Atherosclerosis

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Atherosclerosis, a disease of the large arteries, is the primary cause of heart disease and stroke. In westernized societies, it is the underlying cause of about 50% of all deaths. Epidemiological studies have revealed several important environmental and genetic risk factors associated with atherosclerosis. Progress in defining the cellular and molecular interactions involved, however, has been hindered by the disease's aetiological complexity. Over the past decade, the availability of new investigative tools, including genetically modified mouse models of disease, has resulted in a clearer understanding of the molecular mechanisms that connect altered cholesterol metabolism and other risk factors to the development of atherosclerotic plaque. It is now clear that atherosclerosis is not simply an inevitable degenerative consequence of ageing, but rather a chronic inflammatory condition that can be converted into an acute clinical event by plaque rupture and thrombosis.

Atherosclerosis is a progressive disease characterized by the accumulation of lipids and fibrous elements in the large arteries. The anatomy of a normal artery is shown in Fig. 1. The early lesions of atherosclerosis consist of subendothelial accumulations of cholesterol-engorged macrophages, called 'foam cells'. In humans, such 'fatty streak' lesions can usually be found in the aorta in the first decade of life, the coronary arteries in the second decade, and the cerebral arteries in the third or fourth decades. Because of differences in blood flow dynamics, there are preferred sites of lesion formation within the arteries. Fatty streaks are not clinically significant, but they are the precursors of more advanced lesions characterized by the accumulation of lipid-rich necrotic debris and smooth muscle cells (SMCs). Such 'fibrous lesions' typically have a 'fibrous cap' consisting of SMCs and extracellular matrix that encloses a lipid-rich 'necrotic core'. Plaques can become increasingly complex, with calcification, ulceration at the luminal surface, and haemorrhage from small vessels that grow into the lesion from the media of the blood vessel wall. Although advanced lesions can grow sufficiently large to block blood flow, the most important clinical complication is an acute occlusion due to the formation of a thrombus or blood clot, resulting in myocardial infarction or stroke. Usually, the thrombosis is associated with rupture or erosion of the lesion.

The events of atherosclerosis have been greatly clarified by studies in animal models, including rabbits, pigs, non-human primates and rodents. Mice deficient in apolipoprotein E (apoE) or the low-density lipoprotein (LDL) receptor develop advanced lesions and are the models most used in genetic and physiological studies[1]. Figure 2 shows stages in the development of atherosclerotic plaques in experimental animals. The first observable change in the artery wall following the feeding of a high-fat, high-cholesterol diet is the accumulation of lipoprotein particles and their aggregates in the intima at sites of lesion predilection (Fig. 2a, b). Within days or weeks, monocytes can be observed adhering to the surface of the endothelium. The monocytes then transmigrate across the endothelial monolayer into the intima, where they proliferate, differentiate into macrophages and take up the lipoproteins, forming foam cells (Fig. 2c, d)[2]. With time, the foam cells die, contributing their lipid-filled contents to the necrotic core of the lesion. Some fatty streaks subsequently accumulate SMCs, which migrate from the medial layer. With the secretion of

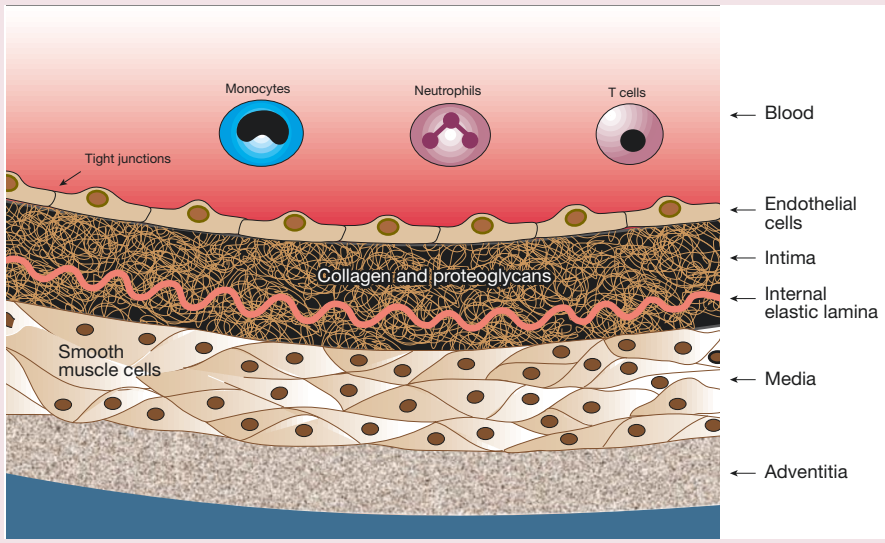

Figure 1 Structure of a normal large artery. A large artery consists of three morphologically distinct layers. The intima, the innermost layer, is bounded by a monolayer of endothelial cells on the luminal side and a sheet of elastic fibres, the internal elastic lamina, on the peripheral side. The normal intima is a very thin region (size exaggerated in this figure) and consists of extracellular connective tissue matrix, primarily proteoglycans and collagen. The media, the middle layer, consists of SMCs. The adventitia, the outer layer, consists of connective tissues with interspersed fibroblasts and SMCs.

fibrous elements by the smooth muscle cells, occlusive fibrous plaques develop and increase in size. Initially, the lesions grow towards the adventitia until a critical point is reached, after which they begin to expand outwards and encroach on the lumen. The lesions continue to grow by the migration of new mononuclear cells from the blood, which enter at the shoulder of the vessel; this is accompanied by cell proliferation, extracellular matrix production and the accumulation of extracellular lipid (Fig. 2e). Atherogenesis can be viewed as a 'response to injury', with lipoproteins or other risk factors as the injurious agents[2,3].

