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# The process of atherogenesis — cellular and molecular interaction: from experimental animal models to humans

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**Summary.** Atherogenesis is a disorder of the artery wall that involves: adhesion of monocytes and lymphocytes to the endothelial cell surface; migration of monocytes into the sub-endothelial space and differentiation into macrophages; ingestion of low density lipoproteins and modified or oxidised low density lipoproteins by macrophages by several pathways, including a scavenger pathway, leading to accumulation of cholesterol esters and formation of "foam cells". These foam cells together with T lymphocytes form the fatty streak. Vascular smooth muscle cells migrate from the media into the intima and proliferate with the formation of atherosclerotic plaques. These processes which involve cell adhesion, migration, differentiation, proliferation and cell interaction with the extracellular matrix are regulated by a complex network/cascade of cytokines and growth regulatory peptides. Thus, atherosclerosis may be the result of a specialised chronic inflammatory fibroproliferative process which has become excessive and in its excess this protective response has become the disease state.

**Key words:** Atherogenesis, risk factors, diabetes mellitus, cytokines, growth factors.

## Introduction

### *Risk factors in vascular disease*

Vascular disease is a major cause of morbidity and mortality in developed countries. Several risk factors have been identified that are associated with large vessel disease [1, 2]. High plasma cholesterol levels are a major, but not the only, causative risk factor [3]. High plasma levels of low density lipoproteins (LDL) and decreased high density lipoproteins (HDL) are associated with accelerated atherogenesis [3]. The first gross lesion that develops in atherogenesis is the fatty streak, characterised by an accumulation of macrophages loaded with cholesterol ester ("foam cells") just beneath the endothelium. Most foam cells arise from circulating monocytes that adhere to the endothelium and then migrate beneath the vascular endothelium and accumulate cholesterol ester. How monocytes are targeted to the sub-endothelium is not fully understood, but is presumed to involve the interaction between specific monocyte/ macrophage cell surface proteins and markers on the endothelium or sub-endothelial matrix. Accumulation of LDL by macrophages is mediated by both the classical LDL receptor and by a scavenger receptor that has a high affinity for modified as distinct from native LDL [3, 4].

Modification of LDL can occur either intracellularly as it is transcytosed across the endothelium or by extracellular oxidation. The relative roles of intracellular modification as distinct from extracellular oxidation remain an unresolved issue. Oxidatively modified LDL is immunogenic and may therefore contribute to the inflammatory response. Non-enzymatic glycation of proteins in LDL by high circulating glucose concentrations may also affect LDL clearance by different receptors and thereby its atherogenicity.

Lipoprotein (a) [Lp(a)] is an LDL-like particle that is present in very low levels in the normal population but its incidence is increased in some individuals with thrombotic events. Several studies have demonstrated significant correlation between Lp(a) levels, coronary heart disease and atherosclerosis. The presence of Lp(a) is therefore considered to be a high-risk factor for coronary artery disease and atherosclerosis. The protein components of Lp(a) consist of one molecule of apolipoprotein B-100 (apoB-100) and two molecules of apolipoprotein (a) [apo(a)], which are disulphide-linked to apoB-100. Several

immunologically related forms of apo(a) have been identified. Apo(a) shares a strong homology with plasminogen, a plasma serine protease zymogen that consists of five homologous repeated domains (kringles) and a trypsin-like protease domain [5]. Circulating plasminogen interacts with the endothelial cell surface and is converted to a catalytically active state via a plasminogen activator synthesised and secreted by the endothelial cells. Lp(a) is present in the microvasculature of inflamed tissue. It can competitively inhibit plasminogen binding to the endothelial surface, thereby interfering with surface-associated plasmin generation. Lp(a) also induces synthesis and secretion of the major physiological inhibitor of plasminogen activator (plasminogen activator inhibitor-1) [6]. One hypothesised effect of Lp(a) is inhibition of plasminogen activation and promotion of a hypercoagulable thrombotic state.

It is controversial whether hypertriglyceridaemia is a risk factor in vascular disease. Generally, very low density lipoprotein (VLDL) levels are inversely related to levels of HDL which has a negative association with vascular disease [4]. Hypertriglyceridaemia is frequently associated with hyperinsulinaemia which appears to be an important risk factor in atherogenesis [7]. Several studies have reported an increased insulin response to oral glucose in non-diabetic patients with ischaemic disease of the heart, lower limbs and brain, and population studies have also shown that insulin responses are higher in populations with high incidence of cardiovascular disease [7].

