque ruptures and clots inside the artery lumen over the ruptures. The clots heal and usually shrink but leave behind stenosis (narrowing) of the artery (both locally and in smaller downstream branches), or worse, complete closure, and, therefore, an insufficient blood supply to the tissues and organ it feeds. Second, if the compensating artery enlargement process is excessive, then a net aneurysm results.

These complications of advanced atherosclerosis are chronic, slowly progressive and cumulative. Most commonly, soft plaque suddenly ruptures (see vulnerable plaque), causing the formation of a thrombus that will rapidly slow or stop blood flow, leading to death of the tissues fed by the artery in approximately 5 minutes. This catastrophic event is called an infarction. One of the most common recognized scenarios is called coronary thrombosis of a coronary artery, causing myocardial infarction (a heart attack). Even worse is the same process in an artery to the brain, commonly called stroke. Another common scenario in very advanced disease is claudication from insufficient blood supply to the legs, typically due to a combination of both stenosis and aneurysmal segments narrowed with clots. Since atherosclerosis is a body-wide process, similar events occur also in the arteries to the brain, intestines, kidneys, legs, etc.

CAUSES

Atherosclerosis develops from low-density lipoprotein molecules (LDL) becoming oxidized (ldl-ox) by free radicals, particularly oxygen free radicals (ROS). When oxidized LDL comes in contact with an artery wall, a series of reactions occur to repair the damage to the artery wall caused by oxidized LDL. The LDL molecule is

globular shaped with a hollow core to carry cholesterol throughout the body to generate brain tissues, vitamin D, and so on. Cholesterol does not dissolve in water. Blood is 70% water. Cholesterol can move in the bloodstream only by being transported by LDL.

The body's immune system responds to the damage to the artery wall caused by oxidized LDL by sending specialized white blood cells (macrophages and T-lymphocytes) to absorb the oxidized-LDL forming specialized foam cells. Unfortunately, these white blood cells are not able to process the oxidized-LDL, and ultimately grow then rupture, depositing a greater amount of oxidized cholesterol into the artery wall. This triggers more white blood cells, continuing the cycle.

Eventually, the artery becomes inflamed. The cholesterol plaque causes the muscle cells to enlarge and form a hard cover over the affected area. This hard cover is what causes a narrowing of the artery, reduces the blood flow and increases blood pressure. Some researchers believe that atherosclerosis may be caused by an infection of the vascular smooth muscle cells. Chickens, for example, develop atherosclerosis when infected with the Marek's disease herpesvirus (2). Herpesvirus infection of arterial smooth muscle cells has been shown to cause cholesteryl ester (CE) accumulation (3). Cholesteryl ester accumulation is associated with atherosclerosis. Also, cytomegalovirus (CMV) infection is associated with cardiovascular diseases (4).

SYMPTOMS

Atherosclerosis typically begins in early adolescence, and is usually found in most major arteries, yet is asymptomatic and not detected by most diagnostic methods during life. Atheroma in arm, or more often in leg arteries, which produces decreased blood flow is called peripheral artery occlusive disease (PAOD). According to United States data for the year 2004, for about 65% of men and 47% of women, the first symptom of atherosclerotic cardiovascular disease is heart attack or sudden cardiac death (death within one hour of onset of the symptom). Most artery flow disrupting events occur at locations with less than 50% lumen narrowing (~20% stenosis is average). [The reader might reflect that the illustration above, like most illustrations of arterial disease, overemphasizes lumen narrowing, as opposed to compensatory external diameter enlargement (at least within smaller arteries, e.g., heart arteries) typical of the atherosclerosis process.

Cardiac stress testing, traditionally the most commonly performed non-invasive testing method for blood flow limitations, in general, detects only lumen narrowing of ~75% or greater, although some physicians claim that nuclear stress methods can detect as little as 50%.

The list of signs and symptoms mentioned in various sources for Atherosclerosis includes the 20 symptoms listed below:

- No early symptoms
- Symptoms of coronary artery disease
 - Angina
 - Heart attack
 - Coronary thrombosis
- Symptoms of brain artery disease
 - Stroke
 - Transient ischemic attack

- Symptoms of leg artery disease
 - Leg blood clot
 - Leg pain
 - Leg cramps
 - Intermittent claudication
- Other symptoms
 - Erectile dysfunction
- Atherosclerosis usually doesn't cause signs and symptoms until it severely narrows or totally blocks an artery.
- Angina may feel like pressure or a squeezing pain in your chest. You also may feel it in your shoulders, arms, neck, jaw, or back. This pain tends to get worse with activity and go away when you rest. Emotional stress also can trigger the pain.
- Shortness of breath
- Arrhythmias (irregular heartbeats).
- Sudden numbness, weakness, and dizziness.

Signs and symptoms of atherosclerosis are not visible until the arteries are severely narrowed or blocked. Signs and symptoms differ depending on the arteries affected by atherosclerosis.

Coronary Artery Disease (CAD):

Once the arteries supplying blood to heart are blocked, the cells in the heart begin to die and a heart attack may occur. The most common symptoms of Coronary Artery Disease (CAD) are:

1. Chest pain with a heavy, squeezing or crushing sensation with possible burning or stabbing pain
2. Abdominal, neck, back, jaw or shoulder and arm pain
3. Nausea and vomiting sensation
4. Fatigue
5. Weakness
6. Perspiration
7. Shortness of breath
8. Depression and anxiety

Carotid Artery Disease or Cerebrovascular Disease:

Cerebrovascular disease is caused due to reduced supply of oxygen rich blood to brain leading to transient ischemic attack (meaning sudden loss of brain function and complete recovery within a day) and stroke. Some of the symptoms include:

1. Paralytic stroke on one side of the body
2. Inability to comprehend speech, or to have garbled speech
3. Loss of vision in one of the eyes
4. Muscle weakness
5. Impairment of facial muscles
6. Poor coordination
7. Involuntary and jerky movements on one side of the body
8. Rapid and iterative involuntary eye movement
9. Vertigo

Peripheral Artery Disease (PAD):

Accumulation of plaque in the arteries supplying blood to the hands and feet lead to PAD. Some of the symptoms of PAD include:

1. Pain, cramps, numbness and sense of fatigue in muscles of limbs
2. Diminished pulses in the hands and feet
3. Reduced muscle mass
4. Blowing sounds that can be heard with the help of a stethoscope indicating turbulence in blood flow (also called as “Bruits”)
5. Loss of hair
6. Thickening of nails
7. Smooth and shiny skin surface
8. Gangrene

Abdominal Angina and Bowel Infarction:

Narrowing of intestinal arteries leads to abdominal angina and bowel infarction. Some of the symptoms are:

1. Cramping pain in the middle of the abdomen
2. Severe abdominal pain with vomiting and diarrhea or abdominal swelling

RISK FACTORS FOR ATHEROSCLEROSIS

Coronary artery disease is the leading cause of death in the United States and while the exact cause of atherosclerosis remains unknown, certain traits, conditions, or habits may raise a person's chance of developing it.

These conditions are known as risk factors and a person's chances of developing atherosclerosis increase with the number of risk factors they have - most risk factors can be controlled and atherosclerosis can be prevented or delayed - these include high Cholesterol and low-density lipoprotein (LDL) in the blood, low level of high-density lipoprotein (HDL) in the blood, Hypertension (high blood pressure), tobacco smoke, Diabetes Mellitus, Obesity, inactive lifestyle, age - a family history of heart disease is also a risk factor and the one which cannot be controlled.

Unhealthy blood cholesterol levels - this includes high LDL cholesterol (sometimes called bad cholesterol) and low HDL cholesterol (sometimes called good cholesterol).

High blood pressure - blood pressure is considered high if it stays at or above 140/90 mmHg over a period of time.

Smoking - this can damage and tighten blood vessels, raise cholesterol levels, and raise blood pressure - smoking also doesn't allow enough oxygen to reach the body's tissues.

Insulin resistance - Insulin is a hormone that helps move blood sugar into cells where it's used and insulin resistance occurs when the body cannot use its own insulin properly.