A very complex aetiology

Epidemiological studies over the past 50 years have revealed numerous risk factors for atherosclerosis (Table 1). These can be grouped into factors with an important genetic component, and those that are largely environmental. The relative abundance of the different plasma lipoproteins appears to be of primary importance, as raised levels of atherogenic lipoproteins are a prerequisite for most forms of the disease. With the exception of gender, and the level of lipoprotein(a), each of the genetic risk factors involves multiple genes. This complexity can be clearly observed in genetic crosses in animals maintained under similar environmental conditions; such studies in rodents have revealed dozens of genetic loci that contribute to lipoprotein levels, body fat and other risk factors[4]. Another level of complexity involves the interactions between risk factors. Frequently, these are not simply additive; for example, the effects of hypertension on coronary heart disease (CHD) are considerably amplified if cholesterol levels are high[5].

The importance of genetics and environment in human CHD has been examined in many family and twin studies[6]. Within a population, the heritability of atherosclerosis (the fraction of disease explained by genetics) has been high in most studies, frequently exceeding 50%. Population migration studies, on the other hand, clearly show that the environment explains much of the variation in disease incidence between populations. Thus, the common forms of CHD result from the combination of an unhealthy environment, genetic susceptibility and our increased lifespan[5].

Cellular and molecular interactions

Pathological studies have revealed a defined series of changes in the vessel during atherogenesis (Fig. 2) and showed that blood-derived inflammatory cells, particularly monocytes/macrophages, have a key role. Tissue culture studies with vascular cells and monocytes/macrophages suggested possible pathways of disease initiation and progression. They provided evidence for the central role of the endothelium in mediating inflammation, and suggested that accumulation of oxidatively modified LDL in the intima contributes significantly to monocyte recruitment and foam-cell formation. During the past decade, understanding of the molecular mechanisms in atherogenesis has been revolutionized by studies in transgenic and gene-targeted mice[7]. These have allowed *in vivo* testing of hypotheses, although it should be noted that studies in mice are limited by significant species differences compared with humans, and that reliable mouse models for thrombosis involving lesion rupture have not been developed.

Lesion initiation

The endothelium, with its intercellular tight junctional complexes, functions as a selectively permeable barrier between blood and tissues. It has both sensory and executive functions, and can generate effector molecules that regulate thrombosis, inflammation, vascular tone and vascular remodelling. For example, removal of the endothelium results in a burst of SMC migration and proliferation, which subsides when the endothelium regenerates[8]. Among the important physical forces acting on endothelial cells (ECs) is fluid shear stress, which has effects on EC morphology. Cells in the tubular regions of arteries, where blood flow is uniform and laminar, are ellipsoid in shape and aligned in the direction of flow. Cells in regions of arterial branching or curvature, where flow is disturbed, have polygonal shapes and no particular orientation. These latter areas show increased permeability to macromolecules such as LDL and are preferential sites for lesion formation[8].

As shown in Fig. 3, a primary initiating event in atherosclerosis is the accumulation of LDL in the subendothelial matrix. Accumulation is greater when levels of circulating LDL are raised, and both the transport and retention of LDL are increased in the preferred sites for

Table 1 Genetic and environmental factors associated with atherosclerosis and coronary heart disease (CHD)

Factors with a strong genetic component	
Elevated levels of LDL/VLDL	Associations demonstrated in epidemiological studies and supported by studies of genetic disorders and animal models. Clinical trials have shown benefits of cholesterol reduction[54].
Reduced levels of HDL	Associations demonstrated by numerous epidemiological studies and supported by studies of genetic diseases and animal models[58].
Elevated levels of lipoprotein(a)	Associations observed in many, but not all, epidemiological studies. Animal studies have been contradictory[59].
Elevated blood pressure	Associations observed in epidemiological studies. Clinical trials have demonstrated benefits of blood pressure reduction, with particularly strong effects on stroke[54,60].
Elevated levels of homocysteine	Associations have been observed in epidemiological studies, and homocystinuria results in severe occlusive vascular disease[36].
Family history	When all known risk factors are controlled for, family history remains a very significant independent factor[6].
Diabetes and obesity	Associations observed in epidemiological studies and in studies with animal models[54].
Elevated levels of haemostatic factors	Significant independent associations have been observed with elevated levels of fibrinogen, plasminogen activator inhibitor type 1 and platelet reactivity[54].
Depression and other behavioural traits	Associations observed in several population studies[61].
Gender (male)	Below age 60, men develop CHD at more than twice the rate of women[38].
Systemic inflammation	Elevated levels of inflammatory molecules such as C-reactive protein are associated with CHD, as are inflammatory diseases such as rheumatoid arthritis[62].
Metabolic syndrome	This cluster of metabolic disturbances, with insulin resistance as a central feature, is strongly associated with CHD[5].
Environmental factors	
High-fat diet	Population migration and epidemiological studies indicate strong associations with lifestyle, and diet appears to be the most significant factor. High-fat, high-cholesterol diets are usually required for development of atherosclerosis in experimental animals[54].
Smoking	Strong associations observed in numerous epidemiological studies. Clinical trials have demonstrated the benefit of stopping smoking[54].
Low antioxidant levels	Results of clinical trials with antioxidants have not been conclusive. Fat-soluble antioxidants protect against atherosclerosis in experimental animals, however[63].
Lack of exercise	Significant independent associations with CHD[54].
Infectious agents	Epidemiological studies provide suggestive evidence for associations with various infectious agents, such as *Chlamydia pneumoniae*. Preliminary animal studies support the relationship[64].

