

CORONARY ATHEROSCLEROSIS
PATHOPHYSIOLOGY AND THERAPEUTIC TARGETS

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INTRODUCTION

This monograph provides a novel systematic approach for the management of coronary patients. The approach is based upon our current modern understanding of the pathophysiology of coronary artery disease. There is an outline of the molecular, cellular and structural basis of the atherosclerotic process and its sequela, starting from the atherosclerotic plaque and ending in the myocardium.

Clarification of the mechanisms underlying the development of coronary atherosclerosis, its progression and finally the whole spectrum of acute coronary syndromes became possible only in the last two decades. Understanding the pathways leading to the development of ACS helped in the introduction of new therapies and the development of a more rational approach for the management of coronary patients.

Realizing that coronary atherosclerosis is a diffuse inflammatory disorder which runs unpredictable course and the fact that major acute coronary events are the results of rupture or erosion of unstable atherosclerotic plaques introduced new concepts in treatment of coronary disease. An important one is the concept of atherosclerotic plaque stabilization or passivation. Changing unstable, vulnerable plaque, liable to rupture, thrombosis and precipitation of on acute coronary event into a stable, less active and more benign plaque is a novel therapeutic policy.

The discovery of the presence of systemic proinflammatory and procoagulant states in patients with ACS and the persistence of this procoagulant state months after the acute event provided a rationale for prolonged anticoagulant, antiplatelet and anti-thrombotic therapy.

Our concept of atherosclerosis has changed and instead of being considered a degenerative, inevitable and irreversible process, now we know that it is inflammatory, preventable and reversible. It is possible through drug therapy to induce regression of atherosclerotic plaque. Also, it is possible through pharmacologic therapy to attenuate and even correct the adverse myocardial changes and damage following coronary occlusion and myocyte necrosis.

In this monograph four therapeutic targets were identified for drug therapy:

1. Coronary atherosclerotic plaque.
2. Coronary thrombus.
3. Systemic internal milieu.
4. Myocardium.

The first target is the ASO plaque which is responsible for the majority of clinical manifestations of CAD.

Pharmacologic therapy has two goals:

1. Regress or prevent further progression of ASO plaque.
2. Stabilize or passivate the ASO plaque i.e. make it less liable to rupture and, thrombosis. This will prevent acute coronary events.

If therapy fails to stabilize the vulnerable plaque and if the plaque is disrupted or eroded, there is subsequent formation of intracoronary thrombus which is the second target of therapy. The objectives of therapy are to dissolve the thrombus if it occludes completely the coronary artery or prevent its progression if there is partial occlusion and prevent its reoccurrence.

The third target is the systemic milieu in patients with CAD particularly in ACS. In this situation there are generalized proinflammatory and prothrombotic states. The proinflammatory state will invite progression, activation, instability and rupture of the ASO plaque.

The prothrombotic state will help development and will favour the persistence and progression of the intracoronary thrombus. The goals here are to attenuate the inflammatory activity and the prothrombotic state.

The myocardium is the last therapeutic target. Failure to stabilize the plaque and failure to prevent coronary thrombus formation or maintain adequate coronary perfusion will result in myocardial ischemia and myocyte loss. If necrosis is extensive cardiac remodeling and deterioration of LV function develops. Therapy at this stage aims at minimizing the effects of ischemia, protecting cardiac myocytes, preventing or attenuating cardiac remodeling.

Pharmacologic therapy, when achieving, the previous therapeutic goals will help relieving symptoms and preventing complications of coronary atherosclerosis namely MI, deterioration in LV function and sudden death. Modern pharmacologic therapy can improve quality of life and prolong survival.

I hope through this monograph to provide some insight, and help understanding a difficult, challenging and rapidly advancing field.

M. Mohsen Ibrahim, MD
Cairo- March 2005

CHAPTER 1

ATHEROSCLEROTIC PLAQUE

- Structure
- Development
- Cellular Players
- LDL-Cholesterol
- Modulators
- Mediators

- Majority of clinical manifestations of CAD are secondary to the presence and to the complications of ASO plaques.
- ASO starts very early in life and it is already diffuse by midlife.
- ASO plaques are silent and asymptomatic in the majority of patients. They produce symptoms when:
 1. Enlarge and encroach on the coronary lumen compromising coronary flow. Chronic coronary stenosis will result in ischemia and angina of effort when there is an imbalance between coronary supply and myocardial oxygen requirements. This type of ischemia is sometimes called ischemia of demand.
 2. Rupture, exposing their strongly thrombogenic lipid core to the blood stream resulting in formation of coronary thrombi (Fig 1). Coronary thrombi occlude partially or totally the coronary lumen for a variable period. The result is an ACS. Ischemia in this situation is sometimes defined as ischemia of supply.
- ASO plaques are extremely heterogeneous, they vary in morphology, structure, composition and distribution. In the same patient, there are plaques of different types, even in the same coronary artery, plaques are variable.
- Inflammation plays an important central role in the initiation, progression, destabilization and rupture of ASO plaques.

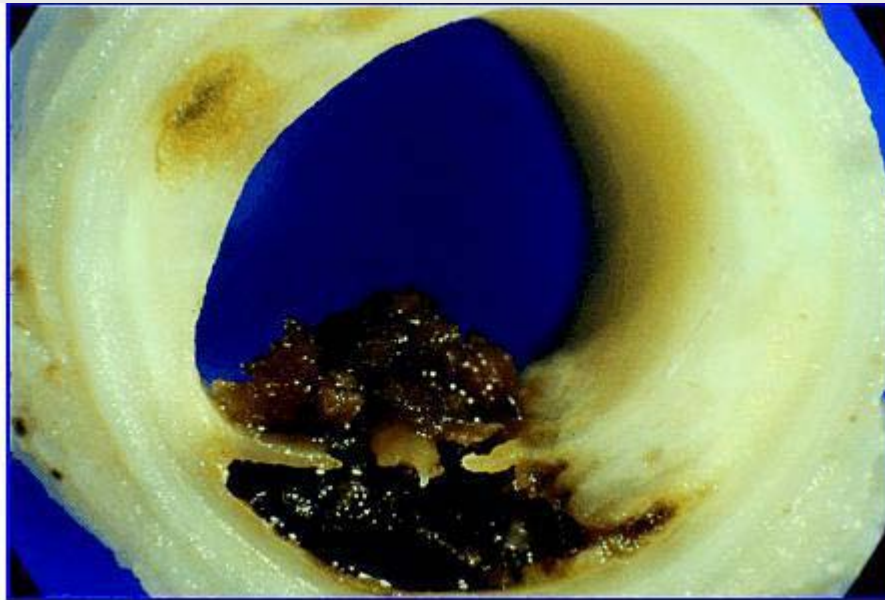


Figure (1): A cross section of autopsy of coronary artery showing a coronary thrombus on top of ruptured atherosclerotic plaque.