Diabetes - this is a disease in which the body's blood sugar level is high because the body doesn't make enough insulin or does not use its insulin properly.

Overweight or obesity - overweight is having extra body weight from muscle, bone, fat, and/or water - obesity is having a high amount of extra body fat.

Lack of physical activity - lack of activity can worsen other risk factors for atherosclerosis.

Age - as the body ages the risk for atherosclerosis increases and genetic or lifestyle factors cause plaque to gradually build in the arteries - by middle-age or older, enough plaque has built up to cause signs or symptoms, in men, the risk increases after age 45, while in women, the risk increases after age 55.

Family history of early heart disease - the risk for atherosclerosis increases if a father or a brother was diagnosed with heart disease before 55 years of age, or if a mother or a sister was diagnosed with heart disease before 65 years of age but though age and a family history of early heart disease are risk factors, it does not mean that you will develop atherosclerosis if you have one or both. Making lifestyle changes and/or taking medicines to treat other risk factors can often lessen the genetic influences and prevent atherosclerosis from developing, even in older adults.

Emerging Risk Factors

Scientists continue to study other possible risk factors for atherosclerosis and have found that high levels of a protein called C-reactive protein (CRP) in the blood may raise the risk for atherosclerosis and heart attack - high levels of CRP are proof of inflammation in the body which is the body's response to injury or infection - damage to the arteries' inner walls appears to trigger inflammation and help plaque grow.

People with low CRP levels may get atherosclerosis at a slower rate than people with high CRP levels and research is currently under way to establish whether reducing inflammation and lowering CRP levels also can reduce the risk of atherosclerosis.

High levels of fats called triglycerides in the blood also may raise the risk of atherosclerosis, particularly in women.

Other Factors That Affect Atherosclerosis

Other risk factors also may raise your risk for developing atherosclerosis include:

Sleep apnoea - a disorder in which the breathing stops or gets very shallow while a person is sleeping - untreated sleep apnoea can raise the chances of high blood pressure, diabetes, and even a heart attack or stroke.

Stress - research shows that the most commonly reported "trigger" for a heart attack is an emotionally upsetting event-particularly one involving anger.

Alcohol - heavy drinking can damage the heart muscle and worsen other risk factors for atherosclerosis - men should have no more than two drinks containing alcohol a day, while women should have no more than one drink containing alcohol a day.

Modifiable

- Having **diabetes** or Impaired glucose tolerance (IGT) +
- **Dyslipoproteinemia** (unhealthy patterns of serum proteins carrying fats & cholesterol): +
 - High serum concentration of low-density lipoprotein (LDL, "bad if elevated concentrations and small"), and / or very low density lipoprotein (VLDL) particles, i.e., "lipoprotein subclass analysis"

- Low serum concentration of functioning high density lipoprotein (HDL "protective if large and high enough" particles), i.e., "lipoprotein subclass analysis"
- An LDL:HDL ratio greater than 3:1
- **Tobacco smoking**, increases risk by 200% after several pack years
- Having **high blood pressure** +, on its own increasing risk by 60%
- Elevated serum **C-reactive protein concentrations**

Nonmodifiable

- Advanced age
- Male sex
- Having close relatives who have had some complication of atherosclerosis (eg. coronary heart disease or stroke)
- Genetic abnormalities, e.g. familial hypercholesterolemia

Lesser or uncertain

The following factors are of relatively lesser importance, are uncertain or nonquantitated:

- Being obese (in particular central obesity, also referred to as *abdominal* or *male-type* obesity) +
- A sedentary lifestyle
- Postmenopausal estrogen deficiency
- High carbohydrate intake
- Intake of trans fat
- Elevated serum levels of triglycerides +
- Elevated serum levels of homocysteine
- Elevated serum levels of uric acid (also responsible for gout)
- Elevated serum fibrinogen concentrations
- Elevated serum lipoprotein(a) concentrations
- Chronic systemic inflammation as reflected by upper normal WBC concentrations, elevated hs-CRP and many other blood chemistry markers, most only research level at present, not clinically done.
- Stress or symptoms of clinical depression
- Hyperthyroidism (an over-active thyroid)
- Elevated serum insulin levels +
- Short sleep duration
- Chlamydia pneumoniae infection

Dietary risk factors

The relation between dietary fat and atherosclerosis is a contentious field. The USDA, in its food pyramid, promotes a low-fat diet, based largely on its view that fat in the diet is atherogenic. The American Heart Association, the American Diabetes Association and the National Cholesterol Education Program make similar recommendations. In contrast, Prof Walter Willett (Harvard School of Public Health, PI of the second Nurses' Health Study) recommends much higher levels, especially of monounsaturated and polyunsaturated fat. Writing in *Science*, Gary Taubes detailed that

political considerations played into the recommendations of government bodies. These differing views reach a consensus, though, against consumption of trans fats.

The role of dietary oxidized fats / lipid peroxidation (rancid fats) in humans is not clear. Laboratory animals fed rancid fats develop atherosclerosis. Rats fed DHA-containing oils experienced marked disruptions to their antioxidant systems, as well as accumulated significant amounts of peroxide in their blood, livers and kidneys.^[16] In another study, rabbits fed atherogenic diets containing various oils were found to undergo the greatest amount of oxidative susceptibility of LDL via polyunsaturated oils. In a study involving rabbits fed heated soybean oil, "grossly induced atherosclerosis and marked liver damage were histologically and clinically demonstrated".

Rancid fats and oils taste very bad even in small amounts; people avoid eating them. It is very difficult to measure or estimate the actual human consumption of these substances.^[20] In addition, the majority of oils consumed in the United States are refined, bleached, deodorized and degummed by manufacturers. The resultant oils are colorless, odorless, tasteless and have a longer shelf life than their unrefined counterparts. This extensive processing serves to make peroxidated, rancid oils much more elusive to detection via the various human senses than the unprocessed alternatives.

PATHOPHYSIOLOGY OF ATHEROSCLEROSIS

ENDOTHELIAL DYSFUNCTION

CAUSE:

- Elevated & modified LDL
- Free radical caused by cigarette smoking
- Elevated plasma homocysteine
- Hypertension
- Diabetes mellitus
- Genetic alterations
- Infections by Herpes virus Chlamydia Pneumonia

Atherosclerosis is a chronic, diffuse disease of large arteries of unknown direct cause but clearly associated with diet and tobacco. In "Westernized" societies the disease begins in childhood and progresses inexorably unless lifestyle is changed. One prevalent hypothesis is that it is caused by damage to the endothelium, the single-cell thick layer lining all blood vessels and the largest organ in the body. Until about 20 years ago the endothelium was thought to be a passive organ whose only job was to prevent clotting of blood on the inside of blood vessels. But since the Nobel Prize winning research of Furchgott, Ignarro and Murad we know that endothelium is a very active organ whose normal function is to keep the arterial wall healthy but which is also very sensitive to substances in the blood and the flow of blood itself. Healthy endothelium releases nitric oxide, a gas that keeps the arterial wall healthy but that is rapidly inactivated by blood. A number of factors can cause endothelial dysfunction, the most important being, diet, tobacco, and inactivity. By as yet unknown mechanisms, possibly related to the production of modified LDL (low density lipoprotein) in the arterial wall, endothelial

dysfunction leads to the build up of atherosclerotic plaque. Most importantly, endothelial dysfunction and plaque are reversible if those damaging factors are removed.