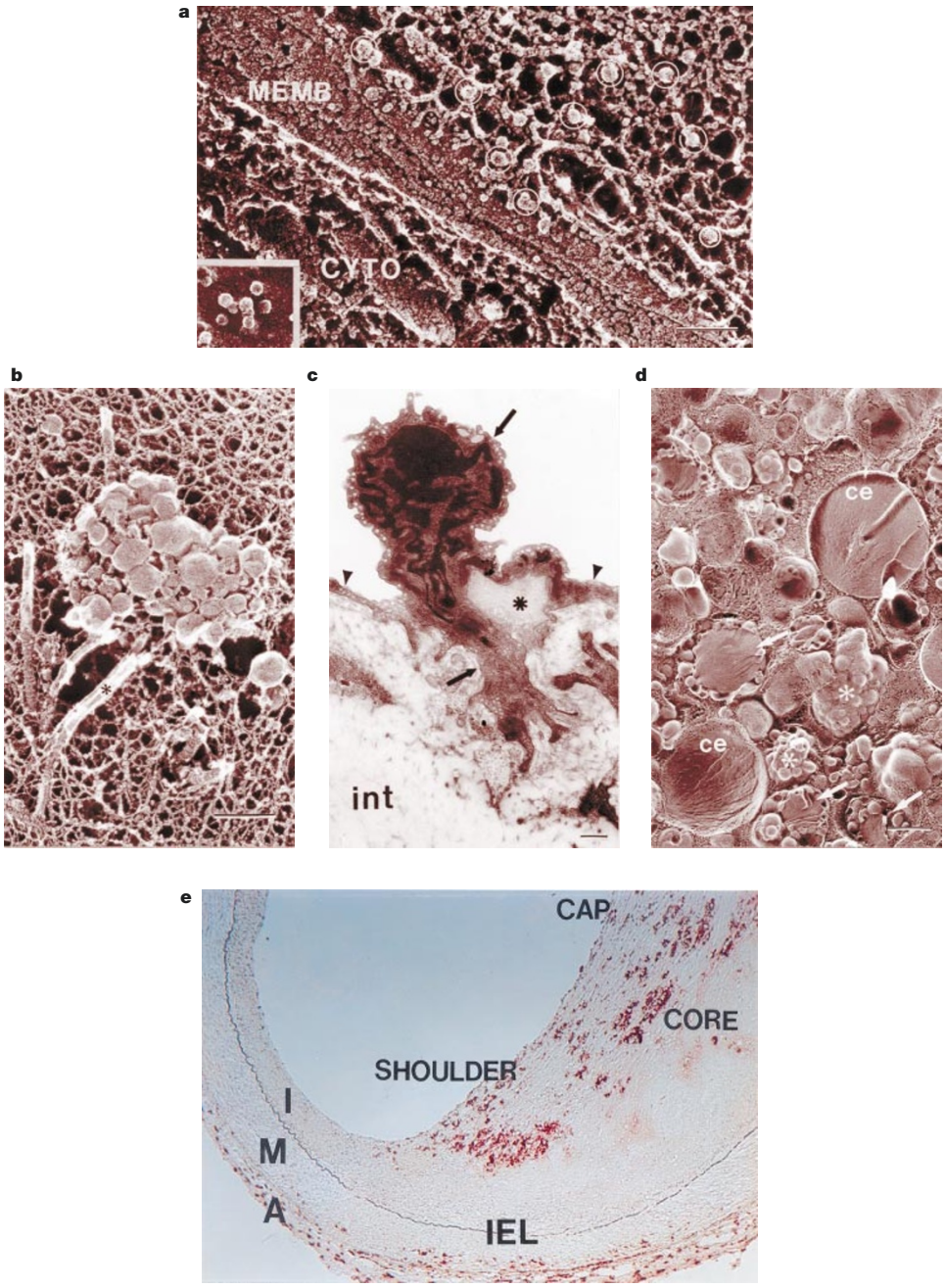

Figure 2 Stages in the development of atherosclerotic plaques. **a**, In the first stages, lipoprotein is trapped in the subendothelial matrix. The freeze-etch electron micrograph shows the accumulation of 23-nm LDL particles (circled) in the matrix of a rabbit atrial-ventricular valve following incubation with LDL (inset). An endothelial cell at lower left shows the plasma membrane (MEMB) and cytoplasma (CYTO)[71]. Magnification ×141,372; scale bar, 0.1 μm. **b**, Lipoprotein aggregation is seen in this freeze-etch electron micrograph of rabbit intima following administration of a bolus of LDL. The aggregated particles are surrounded by matrix and collagen fibrils (asterisk)[72]. Magnification ×52,876; scale bar, 0.2 μm. **c**, Monocyte transmigration. The thin-section electron micrograph of a cross-section of the aorta of a 9-week-old apoE-deficient mouse shows a monocyte (arrow) moving between two endothelial cells (arrowheads) to enter the intima (int). The asterisk denotes a cluster of lipid underneath the endothelial cell[1]. Magnification ×10,078; scale bar, 0.5 μm. **d**, Foam-cell formation. Freeze-etch electron micrograph of the cytoplasm of a macrophage foam cell in the intima of a rabbit fed a high-fat diet for two weeks. Large lipid droplets with the onion skin configuration typical of cholesterol esters (ce) as well as other lipid-filled compartments (arrows) can be recognized. Some compartments contain large aggregated LDL particles (asterisk) resembling those in **b**. Magnification ×21,542; scale bar, 0.5 μm. **e**, Fibrous lesion. Light micrograph (×400) of a section of an advanced human coronary atherosclerotic lesion that has been immunostained for the macrophage-specific antigen EMB-11 (red). A, adventitia; I, intima; IEL, internal elastic lamina; M, media. Photographs courtesy of A. Mottino, J. Frank and T. Drake, UCLA.

lesion formation. LDL diffuses passively through EC junctions, and its retention in the vessel wall seems to involve interactions between the LDL constituent apolipoprotein B (apoB) and matrix proteoglycans[9]. In addition to LDL, other apoB-containing lipoproteins, namely lipoprotein(a) and remnants, can accumulate in the intima and promote atherosclerosis. Lipoprotein(a), a particle resembling LDL but containing an additional polypeptide termed apolipoprotein(a) that is linked to apoB by a disulphide bridge, seems to be particularly atherogenic owing to its additional effects on fibrinolysis and SMC growth[10].