*Diabetes mellitus*

Diabetes mellitus is associated with an increased incidence of vascular disease including coronary heart disease, cerebrovascular disease and peripheral vascular disease. Although this may be accounted for in part by the presence of the well-characterised risk factors, much of the increased incidence remains unexplained. Abnormalities in plasma lipid and lipoprotein levels are commonly observed in both Type 1 (insulin-dependent) and Type 2 (non-insulin-dependent) diabetes mellitus [4].

In well-controlled Type 1 diabetes, LDL metabolism is generally normal. However, poor metabolic control with consequent hyperglycaemia can lead to glycation of LDL, which renders it more atherogenic, since it can be taken up by the macrophage scavenger pathway leading to formation of foam cells. Diabetic LDL may also be more susceptible to oxidative modification which would lead to increased clearance by macrophages. Plasma levels of VLDL are commonly elevated in diabetes and frequently correlate with glycaemic control. Hypertriglyceridaemia is frequently associated with high fatty acid levels and hepatic overproduction of VLDL, although diminished clearance by lipoprotein lipase may also occur. There may also be abnormalities in VLDL composition by way of enrichment of free and esterified cholesterol, which is a major risk factor. Hypertriglyceridaemia and increased production of VLDL is common in Type 2 diabetes. The VLDL particles are enriched with triglyceride and also cholesterol ester and may be more atherogenic.

Hyperinsulinaemia is an important factor in both Type 1 and Type 2 diabetes [3, 7]. Physiologically, insulin is secreted into the hepatic portal circulation and a high proportion of the insulin is cleared during first pass through to the liver. Consequently, the concentration of insulin in the portal circulation is much higher than in the peripheral circulation. In conventional insulin therapy in Type 1 diabetes, insulin is delivered into the peripheral circulation. Consequently, the portal insulin concentration is similar to or lower than the peripheral concentration. In order to achieve the same degree of insulinisation of the liver in conventional insulin therapy, a higher peripheral insulin concentration is necessary than would occur in the physiological situation, with consequent hyperinsulinisation of peripheral vessels.

Type 2 diabetes is associated with impaired clearance of glucose in response to insulin. Plasma insulin levels are higher than in normal subjects and the insulin response to oral glucose loads is also elevated. Studies on patients with cardiovascular disease and diabetes have also suggested increased insulin levels and responses to oral glucose in diabetic patients with cardiovascular disease.

The identification of risk factors does not explain the initiating factors or the underlying mechanism of atherogenesis. Several questions remain unanswered in relation to the process of atherogenesis in the non-diabetic state and the interactions of diabetes with this mechanism.

*Animal models and in vitro studies*

Although hypercholesterolaemia is a major risk factor in human atherogenesis, the commonly studied rodent models do not develop atherosclerotic lesions in response to hypercholesterolaemia. High circulating plasma cholesterol levels can be attained in rabbits, pigs and primates by feeding with a high-fat diet [8, 9]. The rabbit develops atherosclerosis when fed a high cholesterol diet. Hypercholesterolaemia is produced when cholesterol intake exceeds the normal mechanism of cholesterol clearance. The Watanabe heritable hyperlipidaemic rabbit (WHHL) develops atherosclerosis even on a cholesterol-free diet. This rabbit strain has high LDL levels and impaired clearance of LDL because of a genetic defect in the LDL receptor. Clearance of chylomicrons is normal and conversion of VLDL to intermediate density lipoprotein (IDL) is also normal; however, IDL is not cleared by the liver but is converted to LDL in plasma, leading to overproduction of LDL. Since hepatic IDL clearance is mediated by the LDL receptor, overproduction of LDL and impaired clearance can both be accounted for by the LDL receptor defect. Atherosclerosis in the WHHL rabbit resembles human disease histologically. Cholesterol ester is deposited in macrophage-derived foam cells and in smooth muscle cells of the intima and media. The lesion

develops into a full-blown atherosclerotic plaque with necrotic cholesterol ester-filled core and fibrous cap. Cholesterol is deposited earliest at sites where blood vessels bifurcate or branch from the aorta. These sites are involved in early human atherosclerosis. One of the problems with the fat-fed rabbit model is that it has elevated levels of beta-VLDL but not of LDL, whereas the WHHL rabbit has elevated LDL and low HDL [8].