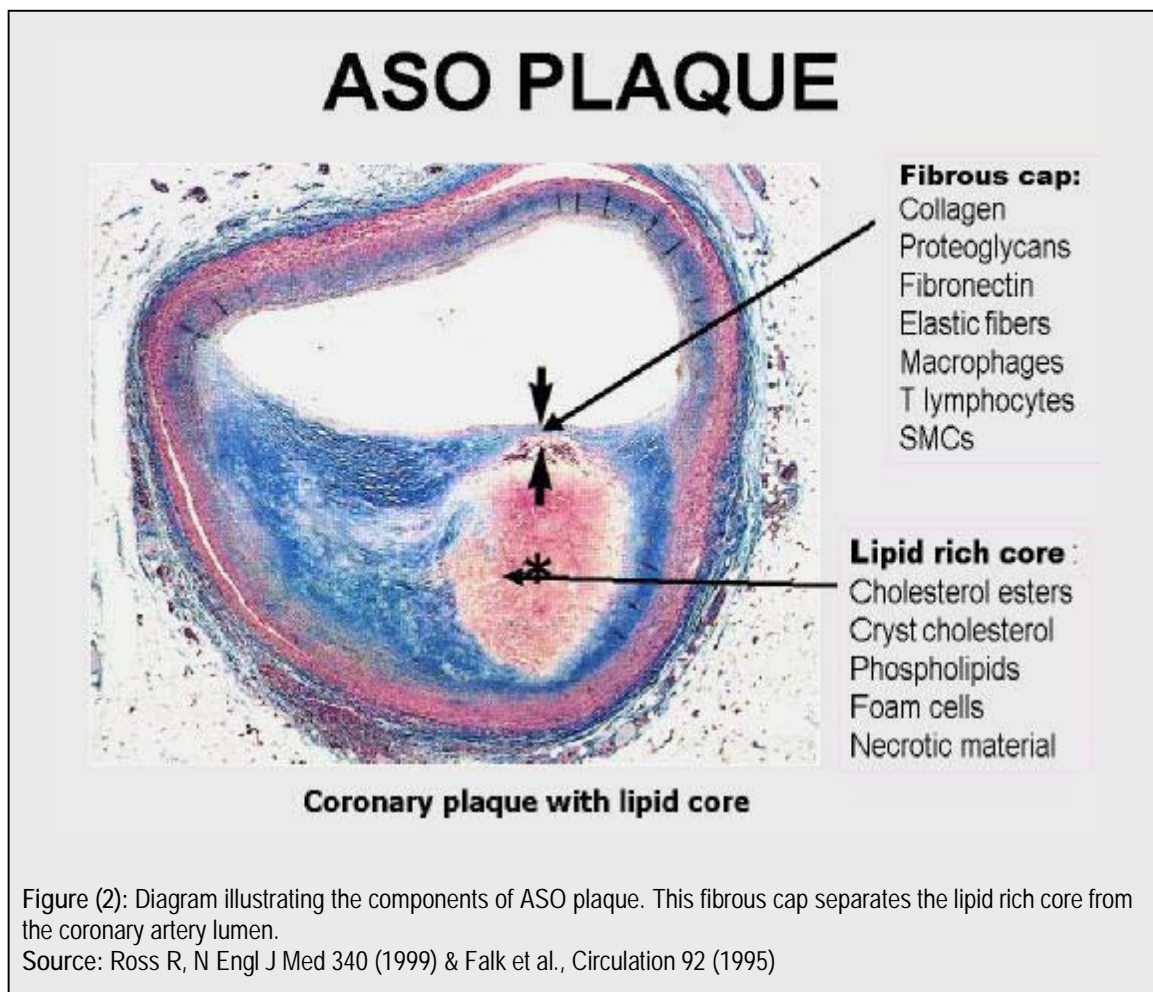
ASO PLAQUES Majority

- **Clinically Silent**
- **Angiographically Invisible – do not encroach on lumen**
- **Heterogenous**

STRUCTURE OF ASO PLAQUE

The two main components of the plaque are:

1. The *fibrosis cap* which separates the lipid core from the endothelium and blood stream (Figs 2,3).
2. The *lipid core* which contains the atherogenic material (Figs 2,3)



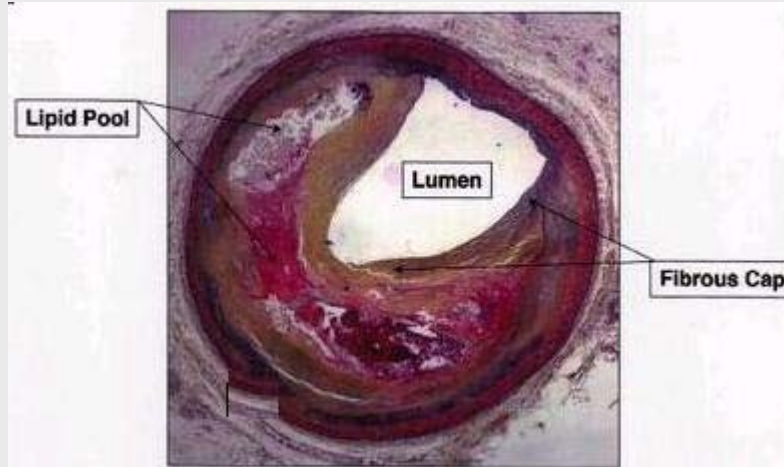


Figure (3): Section in coronary artery showing the heterogeneous nature of ASO plaque which is encroaching on coronary artery lumen. The fibrous cap thickness varies in different parts of the plaque.

The Fibrous Cap

- The cap is made of:
 - Extracellular matrix proteins:
 - § Collagen.
 - § Elastin.
 - § Proteoglycans (protein polysaccharides include chondroitin sulfate and heparin sulfate).
 - Inflammatory and smooth muscle cells.
- The main source of extracellular matrix proteins are the vascular smooth muscle cells (VSMCs).
- The collagen content of the fibrous cap gives the tensile strength and firmness to the cap and its ability to resist stresses, wear and tear.
- A thick fibrous cap rich in collagen fibers is a feature of stable, relatively benign ASO plaques (Fig 4).
- A thin cap with little collagen carries the increased risk of tear, fissuring or rupture. Thin caps are characteristic of vulnerable or unstable ASO plaques.
- Fibrous cap is a dynamic structure, its thickness and collagen content depends upon the balance between collagen formation by VSMCs and collagen degradation by proteolytic enzymes produced by macrophages: space matrix metallo proteinases (MMPs) (Fig 5).

The stable atherosclerotic plaque

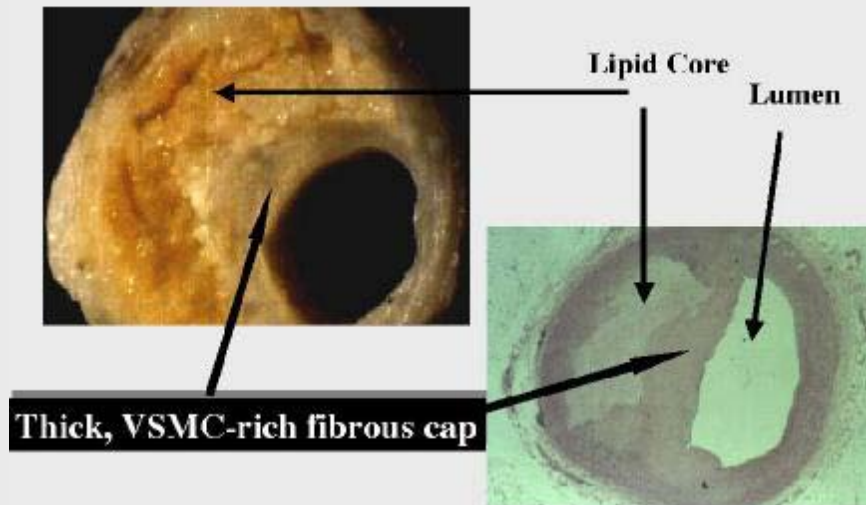


Figure (4): Autopsy specimen of coronary artery segment and a stained section. The fibrous cap is thick rich in collagen and VSMCs which are characteristic of stable plaques.

Source: Weissberg,2001.