Native LDL is not taken up by macrophages rapidly enough to generate foam cells, and so it was proposed that LDL is somehow 'modified' in the vessel wall[11]. It has subsequently been shown that trapped LDL does indeed undergo modification, including oxidation, lipolysis, proteolysis and aggregation, and that such modifications contribute to inflammation as well as to foam-cell formation. One of the modifications most significant for early lesion formation is lipid oxidation as a result of exposure to the oxidative waste of vascular cells. Such modifications initially give rise to 'minimally oxidized' LDL species that have pro-inflammatory activity but may not be sufficiently modified to be recognized by macrophage scavenger receptors. Mice lacking 12/15-lipoxygenase have considerably diminished atherosclerosis, suggesting that this enzyme may be an important source of reactive oxygen species in LDL oxidation[12]. Lipoxygenases insert molecular oxygen into polyenoic fatty acids, producing molecules such as hydroperoxyeicosatetraenoic acid (HPETE), which are likely to be transferred across the cell membrane to 'seed' the extracellular LDL.

High-density lipoprotein (HDL) is strongly protective against atherosclerosis. An important mechanism underlying this protective

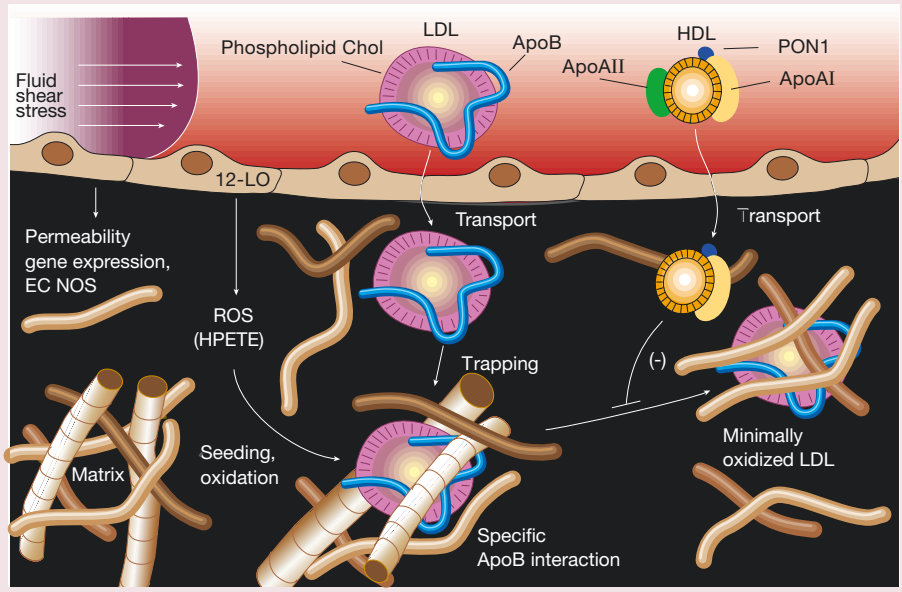

Figure 3 Lesion initiation. Sites of lesion predilection are determined in part by haemodynamic forces acting on endothelial cells. These influence the permeability of the endothelial barrier and expression of endothelial cell (EC) genes such as that for nitric oxide synthase (NOS). An important initiating event is the retention of LDL and other apolipoprotein B (apoB)-containing lipoproteins as a result of interaction with matrix components. The LDL undergoes oxidative modification as a result of interaction with reactive oxygen species (ROS) including products of 12/15 lipoxygenase (12-LO) such as HPETE. Oxidation of LDL is inhibited by HDL, which contains the antioxidant protein serum paraoxonase (PON1).

effect is the role of HDL in the removal of excess cholesterol from peripheral tissues. But in addition, HDL also protects by inhibiting lipoprotein oxidation. The antioxidant properties of HDL are due in part to serum paraoxonase, an esterase carried on HDL that can degrade certain biologically active oxidized phospholipids[13,14].

Inflammation

Atherosclerosis is characterized by the recruitment of monocytes and lymphocytes, but not neutrophils, to the artery wall (Fig. 4). A triggering event for this process is the accumulation of minimally oxidized LDL, which stimulates the overlying ECs to produce a number of pro-inflammatory molecules, including adhesion molecules and growth factors such as macrophage colony-stimulating factor (M-CSF). The biological activity of minimally oxidized LDL is contained primarily in its phospholipid fraction, and three active oxidation products resulting from the scission or rearrangement of unsaturated fatty acids have been identified[15]. Oxidized LDL can also inhibit the production of nitric oxide (NO), a chemical mediator with multiple anti-atherogenic properties, including vasorelaxation. Mice lacking endothelial NO synthase showed enhanced atherosclerosis, due in part to raised blood pressure[16]. In addition to oxidized LDL, a number of other factors are likely to modulate inflammation, including haemodynamic forces, homocysteine levels, sex hormones, and infection. Diabetes may promote inflammation in part by the formation of advanced endproducts of glycation that interact with endothelial receptors[17].

The entry of particular types of leukocytes into the artery wall is mediated by adhesion molecules and chemotactic factors. After cultured ECs are exposed to oxidized LDL, they will bind monocytes but not neutrophils. The first step in adhesion, the 'rolling' of leukocytes along the endothelial surface, is mediated by selectins which bind to carbohydrate ligands on leukocytes. Studies of mice deficient in P- and E-selectins or the cell adhesion molecule ICAM, revealed the role of these adhesion molecules in atherosclerosis[18,19]. The firm adhesion of monocytes and T cells to endothelium can be mediated by the integrin VLA-4 on these cells, which interacts with both VCAM-1 on the endothelium and the CS-1 splice variant of fibronectin. Both *in vitro* and *in vivo* studies suggested that these interactions have a role in atherosclerosis[20]. Finally, mice deficient in monocyte chemotactic protein (MCP-1) or its receptor CCR2 had significantly reduced atherosclerotic lesions, suggesting that