Pigs develop arterial changes similar to the early atherosclerotic lesions in man within the first year of life, and they develop advanced lesions within 8-12 years. Feeding of high-cholesterol diets accelerates development of atherosclerosis and results in non-occlusive thrombi. The structure of the tunica media of the aorta and coronary arteries is similar to that of man as is early intimal proliferation composed of smooth muscle cells. In the miniature pigs fed high cholesterol/fat diets, proliferative lesions can be produced within 30 days, which then progress to advanced atheromatous lesions [9].

Studies in the rabbit, pig and non-human primates [8-10] have helped in understanding the sequence of events with respect to: 1) entry of lipoproteins beneath the endothelium; 2) cytological changes of adherence of lymphocytes and monocytes followed by their entry into the arterial wall; 3) sequestration of LDL-lipid by macrophage-derived foam cells; 4) formation of fatty streak, progressing to advanced lesions of atherosclerosis. The first observable change by electron microscopy is the presence of lipoprotein-like particles of varying size (ranging from the size of LDL to the size of chylomicrons) in the interstitium beneath the endothelial cells. This precedes the cytological changes which include the adhesion of lymphocytes and monocytes. The localisation and adhesion of these cells is indicative of an inflammatory response. However, there is very little evidence for adhesion of neutrophils. This apparent selectivity of monocyte but not neutrophil binding may be indicative of specific chemoattractant or adhesive proteins. Subsequent changes include migration of monocytes between the endothelium and the intima and accumulation of lipid and conversion to foam cells with development of fatty streak sometimes accompanied by changes in the morphology of the endothelium and progression to atherosclerotic plaques.

LDL extracted from atherosclerotic plaques has characteristics of oxidised LDL. The current hypothesis is that oxidised LDL, present in the circulation or more likely formed as LDL, is transcytosed across the endothelial cell. Immunocytochemistry has demonstrated cellular deposits of oxidised LDL in macrophages followed by cell death. The oxidised LDL may be released and accumulate in the surrounding matrix. There is some question as to the extent by which oxidised LDL extracted from atherosclerotic plaques may have formed in the course of the extraction procedure. Additional evidence for the involvement of oxidised lipid in the development of atherosclerotic lesions has been obtained from animal studies with anti-oxidants which act as traps for free radicals including vitamin E, butylated hydroxytoluene and probucol. The latter suppresses the development of lesions in the WHHL rabbit. Probucol may have different effects in rabbit and man. It has been shown to lower HDL levels and is a poor lipid lowering agent. This emphasises the need for primate models to study the effects of anti-oxidants on lipoprotein profiles and development of atherosclerotic lesions in systems closer to humans.

In addition to the native LDL receptor which binds normal LDL, macrophages and smooth muscle cells also have an additional scavenger receptor, described as the acetyl-LDL receptor, that binds oxidised LDL. The scavenger receptor, unlike the native LDL-receptor, does not appear to be regulated and may bind a wide range of ligands. The term "oxidised LDL" encompasses a wide spectrum of lipid oxidation products formed from polyunsaturated fatty acids as well as proteins with modified epsilon-amino groups which have altered electrophoretic mobility.

Uptake of oxidatively modified LDL in intimal macrophages is suggested to be a contributing factor to the inflammatory response. This hypothesis is supported by in vitro studies demonstrating that oxidised LDL: stimulates the release from endothelial cells of chemoattractants to monocytes and also increases the rate of adhesion of monocytes to endothelial cells via induction of receptors in the endothelial cells; stimulates the release of growth regulatory peptides from monocytes; activates T cells by a monocyte-dependent process; accelerates the differentiation of monocytes to macrophages; and alters the sensitivity of muscle cells to growth factors [11, 12]. The effect of oxidised LDL is biphasic with stimulation of growth at low concentrations and cytotoxicity at higher concentrations. The latter toxic effect is suppressed or abolished by superoxide dismutase suggesting that it is mediated via free radicals [11]. Studies in vitro have shown that oxidised LDL, produced by pre-treatment of LDL with $Cu^{2+}$ is cytotoxic to many cells in culture including endothelial cells, macrophages and smooth muscle cells, particularly during incubation in a serum-free medium. The cytotoxicity of oxidised LDL preparations within a study appears to correlate with the degree of oxidation. However, there is considerable variation between studies in the extent of toxicity or induction of growth regulatory peptides at low concentrations of oxidised LDL. This may reflect the heterogeneity of oxidation products in different studies. Variation in the degree of polyunsaturation of the fatty acid composition of LDL would affect the susceptibility to peroxidation. Whether differences in fatty acid composition of LDL in diabetes might account for an increased susceptibility to oxidation is an important issue. HDL may counteract the cytotoxic effect of oxidised LDL, but whether this reflects partitioning of oxidation products into the HDL particle has not been established. Animal models are useful to study the temporal changes in development of lesions in different anatomical sites and

reversibility of changes after lowering of plasma cholesterol levels or for studying the effects of diabetes, insulin, anti-oxidants and other therapeutic agents. Recent data on primates indicates that lesions can be reversed at all stages of development, including advanced lesions.