Fibrous cap

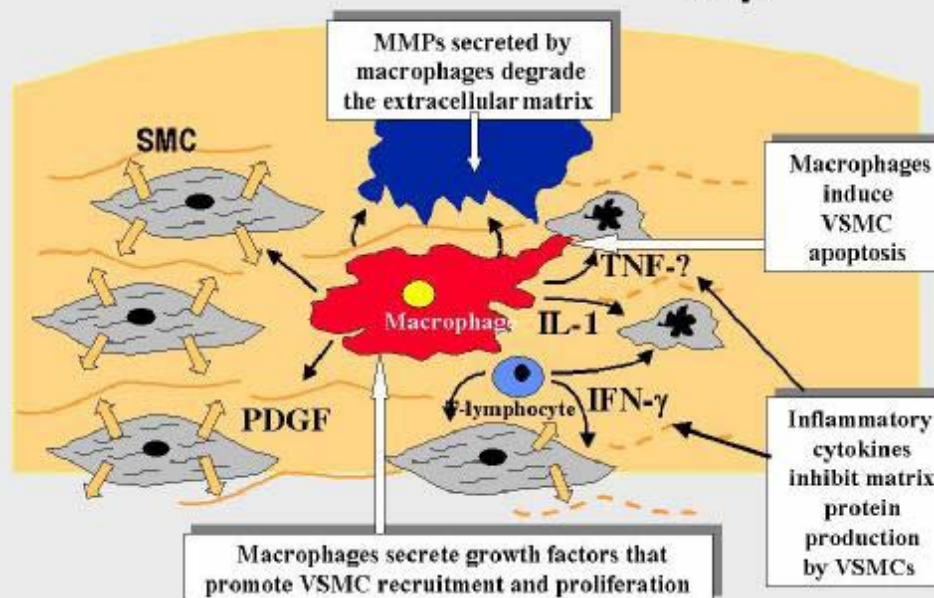


Figure (5): Illustrative diagram showing the components of the fibrous cap and the different cytokines released from inflammatory cells. Macrophages are the chief inflammatory cells and play a central role in plaque instability. Macrophages secrete $\text{TNF-}\alpha$ which induces VSMCs apoptosis and also secretes MMPs which breakdown and degrade extracellular matrix proteins resulting in thinning of the fibrous cap. Macrophages also secrete growth factors (PDGF) that stimulate VSMCs migration from the arterial media and induce their proliferation. Lymphocytes the other inflammatory cells secrete cytokines $\text{IFN-}\gamma$ which inhibits collagen synthesis by VSMCs. SMC: smooth muscle cell. PDGF: platelet derived growth factor.

Source: Weissberg,2001 (modified).

The Lipid Core

- The atherogenic material of the plaque is in the lipid core which is made of:
 - Cholesterol esters, cholesterol crystals, phospholipids, foam cells, macrophages, apoptotic inflammatory and SMCs and necrotic material.
- The lipid core is rich in tissue factor (TF): a major activator of the coagulation process.
- The size and composition of lipid core are variable.
- The core is soft has tooth-paste like consistency at room temperature and becomes softer at body temperature.

Arterial Remodeling

- Growth and enlargement of ASO plaque within coronary arterial wall does not initially encroach on the lumen and is directed externally (abluminal). This is called positive or reversed remodeling (Fig 6).
- Further enlargement of the plaque will proceed internally and the plaque will protrude into the lumen of the coronary artery.
- Thanks to positive remodeling, the majority of plaques do not produce significant coronary stenosis.
- Positive remodeling is very frequent in vulnerable unstable plaques.

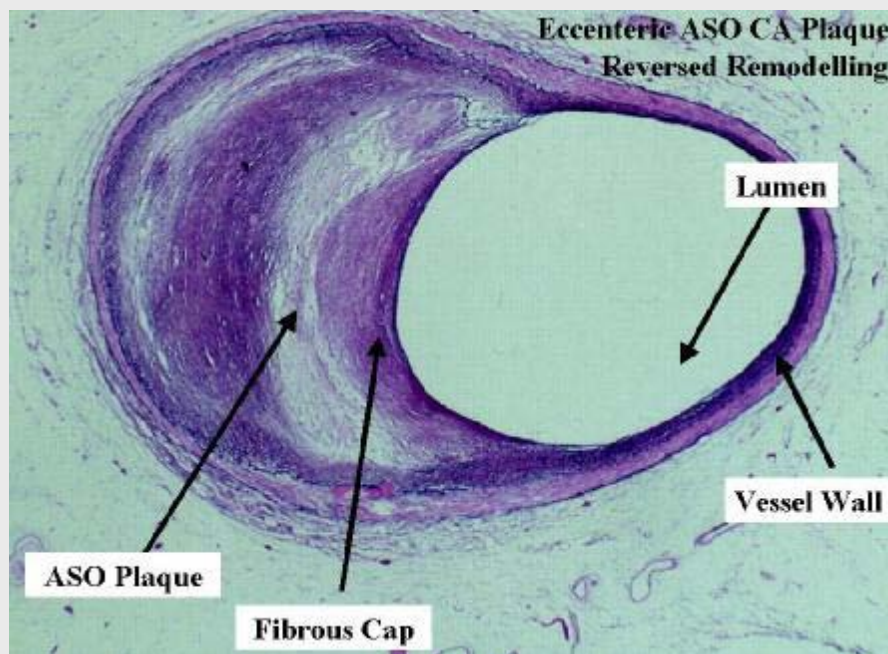


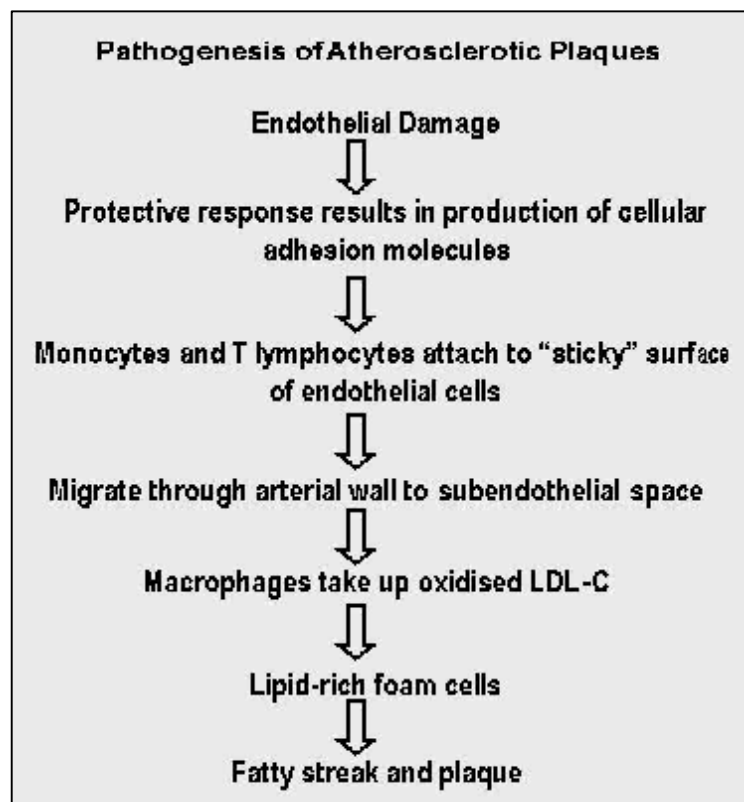
Figure (6): A large ASO plaque in the wall of coronary artery growing away from the coronary artery lumen producing external or reversed remodeling. There is no encroachment on the lumen which looks normal in spite of the large size of the plaque. The fibrous cap is thin.