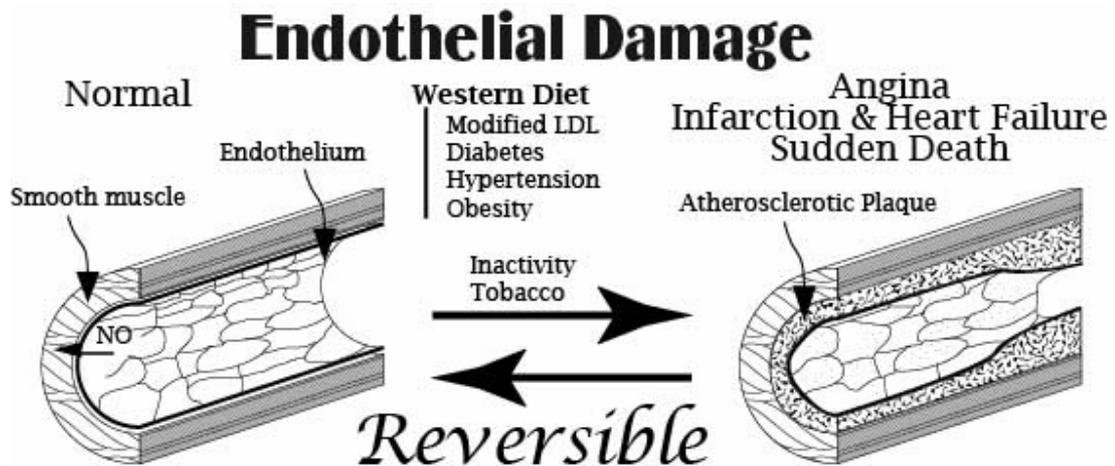


Figure 1 Endothelial Damage

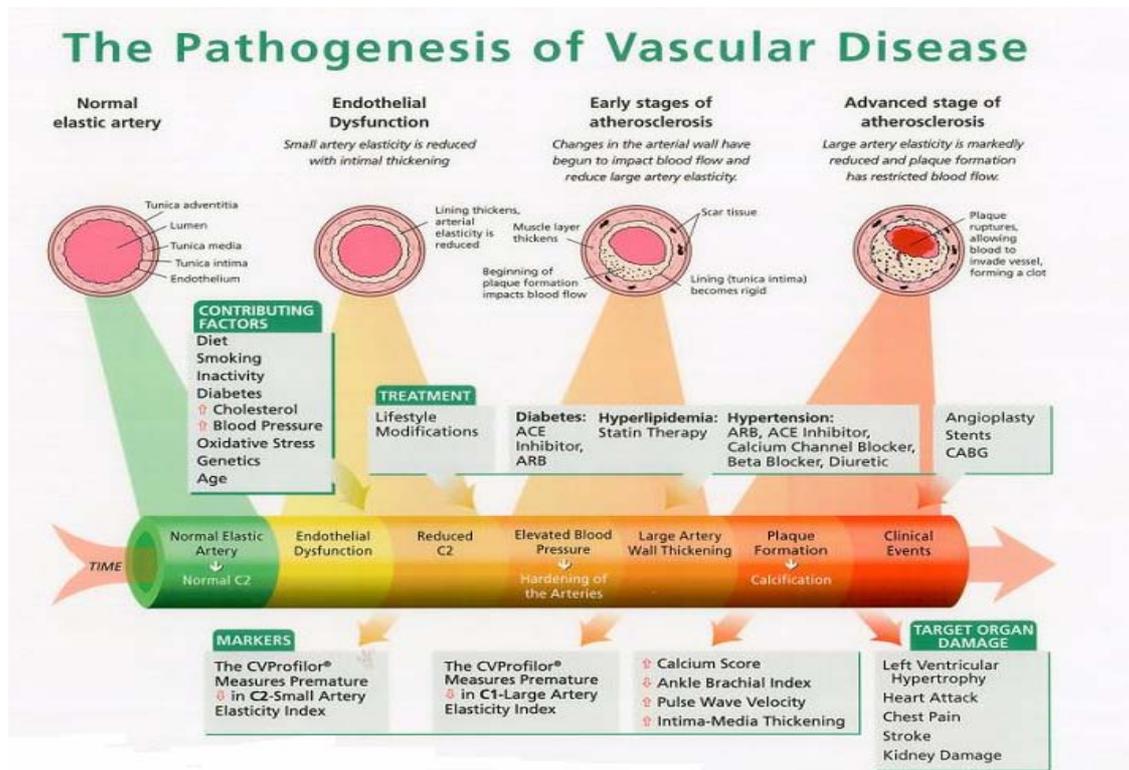


Figure 2 The Pathogenesis of Vascular Disease

The response-to-injury hypothesis of atherosclerosis. In response to mechanical injury or exposure to atherogenic stimuli, such as oxidized LDL, diabetes mellitus, severe

hyperhomocystinemia, and cigarette smoking, endothelial cells express adhesion molecules and elaborate growth factors that lead to recruitment of leukocytes in an inflammatory response to injury. Leukocytes adhere and migrate into the vessel wall, localize subendothelially, and develop into lipid-laden macrophages (foam cells). Foam cells, in turn, release growth factors and cytokines that promote recruitment of smooth muscle cells and stimulate neointimal proliferation, continue to accumulate lipid, and support endothelial cell dysfunction. Collectively, these events promote the development of a lipid-rich atheromatous lesion. Subsequent denudation of the endothelium exposes circulating platelets and coagulants to the underlying matrix, thereby initiating thrombosis, and triggering a cascade of events leading to a fibroproliferative lesion and luminal narrowing.