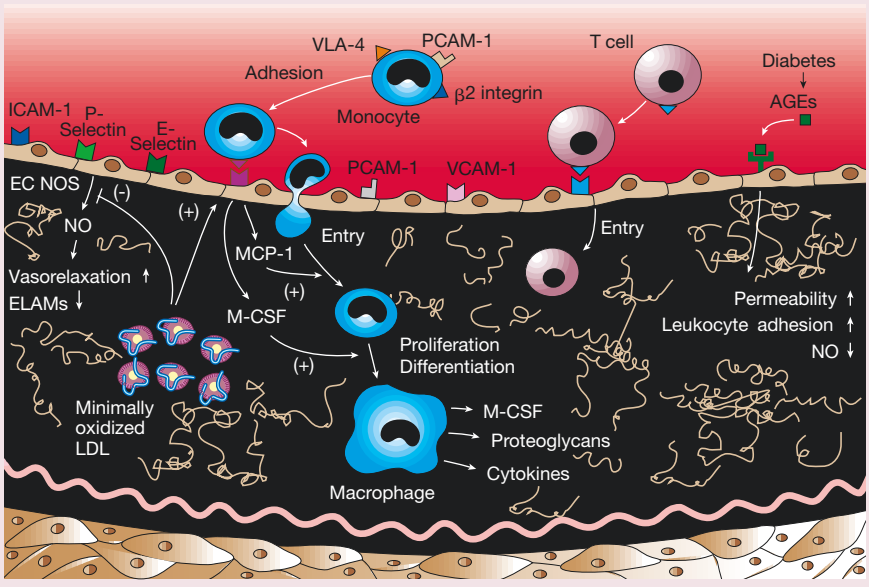

Figure 4 Inflammation. Minimally oxidized LDL stimulates the overlying endothelial cells to produce adhesion molecules, chemotactic proteins such as monocyte chemotactic protein-1 (MCP-1), and growth factors such as macrophage colony-stimulating factor (M-CSF), resulting in the recruitment of monocytes to the vessel wall. Oxidized LDL has other effects, such as inhibiting the production of NO, an important mediator of vasodilation and expression of endothelial leukocyte adhesion molecules (ELAMs). Among endothelial cell adhesion molecules likely to be important in the recruitment of leukocytes are ICAM-1, P-selectin, E-selectin, PCAM-1 and VCAM-1. Important adhesion molecules on monocytes include β2 integrin, VLA-4, and PCAM-1. Advanced glycosylation endproducts (AGEs) are formed in diabetes and these promote inflammation via specific receptors on endothelial cells.

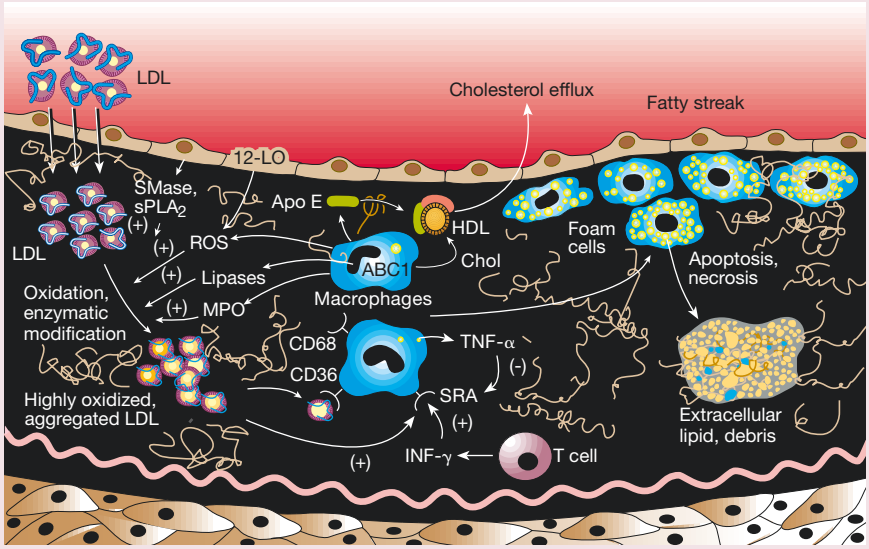

Figure 5 Foam-cell formation. Highly oxidized aggregated LDL is formed in the vessel as a result of the action of reactive oxygen species (ROS) and the enzymes sphingomyelinase (SMase), secretory phospholipase 2 ($sPLA_2$), other lipases, and myeloperoxidase (MPO). The oxidized aggregated LDL is recognized by macrophage scavenger receptors such as SR-A, CD36 and CD68. Scavenger receptor expression is mediated by cytokines such as tumour necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). Foam cells secrete apolipoprotein E (apoE), which may facilitate removal of excess cellular cholesterol. The death of foam cells leaves behind a growing mass of extracellular lipids and other cell debris.

MCP-1/CCR2 interaction has a role in monocyte recruitment in atherosclerosis[21,22].

The cytokine M-CSF stimulates the proliferation and differentiation of macrophages, and influences various macrophage functions such as expression of scavenger receptors. Mice with a spontaneous null mutation of M-CSF had dramatically reduced lesions, suggesting an obligatory role for macrophages in lesion formation[23].

Foam-cell formation

LDL must be extensively modified ('highly oxidized') before it can be taken up sufficiently rapidly by macrophages to form foam cells (Fig. 5). This modification presumably involves reactive oxygen species produced by ECs and macrophages, but several enzymes are also thought to be involved, including myeloperoxidase, sphingomyelinase and a secretory phospholipase, all of which occur in human atherosclerotic lesions. Myeloperoxidase generates highly reactive species such as hypochlorous acid and tyrosyl radical, and myeloperoxidase-modified LDL binds to macrophage scavenger receptors[24]. Sphingomyelinase may promote lipoprotein aggregation, leading to increased retention and enhanced uptake by macrophages[25]. Finally, a secretory phospholipase (group II sPLA2) can promote LDL oxidation, and transgenic mice overexpressing the enzyme show increased atherosclerosis[26].

The rapid uptake of highly oxidized (and otherwise modified) LDL particles by macrophages, leading to foam-cell formation, is mediated by a group of receptors that recognize a wide array of ligands. Two such 'scavenger' receptors, SR-A and CD36, appear to be of primary importance, and mice lacking either receptor show a modest reduction in atherosclerotic lesions[27,28]. The expression of scavenger receptors is regulated by peroxisome proliferator-activated receptor-γ, a transcription factor whose ligands include oxidized fatty acids, and by cytokines such as tumour necrosis factor-α and interferon-γ (IFN-γ)[29].