Source: Varnava, Mills and Davies

THE MAJOR PLAYERS IN THE ATHEROSCLEROTIC PROCESS

1. Cellular players:
 - § Endothelial cells.
 - § Monocytes/macrophages.
 - § Vascular smooth muscle cells (VSMCs).
 - § Lymphocytes.
2. LDL-cholesterol
3. Modulators: cytokines (IL-6, TNF- α , interferon - γ)
4. Mediators:
 - § Chemokines: chemoattractant proteins (MCP-1)
 - § Cellular adhesion molecules (ICAM-1, VCAM-1, selectins)
 - § Growth factors (PDGF).
 - § Coagulation factors (TF)
 - § Enzymes: MMP, caspases, elastases
 - § Superoxide anions (oxidative stress).

DEVELOPMENT OF ASO PLAQUE



- ASO is an inflammatory process initiated by vascular endothelial cell injury.
- Cardiovascular atherosclerotic risk factors e.g. hypertension, smoking, diabetes, dyslipidemia cause endothelial injury leading to endothelial cells dysfunction.
- The following are the major players in the atherosclerotic process:
 1. Cellular players:
 - § Endothelial cells.
 - § Monocytes/macrophages.
 - § Vascular smooth muscle cells (VSMCs).
 - § Lymphocytes.
 2. LDL-cholesterol
 3. Modulators: cytokines (IL-6, TNF- α , interferon γ)
 4. Mediators:
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 - § Growth factors (PDGF).
 - § Coagulation factors (TF)
 - § Enzymes: MMP, caspases, elastases
 - § Superoxide anions (oxidative stress) (Figs 7,8).

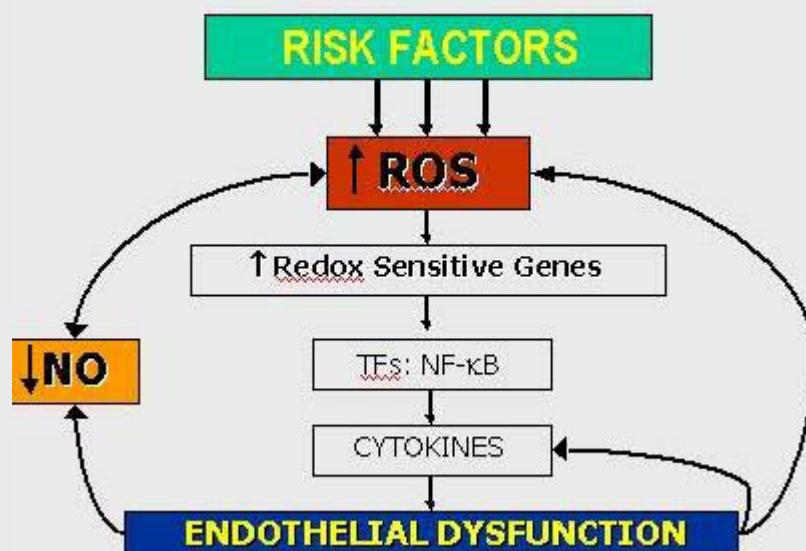
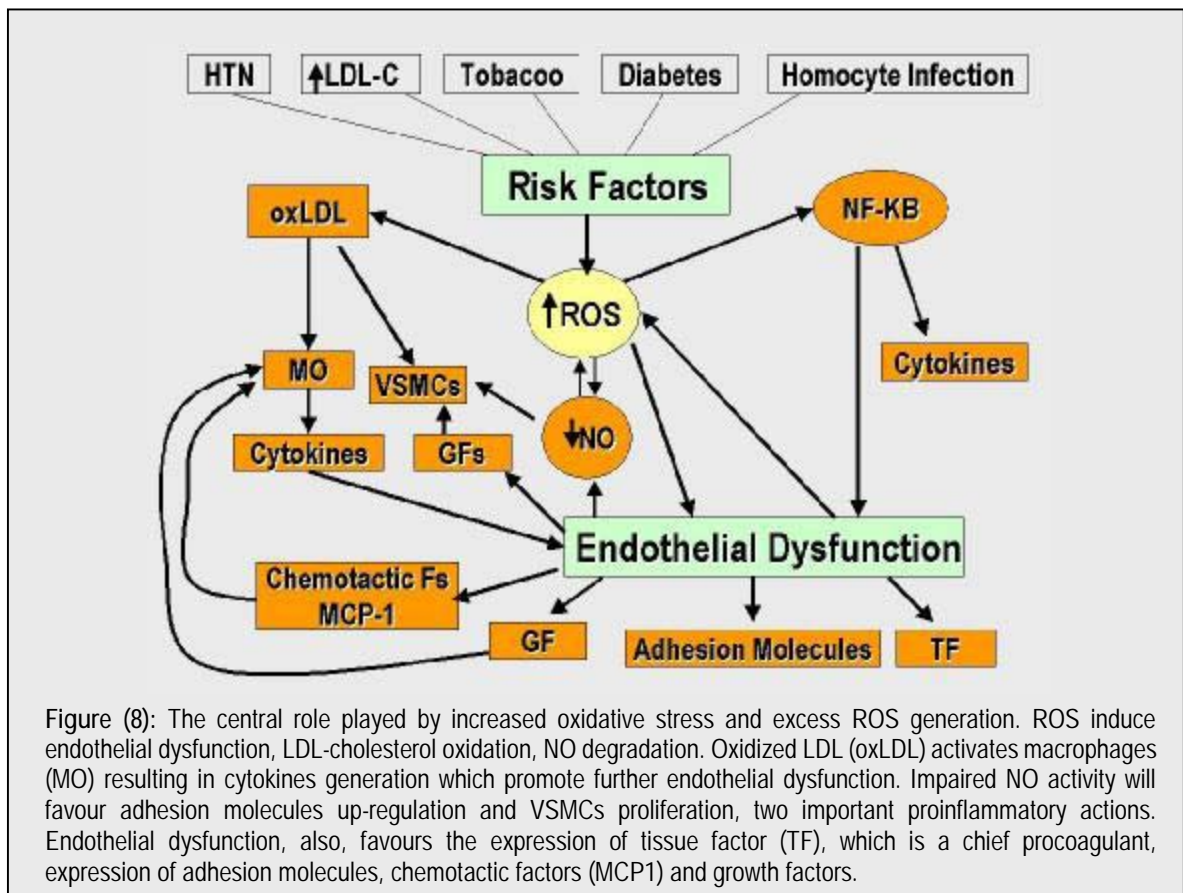


Figure (7): Risk factors e.g. smoking, hypertension, diabetes, dyslipidemia produce their harmful proatherogenic effect through vascular endothelial injury and excessive generation of reactive oxygen species (ROS) such as superoxide anions. Increased ROS will activate redox sensitive genes (genes that are activated by increased oxidative stress) and ROS will inactivate NO. Transcription factor KB (TF-kB) is activated by increased ROS and translocates to the nucleus where it stimulates the transcription of genes coding for proinflammatory proteins e.g. adhesion molecules, MCP-1 and cytokines (TNF- α , IL-6). Cytokines induce endothelial dysfunction resulting in excess ROS production and impaired NO generation.



CELLULAR PLAYERS

Endothelial Cells

- These cells are strategically situated at the interface of blood stream with the extravascular compartment (Fig 9).
- Endothelial cell dysfunction is the earliest manifestation of ASO and is manifest before any structural changes. It is an independent predictor of future cardiovascular events.