*Human diabetes*

In human diabetes atherosclerosis prominently affects peripheral vessels as well as cerebral and carotid arteries, which may indicate different initiating factors, the site of insulin administration, or a different tissue response. There has been little in the way of comparative histological studies of atherosclerotic lesions in diabetic patients and normal subjects. Although there are some indications that oxidised LDL levels may be elevated in the circulation in diabetes, there is no information on the role of endothelial cell oxidation of LDL, which may be an important contributor.

*Hypothesis:* Atherosclerosis involves a specialised chronic inflammatory fibroproliferative process which is excessive and, in its excess, this protective response has become the disease state.

**State-of-the-art and future perspectives**

*Cytokines and secondary gene expression*

Adherent monocytes and lymphocytes that accumulate as a result of the inflammatory response produce a variety of cytokines [13]. Monocytes produce interleukin-1 (IL-1), interleukin-6 and tumour necrosis factor alpha (TNF). T-lymphocytes produce interleukin-2 and interferon gamma. These cytokines can regulate the expression of growth factor receptors and growth factors, and these endogenously produced growth factors in turn affect the differentiation and proliferation state, directly or indirectly, through secondary gene expression. They may also control the production of extracellular matrix proteins and enzymes that affect the matrix as well as production of cell adhesion molecules [14].

*Platelet-derived growth factor* (PDGF) is a major mitogen of smooth muscle cells. It is a 30 kDa disulphide-linked dimeric protein composed of two homologous polypeptide chains denoted A and B. The different isoforms of PDGF (PDGF-AA, -AB and -BB) bind with different affinities to two structurally related receptors. The A subunit of PDGF binds to alpha receptors, whereas the B subunit binds to alpha or beta receptors. Studies in vitro using antibodies to PDGF receptors and anti-sense RNA to PDGF A and B chains have indicated that the effects of various cytokines on smooth muscle cell proliferation are mediated by secondary changes in the expression of PDGF A and B chains or changes in receptor expression. Transforming growth factor beta (TGFβ) and IL-1 have bimodal effects on DNA synthesis in smooth muscle cells, with maximum stimulation at low concentrations and diminished stimulation at higher concentrations [15]. This bimodal response with respect to concentration suggests that there are built-in controls that can limit the cellular proliferative response. The stimulatory component at low concentrations of TGFβ and IL-1 results in increased PDGF-A mRNA and PDGF secretion, which is blocked by neutralising antibodies to PDGF. This is the result of secondary expression of PDGF-A while the diminished stimulation at high concentrations of TGFβ is associated with decreased expression of PDGF alpha receptor. Stimulation of DNA synthesis by low concentrations of TGFβ is delayed relative to that induced by exogenous PDGF consistent with an indirect mechanism. Thus, low concentrations of TGFβ induce proliferation by stimulating production of PDGF-AA. Autocrine control by PDGF-AA is inhibited at high concentrations of TGFβ because of down regulation of the alpha receptors and possibly also by other mechanisms. Similarly, TGFβ, TNF and IL-1 induce PDGF-B chain formation in vascular endothelial cells. Thus, these cytokines indirectly affect autocrine and paracrine cell proliferation via induction of PDGF gene expression and secretion of PDGF.

*Basic fibroblast growth factor* (bFGF) is a potent mitogen for vascular endothelial cells and smooth muscle cells. It is synthesised by smooth muscle cells and endothelial cells and sequestered in sub-endothelial cell extracellular matrix. It is abundant in large blood vessels such as the carotid and aorta. A role for bFGF in vivo has been demonstrated by observations that bFGF is responsible for smooth muscle cell proliferation in the carotid induced by balloon catheter de-endothelialisation [16].