Macrophages actively secrete apoE, and this may promote cholesterol efflux to HDL, thereby inhibiting the transformation of macrophages to foam cells. Evidence for this role of apoE comes from bone marrow transplantation studies showing that mice transplanted with marrow from apoE-null mice develop much larger lesions than mice receiving marrow from control mice[30]. Interestingly, mice deficient in ACAT1, the enzyme responsible for cholesterol esterification in macrophages, are still able to develop significant lesions[31].

Fibrous plaques

Fibrous plaques are characterized by a growing mass of extracellular lipid, mostly cholesterol and its ester, and by the accumulation of SMCs and SMC-derived extracellular matrix (Fig. 6). Cytokines and growth factors secreted by macrophages and T cells are important for SMC migration and proliferation and extracellular matrix production.

Recent studies have shown that the interaction of CD40 with its ligand CD40L (CD154) makes an important contribution to the

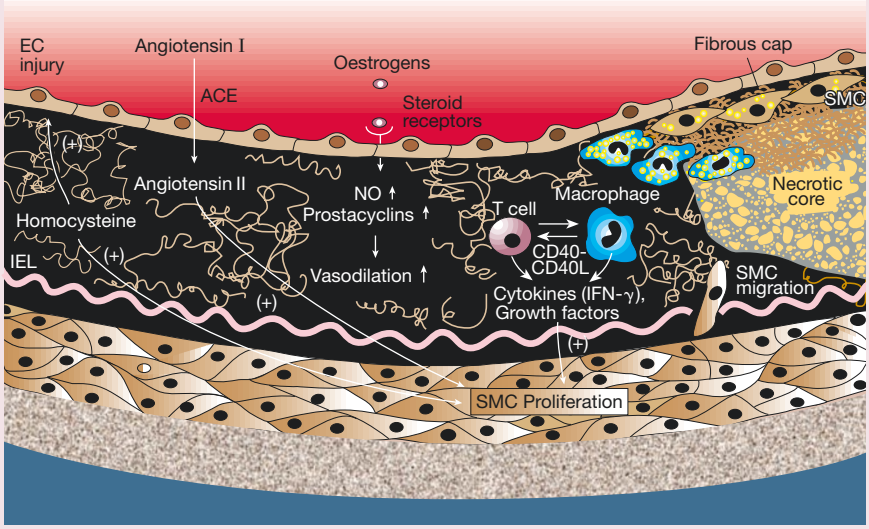

Figure 6 Formation of fibrous plaques. A number of risk factors, including elevated levels of homocysteine and angiotensin II (produced through the action of angiotensin-converting enzyme, ACE), stimulate the migration or proliferation of SMCs. Oestrogens exert beneficial effects on plasma lipoprotein levels and they also stimulate production of NO and prostacyclin by endothelial cells. The interaction of CD40 and CD40 ligand (CD40L) stimulates T lymphocytes (T cells) and macrophages to express cytokines such as IFN-γ that can influence inflammation, SMC growth and matrix accumulation. The intimal SMCs secrete extracellular matrix and give rise to a fibrous cap.

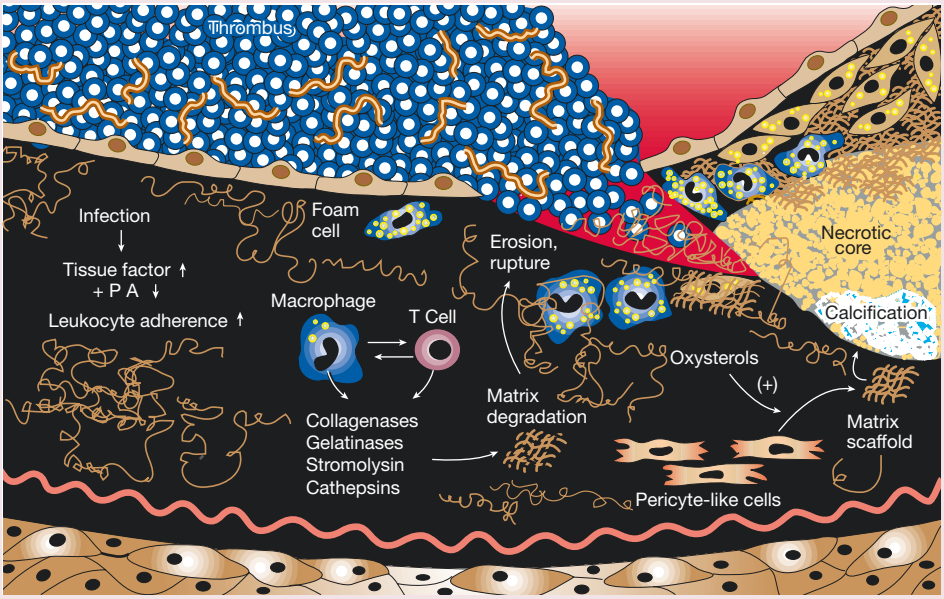

Figure 7 Complex lesions and thrombosis. Vulnerable plaques with thin fibrous caps result from degradation of matrix by various proteinases such as collagenases, gelatinases, stromolysin and cathepsins and by inhibition of matrix secretion. Among various factors that may destabilize plaques and promote thrombosis are infection, which may have systemic effects such as induction of acute phase proteins and local effects such as increased expression of tissue factor and decreased expression of plasminogen activator (PA). The calcification of lesions appears to be an active, regulated process involving the secretion by pericyte-like cells in the intima of a scaffold for calcium phosphate deposition. The formation of a thrombus, consisting of adherent platelets and fibrin crosslinks, usually results from plaque rupture, exposing tissue factor in the necrotic core.

development of advanced lesions[32]. This interaction was first recognized as being essential to major immune reactions involving T and B cells, but it is now clear that CD40 is also expressed on macrophages, ECs and SMCs. The engagement of CD40 and CD40L results in the production of inflammatory cytokines, matrix-degrading proteases and adhesion molecules. Studies using CD40L-null mice or neutralizing antibodies to CD40L have shown that disruption of the interaction results in smaller lesions that are less inflammatory and more fibrous[32]. Although studies with immunodeficient mice originally indicated a modest role of lymphocytes in atherogenesis[33], studies of CD40–CD40L[32], of antibodies to oxidized LDL epitopes[34], and of the T-lymphocyte product IFN-γ[35] are consistent with a major role for lymphocytes.