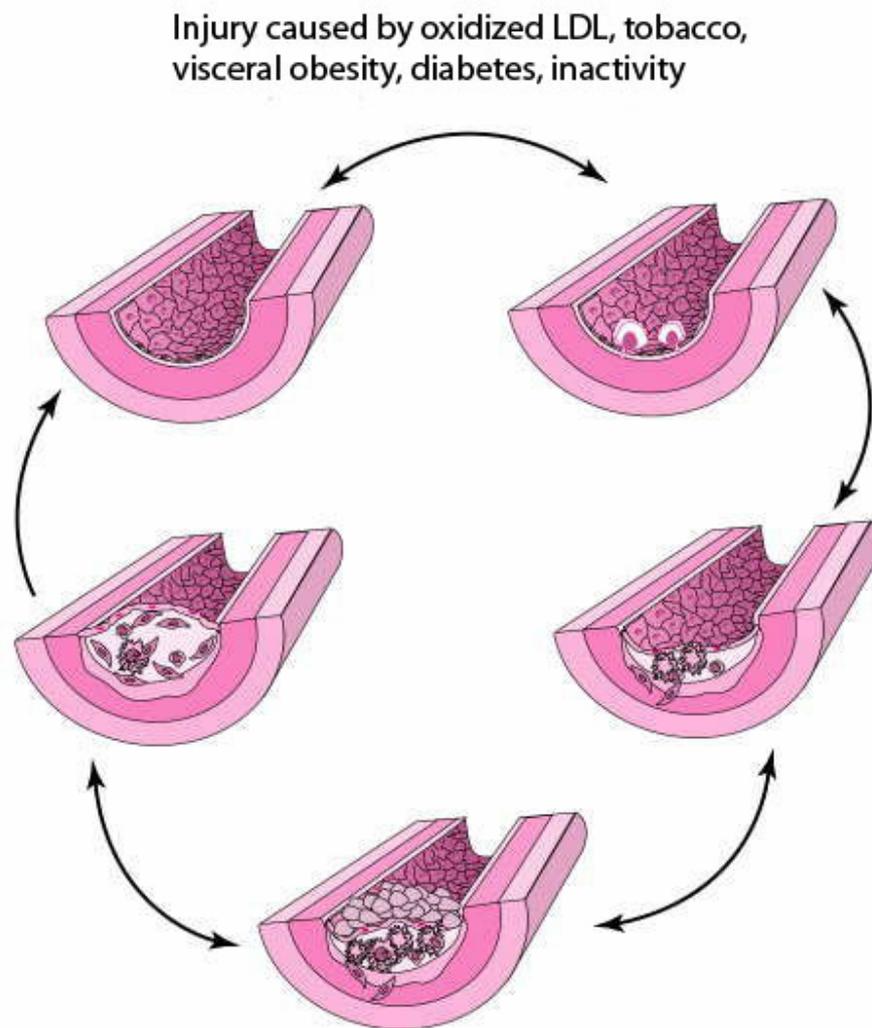


Figure 3. Injury hypothesis of atherosclerosis

FATTY-STREAK FORMATION

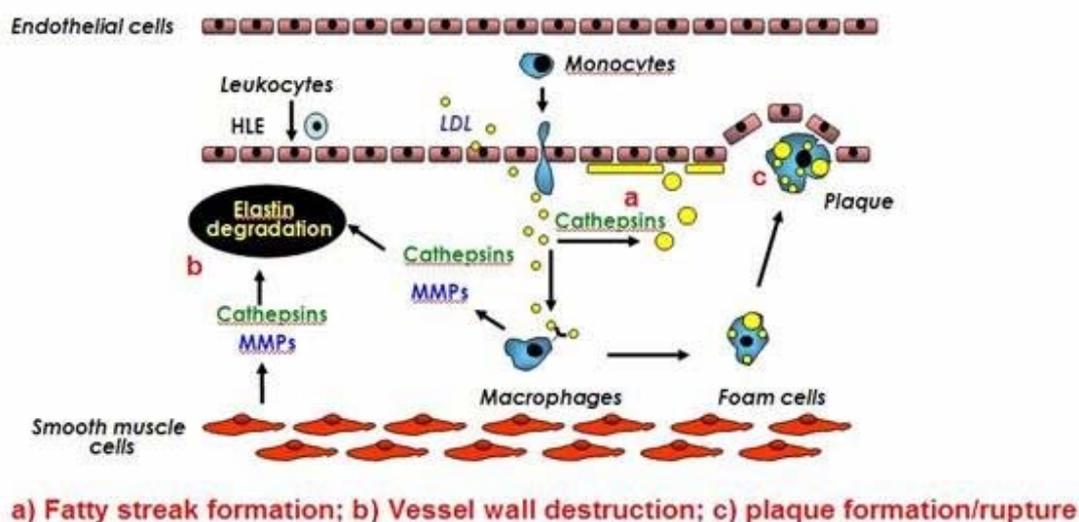


Figure 4. Fatty streak formation

Atherosclerosis (the hardening of arterial blood vessels) is the leading cause of death in North America. Fatty streak formation, blood vessel wall erosion, and plaque formation are hallmarks of the disease. Cathepsins have been implicated in all three steps of atherosclerosis. The scheme below summarizes these pathologies.

To investigate the role of cathepsins in atherosclerosis we use a disease mouse model (ApoE-deficient mice) and various cathepsin-deficient mice strains. Mice are studied for their expression levels of cathepsins, their elastin and collagen turnover in blood vessels, fatty streak and plaque formation as well as their rupture and on their reaction to cathepsin inhibitors.

ATHEROGENESIS

Atherogenesis is the developmental process of atheromatous plaques. It is characterized by a remodeling of arteries involving the concomitant accumulation of fatty substances called plaques. One recent theory suggests that, for unknown reasons, leukocytes, such as monocytes or basophils, begin to attack the endothelium of the artery lumen in cardiac muscle. The ensuing inflammation leads to formation of atheromatous plaques in the arterial tunica intima, a region of the vessel wall located between the endothelium and the tunica media. The bulk of these lesions is made of excess fat, collagen, and elastin. At first, as the plaques grow, only wall thickening occurs without any narrowing, stenosis of the artery opening, called the lumen; stenosis is a late event, which may never occur and is often the result of repeated plaque rupture and healing responses, not just the atherosclerosis process by itself.

Atherosclerosis involves multiple processes including endothelial dysfunction, inflammation, vascular proliferation and matrix alteration. Vascular proliferation contributes to the pathobiology of atherosclerosis and is linked to other cellular processes such as inflammation, apoptosis and matrix alterations. The contribution of vascular

proliferation to the pathophysiology of in-stent restenosis, transplant vasculopathy and vein bypass graft failure is particularly important. Thus, an emerging strategy for the treatment of those conditions is to inhibit cellular proliferation by targeting cell cycle regulation. Here we will review the current understanding of the pathophysiological mechanisms and the status of molecular and gene therapeutic approaches in vascular proliferative diseases. The understanding of the pathophysiology of atherosclerosis and related vascular diseases has changed over the last decade, providing new perspectives for preventive and therapeutic strategies.

Recent studies have emphasized the involvement of inflammation in mediating all stages of atherosclerosis. However, in addition to inflammation, a key process of atherosclerosis involves the proliferation of vascular smooth muscle cells (VSMCs) (Fig. 1). One precursor of lesion development in humans may be focal accumulation of VSMCs within the intima. The exact function of VSMCs in atherosclerosis is, however, still a subject of debate. In early atherosclerosis, VSMCs may contribute to the development of the atheroma through the production of pro-inflammatory mediators such as monocyte chemoattractant protein 1 and vascular cell adhesion molecule, and through the synthesis of matrix molecules required for the retention of lipoproteins. However, VSMCs may also be important in maintaining the stability of the plaque through the formation of a firm fibrous cap. Indeed, in lipid-laden lesions in which the fibrous cap is thin and weak, there is evidence of VSMC apoptosis, especially at the 'shoulder' region, associated with inflammation. In addition, the local inflammatory milieu can induce expression of collagenase and inhibit expression of proteolytic inhibitors, thus rendering the fibrous cap weak and susceptible to rupture. In advanced lesions, fibroblasts and VSMCs with extracellular calcification form a fibrocalcific plaque.

The origin of VSMCs in the atherosclerotic plaque is intriguing. Intimal thickening appears during normal development and aging Intimal VSMCs, including those in atherosclerotic lesions, are reportedly monoclonal in origin. This would indicate that the neointima arises from proliferation of resident pre-existing clonal VSMCs. Although examination of human atherosclerotic lesions has not yielded evidence of extensive replication, it may occur very early or at a low rate throughout the development of atherosclerosis or episodically at a high rate. Indeed, VSMCs have been identified in fatty streaks of young individuals. Experimental data also indicate that intimal VSMCs may originate from the media or the adventitia. Furthermore, embryonic endothelial cells are reportedly able to transdifferentiate into mesenchymal cells expressing smooth muscle cell actin. Animal studies have indicated that neointimal cells may also originate from subpopulations of bone marrow- and non-bone marrow-derived circulating cells. In models of hyperlipidemia-induced atherosclerosis, as well as in post-angioplasty restenosis and graft vasculopathy, bone marrow cells may give rise to a substantial percentage of the VSMCs that contribute to arterial remodeling. The contribution of these cells to human atherosclerosis has not been proven, although circulating smooth muscle progenitor cells have been identified in human peripheral blood.

In summary, the function of the intimal smooth muscle cell in the natural history of the atherosclerotic lesion seems to be to act as a nidus for development of the lesions, perhaps by accelerating lipid accumulation or macrophage chemotaxis. Proliferation is probably an early event, followed by a chronic process that provides an essential fibrous cap that prevents plaque rupture.