Normal Arterial Wall

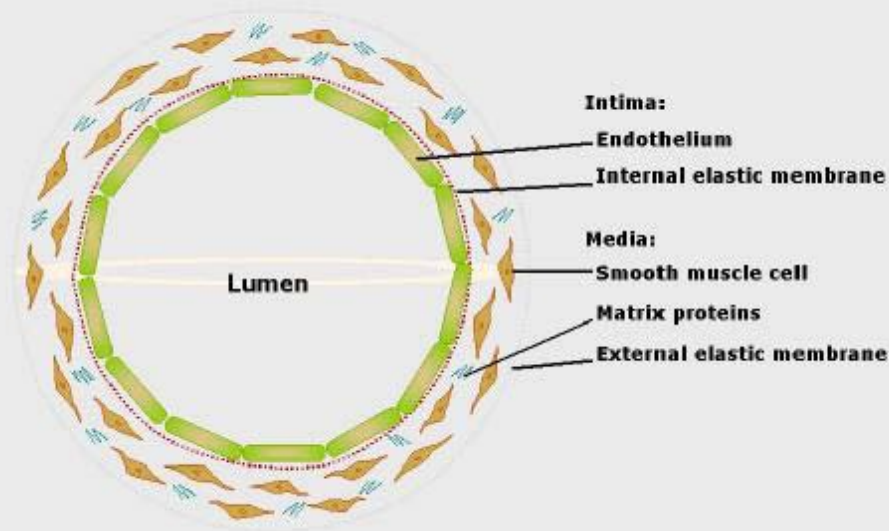


Figure (9): Diagram of a cross section in an artery.

Manifestations of endothelial dysfunction include (Fig 10):

1. Diminished ability to synthesize and release nitric oxide (NO).
NO is a chief vasodilator, anti-inflammatory and antiplatelet agent. The vasomotor balance will favour vasoconstriction when there is decreased production of NO.
2. Expression on the luminal surface of endothelial cells of adhesion molecules that make the surface sticky. Examples are vascular cell adhesion molecules (VCAM-1), intracellular adhesion molecules (ICAM-1) and selectins. Adhesion molecules expression is induced by proinflammatory cytokines (IL-1 β and TNF- α) and by CRP. Adhesion molecules help inflammatory cells (monocytes, lymphocytes) and platelets adhere to the injured endothelium.
3. Increased permeability of vascular endothelium to cells and macromolecules such as LDL-C i.e. the endothelium becomes more leaky.
4. Production and release of chemokines which are chemoattractant proteins that attract and recruit the circulating inflammatory cells to the vessel wall. Examples is monocyte-chemoattractant protein (MCP-1).
5. Release of growth factors such as platelet derived growth factor (PDGF) that stimulate growth, proliferation and change in phenotype (shape, structure and function) of other cellular players namely monocytes, macrophages and VSMC.
6. Increased production of reactive oxygen species (ROS) which include superoxide anion (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH).
7. ROS deplete NO, enhance lipid oxidation and inflammation (Fig 8).

ROS contribute to endothelial dysfunction associated with certain risk factors such as diabetes, hypertension, hypercholesterolemia, smoking and hyperhomocysteinemia.

8. Expression and generation of tissue factor (TF). TF is a glycoprotein and is a chief procoagulant factor. When released in adequate amount in the blood it combines with plasma coagulation factor VIIa increasing several folds its procoagulant activity. TF/VIIa combination activates factors IX and X and stimulating the extrinsic pathway of blood coagulation (Fig 11)).
9. Release of other vasoconstrictor and cellular mitogenic molecules such as endothelin and angiotensin.