Several risk factors seem to contribute to the development of fibrous lesions, including elevated homocysteine, hypertension and hormones. Elevated homocysteine levels appear to injure ECs and to stimulate proliferation of vascular SMCs[36]. Some of the effects of raised blood pressure on atherosclerosis seem to be mediated by components of the renin–angiotensin pathway. For example, angiotensin II directly stimulates SMC growth and the production of extracellular matrix. Studies with spontaneously hypertensive rats (SHR) indicate that raised blood pressure stimulates expression of platelet-derived growth factor, a potent mitogen for SMCs[37]. Oestrogen has multiple anti-atherogenic properties, including effects on plasma lipoprotein levels and stimulation of prostacyclin and NO production[38].

Infection by cytomegalovirus has been linked to atherosclerosis and arterial restenosis (a narrowing of the vessel lumen due to vascular remodelling following angioplasty)[35]. On the basis of *in vitro* studies, a plausible mechanism for this link is stimulation of SMC migration by the virus-coded chemokine receptor US28 (ref. 39). Cytomegalovirus infection is also associated with inactivation of the p53 protein, and p53-null mice exhibited increased SMC proliferation and accelerated atherosclerosis[40].

The monoclonal patchiness of atherosclerotic lesions originally suggested that the disease may involve a nonmalignant transformation of SMCs, but this patchiness has now been shown to result from normal development[41]. Nevertheless, evidence consistent with oncogene activation, loss of heterozygosity and microsatellite instability in human lesions has been reported[5].

Advanced lesions and thrombosis

Pathological studies suggest that the development of thrombus-mediated acute coronary events depends principally on the composition and vulnerability of a plaque rather than the severity of stenosis (Fig. 7). Vulnerable plaques generally have thin fibrous caps and increased numbers of inflammatory cells. Maintenance of the fibrous cap reflects matrix production and degradation, and products of inflammatory cells are likely to influence both processes. For example, T cells produce IFN-γ, which inhibits the production of matrix by SMCs, and macrophages produce various proteases that degrade extracellular matrix, including interstitial collagenase, gelatinases and stromolysin[3]. Rupture frequently occurs at the lesion edges, which are rich in foam cells, suggesting that factors contributing to inflammation may also influence thrombosis. In this regard, it is notable that the incidence of myocardial infarction and stroke increases during acute infections.

The stability of atherosclerotic lesions may also be influenced by calcification and neovascularization, common features of advanced lesions. Intimal calcification is an active process in which pericyte-like cells secrete a matrix scaffold which subsequently becomes calcified, akin to bone formation. The process is regulated by oxysterols and cytokines[42]. The growth of small vessels from the media may provide a conduit for entry of inflammatory cells[43].

The thrombogenicity of the lesion core is likely to depend on the presence of tissue factor, a key protein in the initiation of the coagulation cascade. The production of tissue factor by ECs and macrophages is enhanced by oxidized LDL, infection or the ligation of CD40 on ECs to CD40L on inflammatory cells[44]. The expression of other molecules mediating thrombosis, such as plasminogen activator, may also be important.

Genetic dissection of atherosclerosis

Although the common forms of atherosclerosis are multifactorial, studies of rare mendelian forms have provided the most important insights into the disease (Table 2). Studies of familial hypercholesterolaemia helped unravel the pathways that regulate plasma cholesterol metabolism, knowledge of which was important for the development of cholesterol-lowering drugs. In the past year, Tangier disease, a rare recessive disorder characterized by the virtual absence of circulating HDL, was shown to be due to mutations in the gene for the ATP-binding-cassette (ABC) transporter 1, providing an excellent candidate cause for more common forms of HDL deficiency[45,46]. Recently found mutations in the mineralocorticoid receptor, a kidney protein that is involved in the body's handling of salt, explain why some women have a sharp rise in blood pressure during pregnancy[47].

Table 2 Mendelian disorders relevant to atherosclerosis

Trait	Disease (gene)	Characteristics
Elevated LDL/VLDL levels	Familial hypercholesterolaemia (LDL receptor)[5]	Dominant disorder characterized by very high LDL-cholesterol levels and early CHD
	Familial defective apoB-100 (apoB)[5]	Dominant disorder due to apoB mutations that affect binding to LDL receptor; less severe than FH
Low HDL levels	ApoAI deficiency (apoAI)[5]	In the homozygous state, null mutations of apoAI result in the virtual absence of HDL and early CHD
	Tangier disease (ABC1 transporter)[45,46]	This recessive disorder results in the inability of cells to export cholesterol and phospholipids, resulting in very low levels of HDL
Coagulation	Various genetic disorders of haemostasis[5]	Unlike rare disorders of lipid metabolism where atherosclerotic disease is a primary manifestation, genetic disorders of haemostasis usually present either as increased risk of bleeding or thrombosis (usually venous), with no outstanding effect on atherogenesis
Elevated homocysteine	Homocystinuria (cystathionine β-synthetase)[36]	Recessive metabolic disorder resulting in very high levels of homocysteine and severe occlusive vascular disease
Diabetes, type 2	MODY1 (hepatocyte nuclear factor 4α)[5], MODY2 (glucokinase)[5], MODY3 (hepatocyte nuclear factor 1α)[5]	MODY1, 2, and 3 are characterized by the development of non-insulin dependent diabetes mellitus in young adults
Hypertension	Glucorticoid-remediable aldosteronism (hybrid gene from crossover of 11-β-hydroxylase and aldosterone synthase)[60]	Dominant disorder with early-onset hypertension and stroke
	Liddle's syndrome (epithelial sodium channel)[60]	Dominant disorder with hypertension and metabolic alkalosis
	Mineralocorticoid receptor[47]	Early-onset hypertension associated with pregnancy

In contrast to the mendelian disorders, attempts to identify genes for the common, complex forms of atherosclerosis have met with mixed success. Studies of candidate genes have revealed a number that show significant or suggestive association or linkage with traits relevant to atherosclerosis, but our understanding remains incomplete (Table 3). Large-scale sequencing is now underway to identify polymorphisms for many other candidate genes for hypertension, diabetes and other traits relevant to atherosclerosis[48]. In an attempt to identify atherosclerosis genes, whole-genome scans for loci associated with diabetes, hyperlipidaemia, low HDL levels and hypertension have been performed[49]. But few loci with significant evidence of linkage have been found, emphasizing the complexity of these traits.