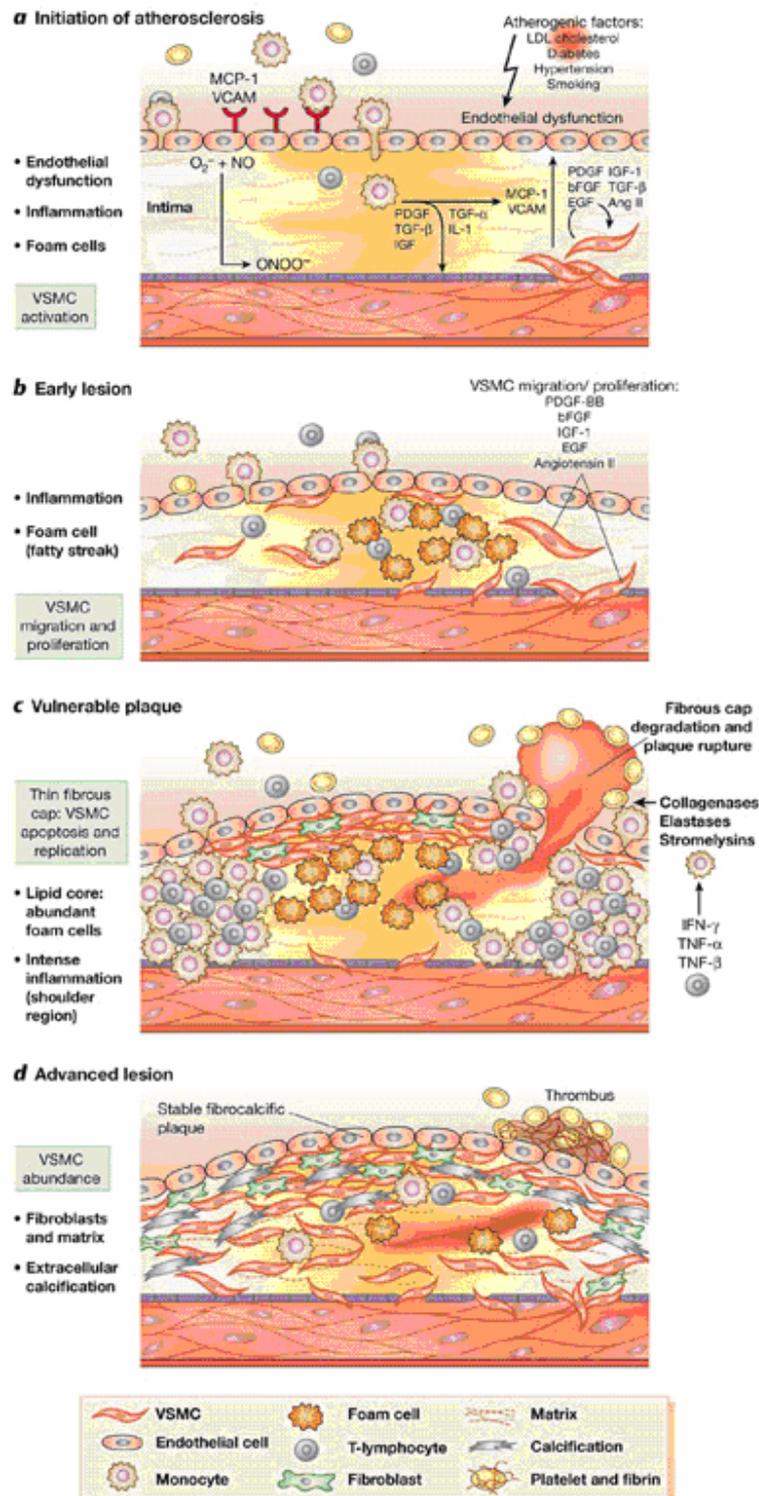


Figure 5. Function of VSMCs during different stages of atherosclerosis.

Cardiovascular risk factors alter the vascular endothelium (EC), which triggers a cascade of events, including the recruitment of leukocytes. Cytokines and growth factors are released by inflammatory cells and vascular cells, generating a highly mitogenic

milieu. VSMCs migrate, proliferate and synthesize extracellular matrix components on the luminal side of the vessel wall, forming the fibrous cap of the atherosclerotic lesion. Inflammatory mediators ultimately induce thinning of the fibrous cap by expression of proteases, rendering the plaque weak and susceptible to rupture and thrombus formation. In advanced disease, fibroblasts and VSMCs with extracellular calcification give rise to fibrocalcific lesions. LDL, low-density lipoprotein; MCP, monocyte chemoattractant protein; VCAM, vascular cell adhesion molecule; PDGF-BB, platelet-derived growth factor (BB, -chain homodimer); TNF, tumor necrosis factor; TGF, transforming growth factor; IL, interleukin 1; IGF, insulin-like growth factor; bFGF, basic fibroblast growth factor; Ang II, angiotensin II; EGF, epidermal growth factor; IFN, interferon.

Several vascular diseases involve VSMC proliferation as the primary pathophysiologic mechanism. These clinical conditions include in-stent restenosis, transplant vasculopathy and vein bypass graft failure. Ironically, these conditions develop as consequences of the procedures used to treat occlusive atherosclerotic diseases. Indeed, 30-40% of patients undergoing percutaneous balloon angioplasty will develop restenosis within the first 6 months.[With the recent deployment of stents, this incidence is now about 20%, still an unacceptably high rate. Vein graft failure ranges from 10 to 30% per year. These vascular proliferative diseases are initiated by mechanical, biochemical or immunological injury to the vessel wall. Vascular injury triggers a cascade of events that includes endothelial denudation or dysfunction, inflammation and VSMC activation and proliferation. Myriad growth factors and cytokines can be detected in human vascular lesions. These mediators may be released by dysfunctional endothelial cells, inflammatory cells, platelets and VSMCs, mediating chemoattraction, cell migration, proliferation, apoptosis and matrix modulation.

Understanding of the responses of growth factors and VSMC proliferation to vascular injury is derived mainly from studies involving animal models of arterial injury. Direct data are difficult to obtain from human disease. In the rat model, basic fibroblast growth factor, released from dying vascular cells, can initiate medial proliferation of VSMCs, whereas platelet-derived growth factor may induce subsequent migration of VSMCs toward the intima. Intimal proliferation and matrix accumulation occur under the influence of platelet-derived growth factor, transforming growth factor-, angiotensin II, epidermal growth factor and insulin-like growth factor 1. Furthermore, loss of growth-inhibitory factors, occurring as a result of decreased endothelial cell secretion of nitric oxide (NO), inactivation of NO by reactive oxygen species or altered heparan sulfate proteoglycan synthesis, may also contribute to the migration and proliferation of VSMCs and to the increased inflammatory response.

With the recognition of the essential involvement of VSMC proliferation in the conditions described above and the improved understanding of the molecular and cellular mechanisms of cellular proliferation, antiproliferative therapeutic modalities have become a focus of research and development. In the following sections, we review the mechanisms of vascular proliferation and cell cycle regulation and examine the therapeutic potential of targeting these processes.

CELLULAR

The first step of atherogenesis is the development of so called "fatty streak"s, which are small sub-endothelial deposits of monocyte-derived macrophages. The primary documented driver of this process is oxidized Lipoprotein particles within the wall, beneath the endothelial cells, though upper normal or elevated concentrations of blood glucose also plays a major role and not all factors are fully understood. Fatty streaks may appear and disappear.

Low Density Lipoprotein particles in blood plasma, when they invade the endothelium and become oxidized creates a risk for cardiovascular disease. A complex set of biochemical reactions regulates the oxidation of LDL, chiefly stimulated by presence of enzymes, e.g. Lp-LpA2 and free radicals in the endothelium or blood vessel lining. Micrograph of an artery that supplies the heart with significant **atherosclerosis** and marked luminal narrowing. Masson's trichrome.