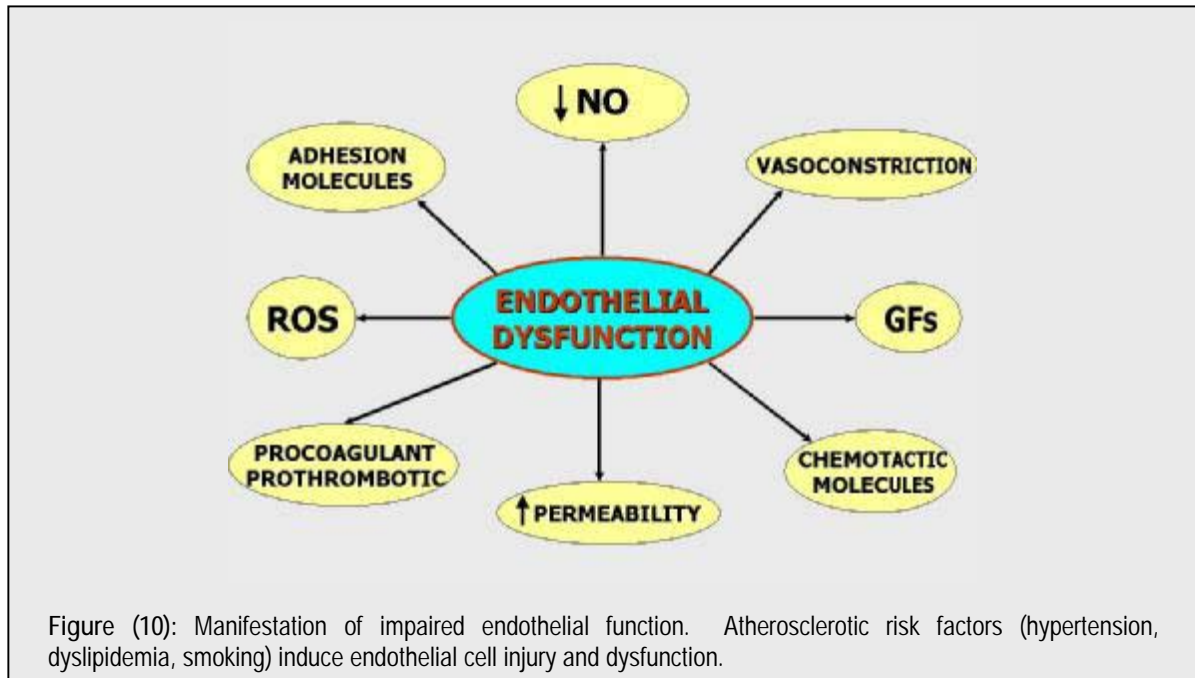
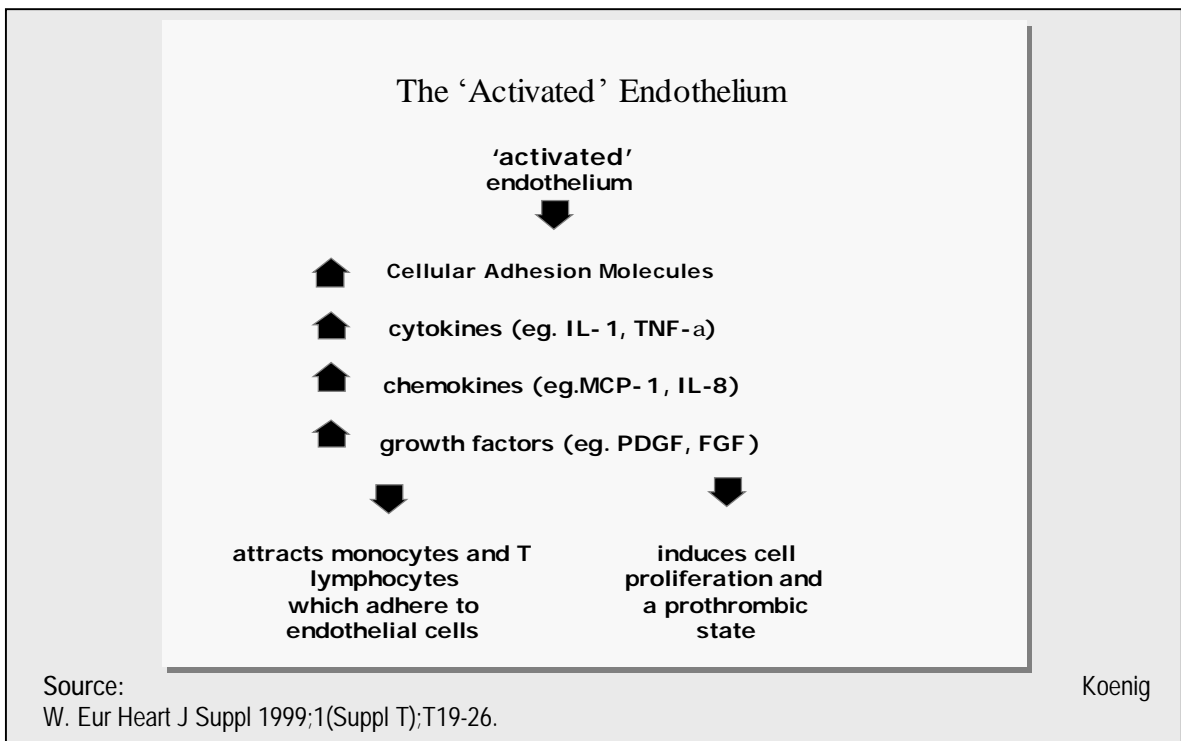
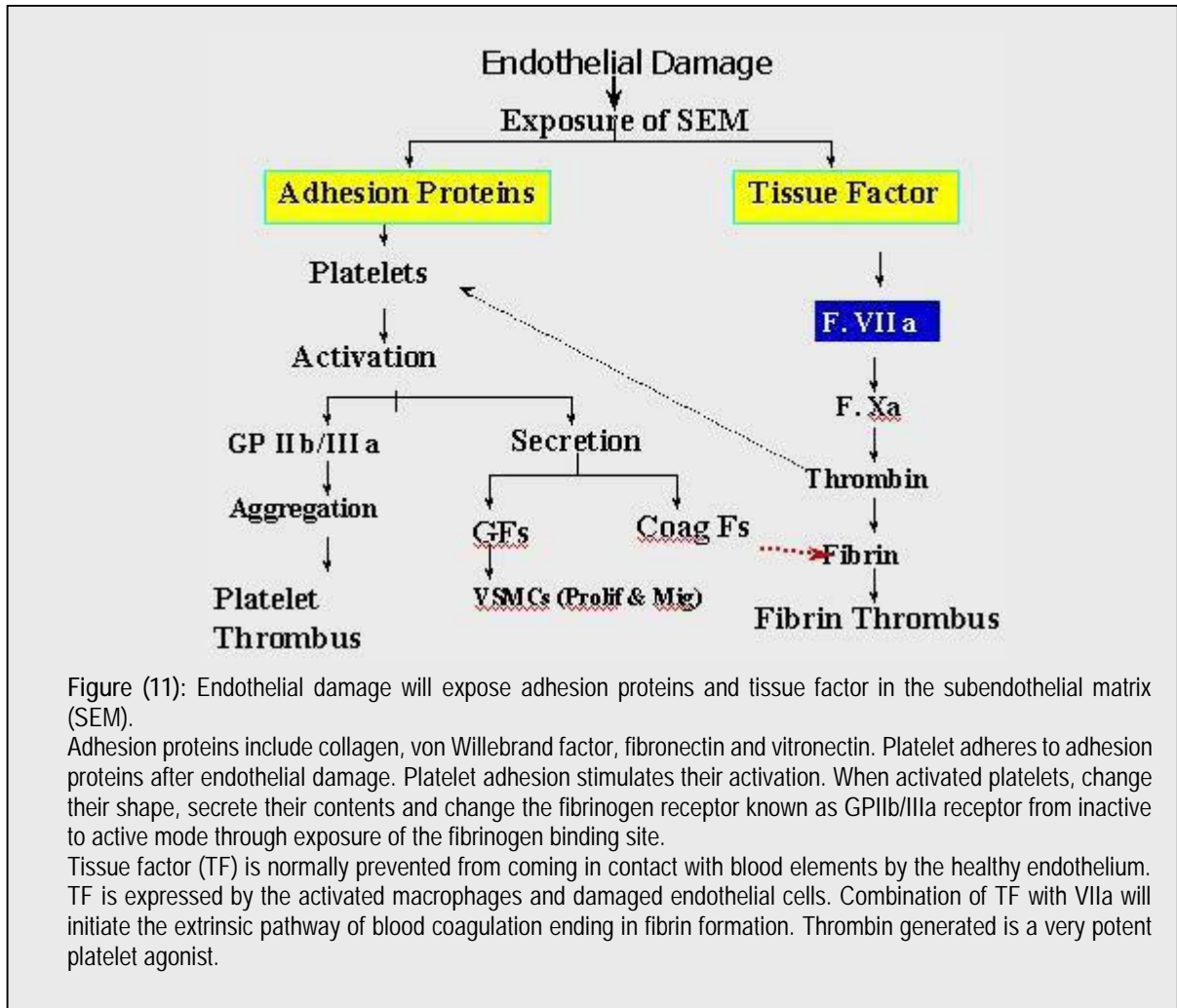


Figure (10): Manifestation of impaired endothelial function. Atherosclerotic risk factors (hypertension, dyslipidemia, smoking) induce endothelial cell injury and dysfunction.



Monocyte/Macrophages

- Monocytes attraction and adherence to the dysfunctional endothelium is the first step in ASO plaque formation (Fig 12).
- Once adherent, the monocytes transmigrate through the intima to the subintimal space by the action of MCP-1 (Fig 12).
- Once within the arterial intima in the subendothelial space, the monocytes develop into macrophages and begin to express scavenger receptors that phagocytose and internalize modified lipoproteins.
- Macrophages avidly take up cholesterol from apolipoprotein B-containing lipoproteins and become trapped in the subendothelial space, forming the lipid core.
- Accumulation of lipoprotein particles in macrophages gives rise to foam cells which characterize early atherosclerotic lesion.
- The uptake of oxidized LDL by macrophages stimulates the expression of cytokines, proteolytic enzymes and other molecules.
- Products of activated macrophages and foam cells include:
 - Proteolytic enzymes: MMPs, caspase, elastases.
 - Superoxides and other ROS.
 - Coagulation factor: TF.
 - Arachidonic acid derivatives.
 - Cytokines: IL-1, IL-6, TNF- α .
- Matrix metallo proteinases (MMP) are proteolytic enzymes released from the activated macrophages. They digest and breakdown the collagen and other proteins of the extracellular matrix.

Endothelial Dysfunction in Atherosclerosis

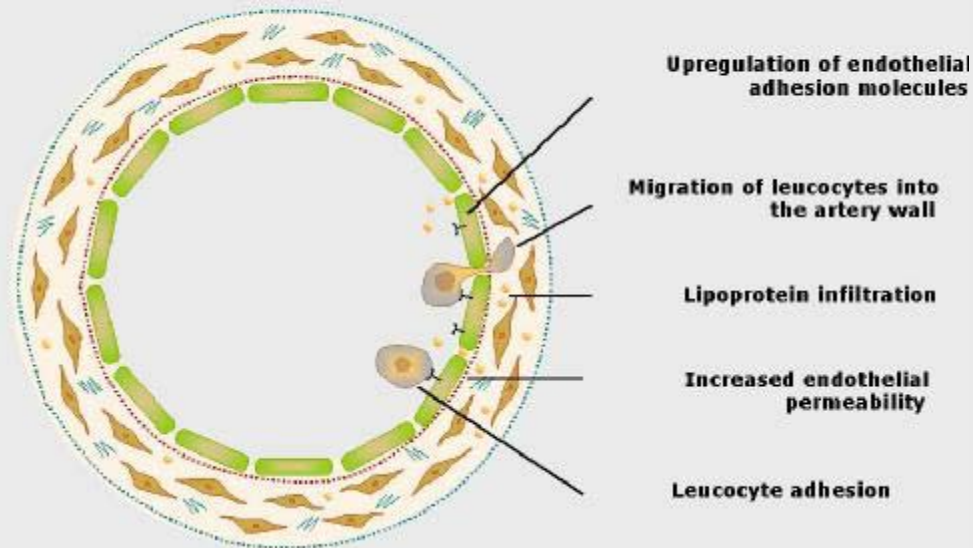


Figure (12): Diagram of a cross section of coronary artery showing endothelial dysfunction in early stages of development of plaque, expression of adhesion molecules and migration of monocytes through endothelial layer.