The use of animal models is a potentially powerful way of identifying genes that contribute to common forms of atherosclerosis. Mice and rats—the most useful mammals for genetic studies—have common variations in many traits relevant to atherosclerosis, and orthologous genes frequently contribute to a trait in rodents and humans[50]. Mapping and identification of genes contributing to complex traits is easier in rodents than in humans, as shown by the recent identification of a diabetes gene in the SHR rat model[51]. Studies in animal models should be particularly useful for the identification of genetic factors influencing vascular cell functions; for example, differences in susceptibility to atherosclerosis between certain strains of mice seem to be due to variation that affects EC responses to oxidized LDL[52]. During this decade it is likely that genome-wide approaches, such as expression array studies and large-scale animal mutagenesis studies, will become widely used in atherosclerosis research.

As a result of the genome projects and large-scale sequencing, tens of thousands of single-nucleotide polymorphisms are being identified and a catalogue of all common variations in humans will be generated over the next few years. This raises the possibility of whole-genome association studies. Given the rapid development of DNA chip technology, it should be possible to type large numbers of polymorphisms in many thousands of individuals. There are, however, significant unresolved issues involving linkage disequilibrium and statistical analysis[53] in this approach.

Table 3 Common genetic variations contributing to CHD and its risk factors

Trait	Gene	Variation
LDL/VLDL	ApoE[5]	Three common missense alleles explain ~5% of variance in cholesterol levels
HDL levels	Hepatic lipase[65]	Promoter polymorphism
	ApoAI-CIII-AIV cluster[65]	Multiple polymorphisms
	Cholesteryl ester transfer protein[5]	Common null mutations (Japanese); missense polymorphisms
	Lipoprotein lipase[66]	Missense polymorphisms
Lipoprotein(a)	Apolipoprotein(a)[59]	Many alleles explain >90% variance
Homocysteine	Methylene tetrahydrofolate reductase[5]	Missense polymorphism
Coagulation	Fibrinogen B[5]	Promoter polymorphism
	Plasminogen activator inhibitor type 1[5]	Promoter polymorphism
	Factor VIII[5]	Missense polymorphism
Blood pressure	Angiotensinogen[60]	Missense and promoter polymorphisms
	β_2-adrenergic receptor[60]	Missense polymorphism
	Alpha-adducin[60]	Missense polymorphism
CHD	Angiotensin-converting enzyme[67]	Insertion–deletion polymorphism
	Serum paraoxonase[13,14]	Missense polymorphism affecting enzymatic activity
	Haemachromatosis-associated gene[68]	Missense polymorphism
	Endothelial nitric oxide synthase[69]	Missense polymorphism
	Factor XIII[70]	Missense polymorphism

Only genes exhibiting evidence of linkage or association in two or more studies are cited.

New therapies

Effective drugs for lowering cholesterol and high blood pressure have been developed. In particular, the statins lower levels of atherogenic lipoproteins and dramatically decrease clinical events and mortality from atherosclerosis[54]. Nevertheless, heart disease and stroke remain by far the most common causes of death in westernized societies, and new weapons, particularly agents that block disease at the level of the vessel wall or that raise anti-atherogenic HDL, are needed.

Over the past decade, a number of promising new targets have been identified, as discussed above and shown in Figs 3–7. For example, interruption of the CD40–CD40L system may have clinical benefits for plaque stability[32]. The identification of the ABC transporter presents exciting new opportunities for treatment of low HDL

levels. It has also become clear that HDLs are functionally very heterogeneous[55]. Thus, rather than attempting to increase levels of HDL, it may be more productive to focus on functional properties such as its antioxidant activity. Preliminary studies in animals suggest that it may be possible not only to block the development of atherosclerosis but also to achieve significant regression[56]. The most critical clinical aspect of atherosclerosis is plaque rupture and thrombosis. Although useful mouse models for this have not been developed, a transgenic hypertensive and hyperlipidaemic rat model showed evidence of myocardial infarction[57].

Diagnosis and risk assessment

Catheterization is the gold standard for diagnosis of atherosclerosis, but it is expensive and carries significant risk. Reliable noninvasive methods of diagnosis are urgently needed. Certain biochemical markers for the disease, such as C-reactive protein, and some noninvasive procedures, such as extravascular ultrasound and ultrafast computerized tomography, should prove useful but have limitations.

As our understanding of the genetics of atherosclerosis increases, genetic diagnosis will become increasingly important. The anticipated 'biallelic map' of the genome is likely to drive the evolution of new technologies for gene screening, from high-throughput, genome-wide methods to testing for particular gene variants in individuals. One application of screening will be to distinguish different forms of the disease so that pharmacological intervention can be better targeted. Atherosclerosis is heterogeneous, and the most appropriate therapy will depend on the particular variety of disease. Classification is already used clinically, as patients are grouped according to the variety of risk factors they display, but genetic testing should greatly expand the subdivisions of the disease.

Another potential benefit of genetic studies is testing for susceptibility. Because CHD and stroke are disorders of adults, knowledge of a propensity to disease could be available many years before clinical disease develops, permitting early intervention. Testing for LDL, HDL and blood pressure have long been advocated as a way of identifying individuals at increased risk, and other factors have emerged more recently as risk indicators (Table 1). Once the genes contributing to common forms of the disease have been identified, along with the particular mutations involved, DNA-based tests may add greatly to our ability to assess risk. But given the importance of environmental influences and the complex genetic aetiology of atherosclerosis, efficient screening procedures are unlikely to be available in the near future. □

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