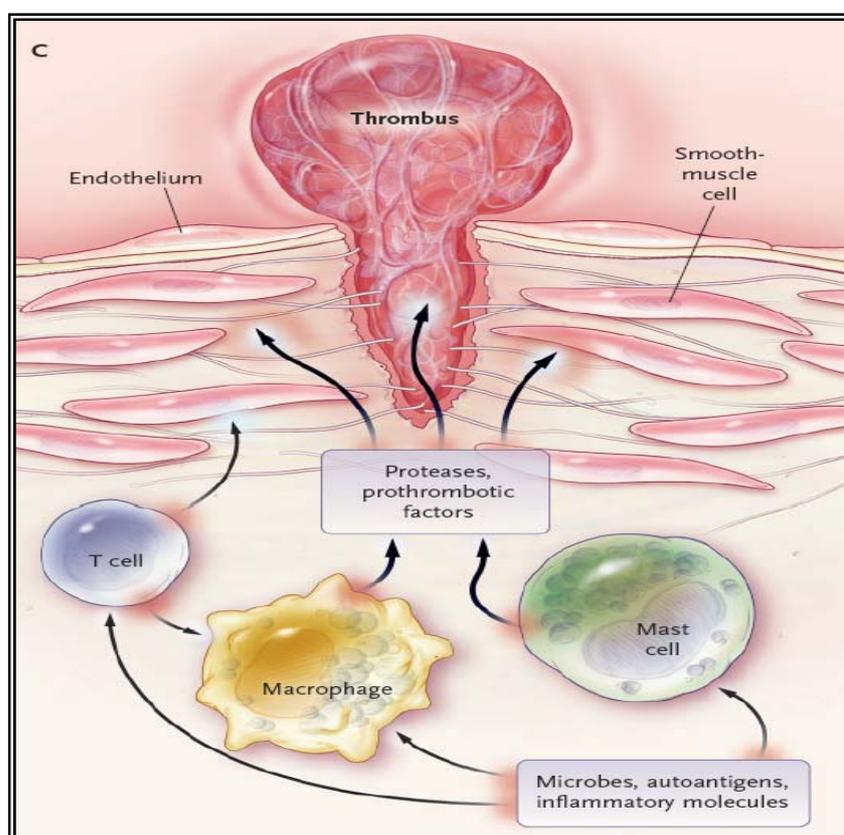


Figure 6. Cellular players in atherogenesis and plaque rupture

The initial damage to the blood vessel wall results in a "call for help," an inflammatory response. Monocytes (a type of white blood cell) enter the artery wall from the bloodstream, with platelets adhering to the area of insult. This may be promoted by redox signaling induction of factors such as VCAM-1, which recruit circulating monocytes. The monocytes differentiate macrophages, which ingest oxidized LDL, slowly turning into large "foam cells" – so-described because of their changed appearance resulting from the numerous internal cytoplasmic vesicles and resulting high

lipid content. Under the microscope, the lesion now appears as a fatty streak. Foam cells eventually die, and further propagate the inflammatory process. There is also smooth muscle proliferation and migration from tunica media to intima responding to cytokines secreted by damaged endothelial cells. This would cause the formation of a fibrous capsule covering the fatty streak.

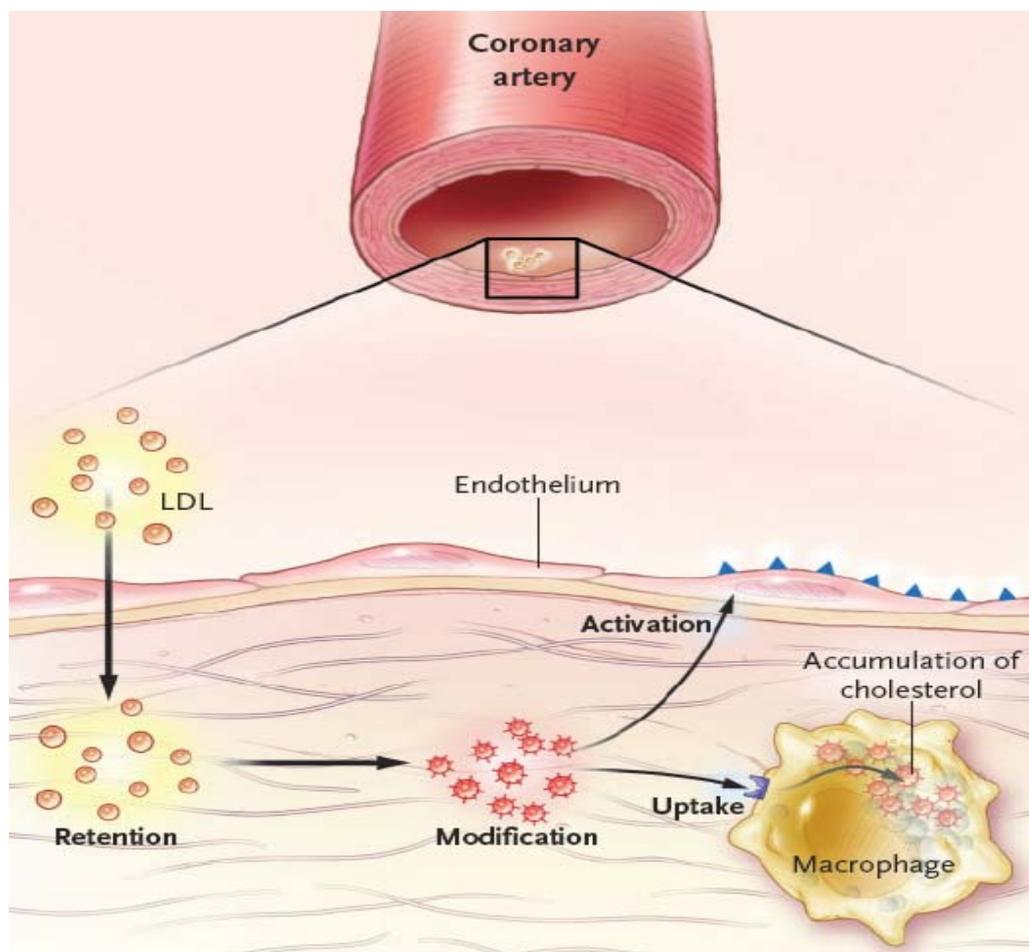


Figure 7. The early lesion: modified lipoproteins and foam cell formation

Role of macrophages

- MCSF induces monocytes entering lesion to differentiate into macrophages
- Macrophage differentiation associated with TLRs and scavenger receptors
- MCSF/apoE KO shows reduced atherosclerosis
- Scavenger receptors bind bacterial endotoxins, apoptotic cells and oxLDL
- SR/apoE KOs show reduced atherosclerosis

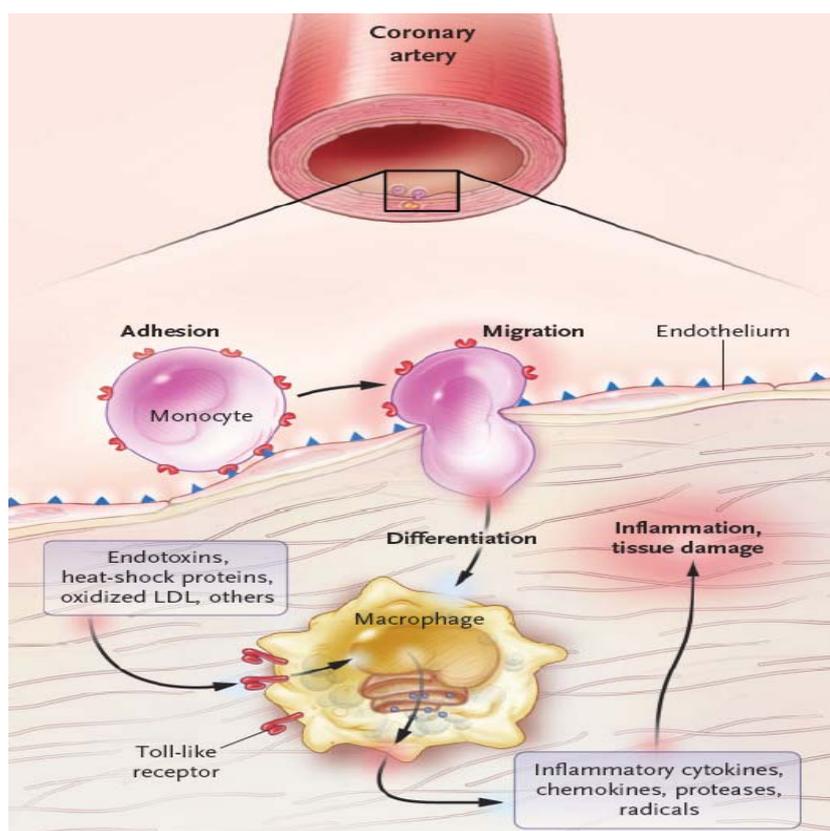


Figure 8. The macrophage as an inflammatory mediator

TLR receptors and atherosclerosis

- 10 family members
- Recognize pathogen associated molecular patterns (e.g. LPS, dsRNA) as well as oxLDL, HSP60 etc.
- Initiate signaling cascades leading to production of inflammatory cytokines, proteases, reactive oxygen species
- In addition to macrophages, expressed by dendritic cells, mast cells, endothelial cells