Vascular Smooth Muscle Cells (VSMCs)

- Under the effect of a number of cytokines, growth factors and ROS, VSMCs cells become activated and migrate from their original residence in the media of the arterial wall to the subintimal space.
- In the subintimal space VSMCs proliferate and change their phenotype from the contractile to the secretory form.
- VSMCs are the chief source of extracellular matrix proteins particularly collagen.
- Collagen produced by VSMCs forms the fibrous cap of the ASO plaque that separates the lipid core from vascular endothelium and vessel lumen.
- Activity of VSMCs and generation of collagen is under control of a number of cytokines. Interferon- γ released by activated T-lymphocytes inhibits collagen synthesis by VSMCs and produces VSMCs apoptosis while transforming growth factor- β stimulates collagen synthesis.

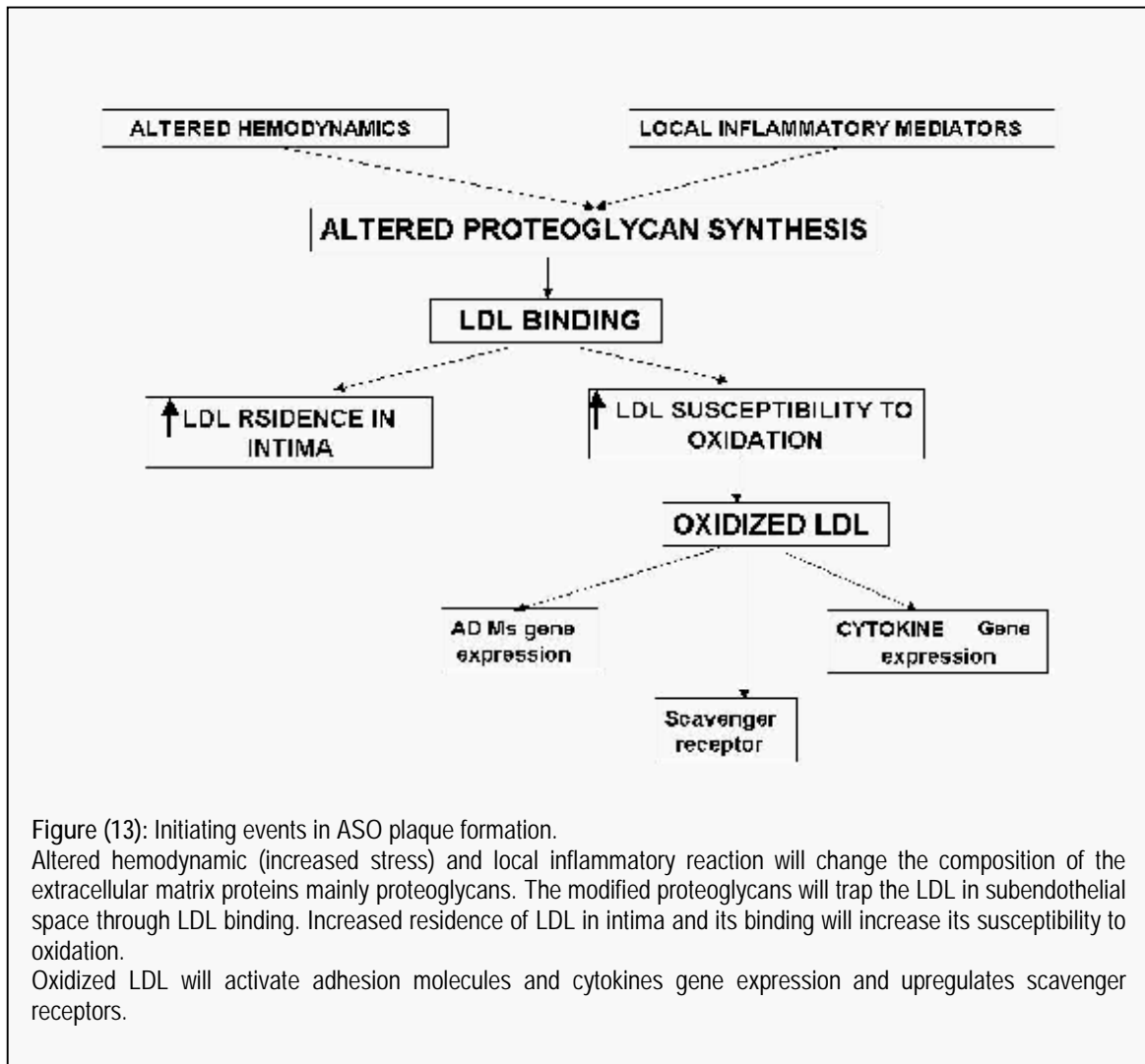
Lymphocytes

- T-lymphocytes form up to 20% of the population of plaque inflammatory cells.
- T cells are cells with important immune regulatory function.
- Attracted and recruited from the blood stream to the subendothelial space under the effect of chemokines and growth factors.
- Once within the arterial intima, T-cells become activated by encountering antigens such as ox-LDL.

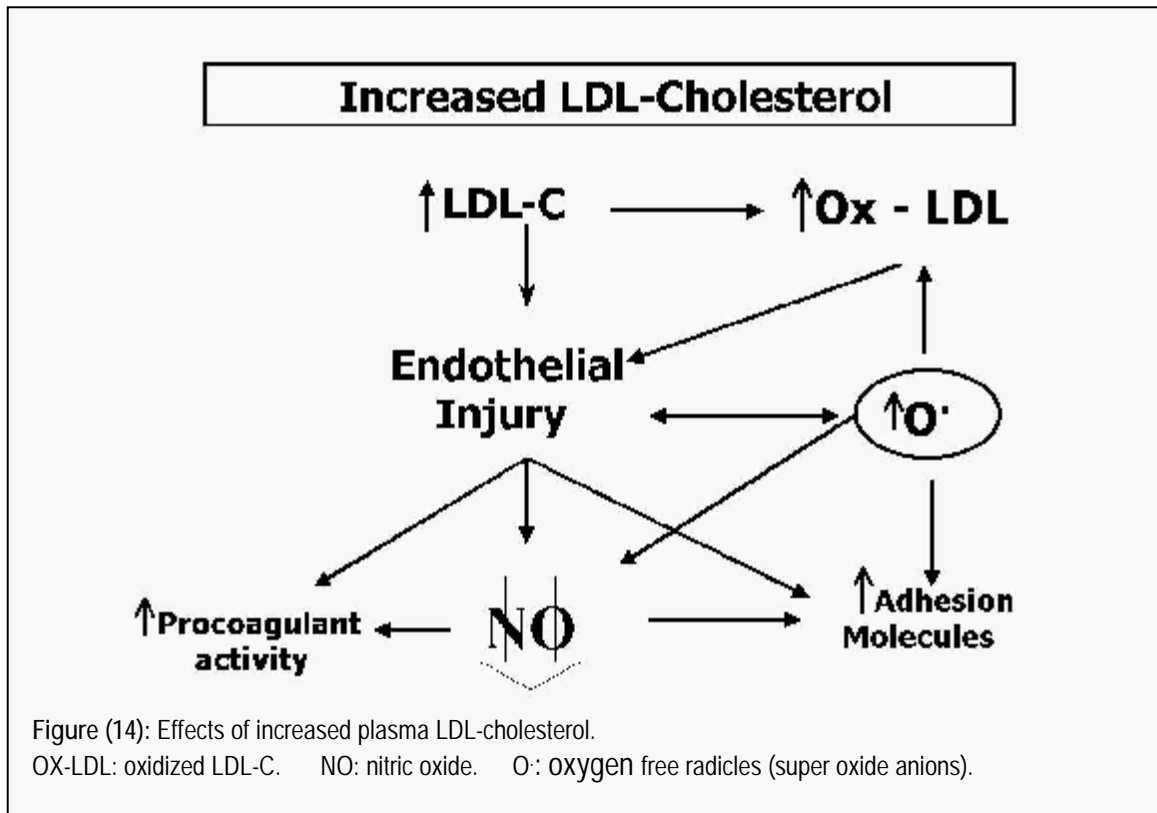
- Activation of T cells is followed by their proliferation and production of cytokines such as CD-40L, TNF and interferon (IFN- γ) that can influence macrophage and VSMCs activity.
- IFN- γ can limit the synthesis of new collagen required for fibrous cap formation. IFN- γ produced by T-lymphocytes acts on both VSMCs and macrophages. It suppresses the production of collagen and induces apoptosis of VSMCs. It stimulates production of MMPs by macrophages.