T cells and atherosclerosis

- Immune cells patrol tissues in search of antigen
- T cell infiltrate is common feature of atherosclerotic lesions
- Predominantly CD4⁺ cells, recognize antigen/MHC II
- CD4⁺ T cells reactive to oxLDL, HSP60, bacterial products detected in human lesions
- NK cells present in early lesions, recognize lipid antigens
- NK activation increases athero in apoE KO mice

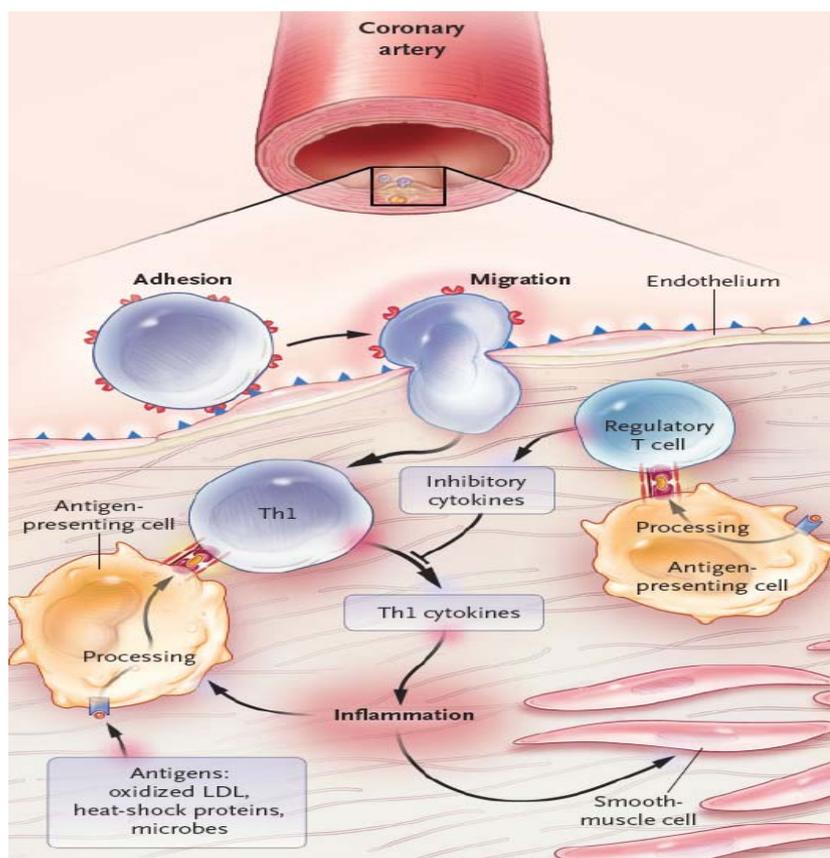


Figure 9 Macrophage-T cell interactions in atherosclerosis

T cell responses

Th1 response activates macrophages and functions in the defense against intracellular pathogens

- Th2 response elicits allergic inflammation
- Atherosclerotic lesions contain cytokines that promote Th1 responses
- Activated Th1 effector cells in lesions produce macrophage activating cytokine IFN γ
- IFN γ improves efficiency of antigen presentation and augments synthesis of TNF α and IL-1
- IFN γ , TNF α and IL-1 in turn stimulate production of many other inflammatory mediators
- apoE mice lacking IFN γ or downstream mediators such as IL-18 or T-bet show reduced atherosclerosis

Anti-inflammatory factors and atherosclerosis

- Anti-inflammatory factors such as TGF β and IL-10 are protective
- IL-10 KO increases atherosclerosis in mice and exacerbates thrombosis
- Abrogation of TGF signaling in T cells leads to large unstable atherosclerotic lesions

Immune cells and plaque rupture

- Preferentially occurs where fibrous cap is thin
- Active immune cells are abundant at site of rupture
- Immune cells produce inflammatory molecules and proteolytic enzymes that weaken cap, activate cells in the core and transform stable plaque into vulnerable, leading to plaque rupture
- MMPs likely to play important roles

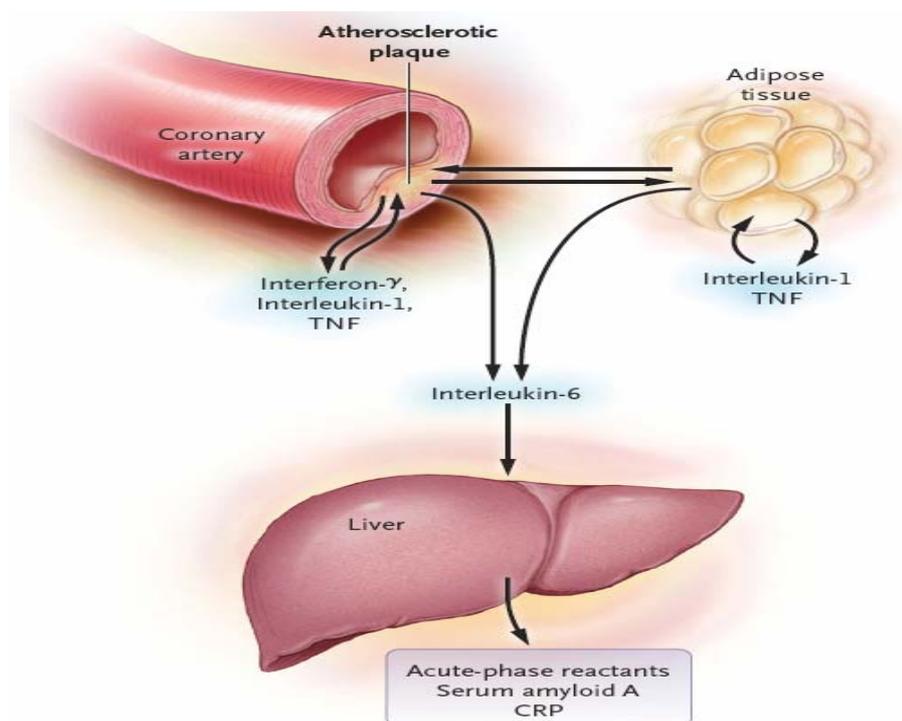


Figure 10 Systemic inflammatory markers in atherosclerosis

CALCIFICATION AND LIPIDS

Intracellular microcalcifications form within vascular smooth muscle cells of the surrounding muscular layer, specifically in the muscle cells adjacent to the atheromas. In time, as cells die, this leads to extracellular calcium deposits between the muscular wall and outer portion of the atheromatous plaques. A similar form of an intramural calcification, presenting the picture of an early phase of arteriosclerosis, appears to be induced by a number of drugs that have an antiproliferative mechanism of action.

Cholesterol is delivered into the vessel wall by cholesterol-containing low-density lipoprotein (LDL) particles. To attract and stimulate macrophages, the cholesterol must be released from the LDL particles and oxidized, a key step in the ongoing inflammatory process. The process is worsened if there is insufficient high-density lipoprotein (HDL), the lipoprotein particle that removes cholesterol from tissues and carries it back to the liver. The foam cells and platelets encourage the migration and proliferation of smooth muscle cells, which in turn ingest lipids, become replaced by collagen and transform into

foam cells themselves. A protective fibrous cap normally forms between the fatty deposits and the artery lining.

These capped fatty deposits (now called 'atheromas') produce enzymes that cause the artery to enlarge over time. As long as the artery enlarges sufficiently to compensate for the extra thickness of the atheroma, then no narrowing ("stenosis") of the opening ("lumen") occurs. The artery becomes expanded with an egg-shaped cross-section, still with a circular opening. If the enlargement is beyond proportion to the atheroma thickness, then an aneurysm is created.^[5]

VISIBLE FEATURES

Severe atherosclerosis of the aorta. Autopsy specimen.

Although arteries are not typically studied microscopically, two plaque types can be distinguished:

1. The **fibro-lipid (fibro-fatty) plaque** is characterized by an accumulation of lipid-laden cells underneath the intima of the arteries, typically without narrowing the lumen due to compensatory expansion of the bounding muscular layer of the artery wall. Beneath the endothelium there is a "fibrous cap" covering the atheromatous "core" of the plaque. The core consists of lipid-laden cells (macrophages and smooth muscle cells) with elevated tissue cholesterol and cholesterol ester content, fibrin, proteoglycans, collagen, elastin, and cellular debris. In advanced plaques, the central core of the plaque usually contains extracellular cholesterol deposits (released from dead cells), which form areas of cholesterol crystals with empty, needle-like clefts. At the periphery of the plaque are younger "foamy" cells and capillaries. These plaques usually produce the most damage to the individual when they rupture.
2. The **fibrous plaque** is also localized under the intima, within the wall of the artery resulting in thickening and expansion of the wall and, sometimes, spotty localized narrowing of the lumen with some atrophy of the muscular layer. The fibrous plaque contains collagen fibers (eosinophilic), precipitates of calcium (hematoxylinophilic) and, rarely, lipid-laden cells.

In effect, the muscular portion of the artery wall forms small aneurysms just large enough to hold the atheroma that are present. The muscular portion of artery walls usually remain strong, even after they have remodeled to compensate for the atheromatous plaques.

However, atheromas within the vessel wall are soft and fragile with little elasticity. Arteries constantly expand and contract with each heartbeat, i.e., the pulse. In addition, the calcification deposits between the outer portion of the atheroma and the muscular wall, as they progress, lead to a loss of elasticity and stiffening of the artery as a whole.

The calcification deposits, after they have become sufficiently advanced, are partially visible on coronary artery computed tomography or electron beam tomography (EBT) as rings of increased radiographic density, forming halos around the outer edges of the atheromatous plaques, within the artery wall. On CT, >130 units on the Hounsfield scale (some argue for 90 units) has been the radiographic density usually accepted as clearly representing tissue calcification within arteries. These deposits demonstrate

unequivocal evidence of the disease, relatively advanced, even though the lumen of the artery is often still normal by angiographic or intravascular ultrasound.

RUPTURE AND STENOSIS

Although the disease process tends to be slowly progressive over decades, it usually remains asymptomatic until an atheroma ulcerates which leads to immediate blood clotting at the site of atheroma ulcer. This triggers a cascade of events that leads to clot enlargement which may quickly obstruct the lumen (opening) of the artery itself. A complete blockage leads to ischemia of the myocardial (heart) muscle and damage. This process is the myocardial infarction or "heart attack."

If the heart attack is not fatal, fibrous organization of the clot within the lumen ensues, covering the rupture but also producing stenosis or closure of the lumen, or over time and after repeated ruptures, resulting in a persistent, usually localized stenosis or blockage of the artery lumen. Stenoses can be slowly progressive, whereas plaque ulceration is a sudden event that occurs specifically in atheromas with thinner/weaker fibrous caps that have become "unstable."

Repeated plaque ruptures, ones not resulting in total lumen closure, combined with the clot patch over the rupture and healing response to stabilize the clot, is the process that produces most stenoses over time. The stenotic areas tend to become more stable, despite increased flow velocities at these narrowings. Most major blood-flow-stopping events occur at large plaques, which, prior to their rupture, produced very little if any stenosis.

From clinical trials, 20% is the average stenosis at plaques that subsequently rupture with resulting complete artery closure. Most severe clinical events do not occur at plaques that produce high-grade stenosis. From clinical trials, only 14% of heart attacks occur from artery closure at plaques producing a 75% or greater stenosis prior to the vessel closing.

If the fibrous cap separating a soft atheroma from the bloodstream within the artery ruptures, tissue fragments are exposed and released, and blood enters the atheroma within the wall and sometimes results in a sudden expansion of the atheroma size. Tissue fragments are very clot-promoting, containing collagen and tissue factor; they activate platelets and activate the system of coagulation. The result is the formation of a thrombus (blood clot) overlying the atheroma, which obstructs blood flow acutely. With the obstruction of blood flow, downstream tissues are starved of oxygen and nutrients. If this is the myocardium (heart muscle), angina (cardiac chest pain) or myocardial infarction (heart attack) develops.

DIAGNOSIS OF PLAQUE-RELATED DISEASE

Areas of severe narrowing, stenosis, detectable by angiography, and to a lesser extent "stress testing" have long been the focus of human diagnostic techniques for cardiovascular disease, in general. However, these methods focus on detecting only severe narrowing, not the underlying atherosclerosis disease. As demonstrated by human clinical studies, most severe events occur in locations with heavy plaque, yet little or no lumen narrowing present before debilitating events suddenly occur. Plaque rupture can lead to artery lumen occlusion within seconds to minutes, and potential permanent debility and sometimes sudden death.

Plaques that have ruptured are called complicated plaques. The lipid matrix breaks through the thinning collagen gap and when the lipids come in contact with the blood, clotting occurs. After rupture the platelet adhesion causes the clotting cascade to contact with the lipid pool causing a thrombus to form. This thrombus will eventually grow and travel throughout the body. The thrombus will travel through different arteries and veins and eventually become lodged in an area that narrows. Once the area is blocked, blood and oxygen will not be able to supply the vessels and will cause death of cells and lead to necrosis and poisoning. Serious complicated plaques can cause death of organ tissues, causing serious complications to that organ system.

Greater than 75% lumen stenosis used to be considered by cardiologists as the hallmark of clinically significant disease because it is typically only at this severity of narrowing of the larger heart arteries that recurring episodes of angina and detectable abnormalities by stress testing methods are seen. However, clinical trials have shown that only about 14% of clinically-debilitating events occur at locations with this, or greater severity of narrowing. The majority of events occur due to atheroma plaque rupture at areas without narrowing sufficient enough to produce any angina or stress test abnormalities. Thus, since the later-1990s, greater attention is being focused on the "vulnerable plaque."^[7]

Though any artery in the body can be involved, usually only severe narrowing or obstruction of some arteries, those that supply more critically-important organs are recognized. Obstruction of arteries supplying the heart muscle result in a heart attack. Obstruction of arteries supplying the brain result in a stroke. These events are life-changing, and often result in irreversible loss of function because lost heart muscle and brain cells do not grow back to any significant extent, typically less than 2%.

Over the last couple of decades, methods other than angiography and stress-testing have been increasingly developed as ways to better detect atherosclerotic disease before it becomes symptomatic. These have included both (a) anatomic detection methods and (b) physiologic measurement methods.

Examples of anatomic methods include:

- (1) coronary calcium scoring by CT
- (2) carotid IMT (intimal media thickness) measurement by ultrasound
- (3) IVUS.

Examples of physiologic methods include:

- (1) lipoprotein subclass analysis,
- (2) HbA1c,
- (3) hs-CRP,
- (4) homocysteine.

The example of the metabolic syndrome combines both anatomic (abdominal girth) and physiologic (blood pressure, elevated blood glucose) methods. Advantages of these two approaches: The anatomic methods directly measure some aspect of the actual atherosclerotic disease process itself, thus offer potential for earlier detection, including before symptoms start, disease staging and tracking of disease progression. The physiologic methods are often less expensive and safer and changing them for the better may slow disease progression, in some cases with marked improvement.

Disadvantages of these two approaches: The anatomic methods are generally more expensive and several are invasive, such as IVUS. The physiologic methods do not quantify the current state of the disease or directly track progression. For both, clinicians and third party payers have been slow to accept the usefulness of these newer approaches.



- **Blood tests:** Evaluate kidney and thyroid function to check Cholesterol levels and the presence of anemia. Level of c-reactive Protein {>3.0mg/l}
- **Chest X-ray:** shows the size of heart and whether there is fluid build up around the heart and lungs.
- **Echocardiogram:** sound waves are used to image of heart chamber & valves.
- **Electron beam computerized tomography (EBCT):** This test, also called an ultrafast CT scan, can detect calcium within plaques that narrow coronary arteries. If a substantial amount of calcium is discovered, coronary artery disease is likely.
- Get regular medical checkups.
- Control your blood pressure.
- Check your cholesterol.
- Don't smoke.
- Exercise regularly.
- Maintain a healthy weight.
- Eat a heart-healthy diet.
- Manage stress.

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