LDL-CHOLESTEROL

- LDL is a major player in inflammatory injury to the endothelium and underlying SMCs.
- There is a constant traffic of LDL particles between plasma and subendothelial space.
- A number of factors participate in accumulation of LDL in the subendothelial space:
 1. Increase in plasma LDL level.
 2. Endothelial injury favoring increased traffic of LDL from plasma across the endothelial layer to the subendothelial space.
 3. Change in the composition of the extracellular matrix proteins namely proteoglycans due to changing wall stress and due to risk factors. The modified proteoglycans will trap the LDL in the subendothelial space (Fig 13). Augmented wall stress promotes the production by VSMCs of proteoglycans.



- The trapped LDL is more liable to oxidative modification, glycation (in patients with diabetes), or incorporation into immune complexes.
- Production of excess ROS from dysfunctional endothelial cells, activated macrophages and SMCs will increase the LDL peroxidation and production of oxidized LDL (ox LDL).
- Oxidized LDL is a toxic proinflammatory and antigenic molecule. It stimulates a local inflammatory reaction in vessel wall (Fig 14).
- Once formed, ox LDL is taken up by macrophages in the vessel wall by special receptors-scavenger receptors, by a mechanism that is not subject to feedback inhibition.
- ox LDL serves as a chemo-attractant for circulating macrophages and promotes their differentiation by enhancing the release of macrophage colony stimulating factor (MCSF).
- The uptake of ox LDL by macrophages stimulates expression of cytokines and proteolytic enzymes.
- Since there is no feedback inhibition, the uptake of ox LDL by macrophage scavenger receptors continues until foam cell dies and rupture. Release of foam cell contents of cholesterol esters and cholesterol crystals and other necrotic material contributes to the formation of the lipid core of ASO plaque and further accentuates the local inflammatory reaction.



MODULATORS

Cytokines: IL-6

Source

- IL-6 is a cytokine produced by many cell types including lymphocytes, monocytes, fibroblasts, endothelial cells and adipose tissue.

Functions

- It is a regulator of the acute phase response. It is the only cytokine that can stimulate the synthesis of all the acute phase proteins involved in the inflammatory response: C-reactive protein (CRP), serum amyloid A, fibrinogen, haptoglobin and α_1 -chymotrypsin.
- It has a wide range of other actions:
 - Coagulation: increased plasma fibrinogen and increased platelet aggregation.
 - Endothelium: dysfunction and increased adhesion molecules.
 - Leucocyte recruitment.
 - Lipoprotein lipase activity: reduction, leading to reduced triglycerides uptake.

Significance

- IL-6 levels appear to be predictive of future heart disease, and are elevated in patients with unstable angina.

MEDIATORS

C-Reactive Protein (CRP)

Source

- CRP is an acute phase protein produced by liver hepatocytes in response to IL-6 stimulation.

Functions

- It is an innate immune response protein.
- Bind and activates complement.
- Upregulates nuclear factor kB (NF-kB)*
- Induce monocyte recruitment to arterial wall.
- Induce expression of : - cell adhesion molecules.
 - tissue factor.
- Enhance MCP-1 production.
- Mediate LDL uptake by macrophages.

Significance

- Elevated CRP (>3 mg/L) is found in:
 - <10% of normals.
 - <20% of patients with chronic stable angina or variant angina.
 - >65% of patients with unstable angina (UA).
 - >90% of patients with AMI preceded by UA.
 - <50% of patients with AMI without preceded UA.
- Half of patients with ACS have persistently elevated CRP after hospital discharge.
- Estimation of plasma hs-CRP improves the global risk assessment.
- An elevated hs-CRP is a cardiovascular risk factor.
- It has a predictive value of future vascular events.
- It helps in targeting statin therapy in primary prevention in patients with normal LDL level.

CRP appears to be a stronger predictor than LDL-cholesterol.

Other Causes of Elevated CRP Levels:

- Abdominal obesity.
- Metabolic syndrome.
- Type 2 diabetes.
- Cigarette smoking.
- Hormone replacement therapy.
- Renal insufficiency.

* NF-kB is a transcription factor, present inactive in cytoplasm. When activated it translocates to the nucleus where it activates transcription of many genes coding for proinflammatory proteins (adhesion molecules, chemokines, ROS generating enzymes) and cytokines such as TNF- α , IL-6, IL-8 and IL-1b and cyclo oxygenase.

Limitations of CRP Testing:

- Daily fluctuation in basal CRP levels.
- Transiently elevated for 2-3 weeks following a major infection or trauma.
- Chronic inflammation e.g. rheumatoid arthritis and minor inflammation stimuli and some non-inflammatory states (e.g. angina, alcohol, depression) can influence CRP.

Angiotensin-II (AII) AND DEVELOPMENT OF ATHEROSCLEROTIC PLAQUE.

- All through proinflammatory action plays an important role in the initiation and progression of atherosclerotic plaque.
- Interfering and attenuation of the pro-atherogenic AII actions is an important reason for the use of ACE-Is and ARBs in cardiovascular protection.
- AII stimulates the production of ROS (reactive oxygen species: superoxide anions, hydrogen peroxides) through action on cell membrane enzyme nicotinamide adenine dinucleotide phosphate oxidase (NADPH).
- Activation of NADPH oxidase and increased ROS within the vessel wall will:
 - Stimulate transcription of genes sensitive to ROS (redox sensitive genes) (Fig 7).
 - Cause endothelial dysfunction.
- All pro atherogenic actions:
 - Enhancing lipid accumulation and peroxidation in vessel wall.
 - It induces endothelial injury resulting in the expression of adhesion molecules and chemo-attractant proteins and increased permeability.
 - AII stimulates monocytes and macrophage attraction, adhesion and migration. Processes central to both the inflammatory and atherosclerotic processes.
 - AII stimulates VSMCs proliferation and activation. VSMCs are the chief source of extracellular matrix collagen which forms the cap of the atherosclerotic plaque and increases the plaque size.

All favours the development and persistence of intravascular thrombi by:

- Platelet activation.
- Endothelial injury.
- Increased PAI-1 production.