*Angiotensin II* is important in the maintenance of vascular homeostasis. It is activated during intravascular volume contraction and induces vasoconstriction via tissue-specific responses in many target organs. These effects are mediated via specific receptors that are coupled to activation of phospholipase C. In addition to vasoconstriction, angiotensin II also has hypertrophic and proliferative effects in vascular tissue in vivo and in vitro [17]. Angiotensin II enhances neointimal proliferation in vivo following vascular injury and promotes growth of smooth muscle cells in culture and expression of the proto-oncogenes c-fos, c-myc and c-jun as well as PDGF-A [18]. The effects of angiotensin II on smooth muscle hypertrophy are associated with both stimulatory and inhibitory effects on proliferation [19]. The stimulatory component is associated with induction of PDGF-A and FGF and the inhibitory component with expression of TGFβ. Inhibition of synthesis of FGF with the corresponding anti-sense DNA leads to dominance of the inhibitory component while inhibition of TGFβ synthesis favours the dominance of the positive component. Apparently, anti-sense to PDGF-A has no effect on proliferation. Angiotensin II mRNA is expressed in the smooth muscle layer of blood vessels and expression is

increased following injury. It may therefore have an autocrine role in vessel hypertrophy.

*Thrombin* is a component of the coagulation cascade that is present at sites of tissue injury and may be involved in the inflammatory response in atherosclerosis [20]. It is a proteinase that can stimulate multiple responses in endothelial cells, including production of prostacyclin, platelet activating factor, von Willebrand activity and PDGF and adhesion of monocytes [21]. The latter response is less sensitive to thrombin than the induction of PDGF expression and secretion. This difference in sensitivity suggests that these responses are mediated by different mechanisms. Stimulation of monocyte adhesion by thrombin is slower than that caused by activators of protein kinase C, suggesting that it may be mediated by an indirect mechanism [22]. Stimulation of PDGF secretion, though not of mRNA expression, is blocked by amiloride or $Na^+$-free media suggesting that it is dependent on $Na^+$-$H^+$ exchange, presumably through either alkalinisation or changes in cell volume or shape.

*Heparin-binding epidermal growth factor-like peptide* (HB-EGF) is a potent mitogen and chemotactic factor of smooth muscle cells produced by macrophages and also smooth muscle cells [23]. It is also present in wound fluid. Physiologically it may have a role in the macrophage-mediated response to injury, and pathologically it may have a role in smooth muscle cell hyperplasia associated with atherosclerosis, re-stenosis and hypertension. It is an O-glycated polypeptide of about 86 amino acid residues, consisting of a C-terminal epidermal growth factor (EGF)-like domain that shares about 40% homology with EGF or transforming growth factor alpha (TGFα) and a hydrophilic N-terminal extension of 35-45 amino acid residues containing the heparin-binding domain that has no counterpart in EGF and TGFα. The EGF-like C-terminal domain is involved in receptor binding and the heparin-binding domain might be involved in the interaction of this growth factor with cell surface heparin sulphate proteoglycans. HB-EGF binds to the EGF receptor and induces auto-phosphorylation but is more potent than EGF at inducing proliferation and chemotaxis of smooth muscle cells. The different potency could be due to presence of distinct receptors for HB-EGF and EGF, or to a greater affinity of binding of HB-EGF than of EGF to the same receptor because of the heparin-binding domain.

*Extracellular binding of pro-forms of growth factors*

An interesting mechanism of growth factor regulation that may be of particular relevance to atherosclerosis is the extracellular localisation of special forms of growth factors bound to cell surface proteoglycans. The long form of PDGF-A and the proform of PDGF-B which contain exon-6-encoded peptides and are secreted by smooth muscle cells and endothelial cells, respectively, bind to cell surface proteoglycans [24]. Little is known of the physiological significance of extracellular binding: whether it constitutes an active or latent extracellular store requiring cleavage by extracellular proteases; whether it serves as a slow release store or whether it can be released rapidly in response to exogenous proteases during an acute inflammatory response. The identification of the cell surface proteoglycans that are responsible for sequestration of this extracellular store and of the plasma proteins that bind circulating PDGF will be important in understanding these regulatory mechanisms. All dimeric forms of PDGF bind to plasma alpha-2-macroglobulin but only PDGF-AB and PDGF-BB bind to a recently identified 42 kDa protein termed SPARC (a secreted protein that is acidic and rich in cysteine residues) [25]. This protein is produced by endothelial cells in culture and binds to the extracellular matrix. It causes cell rounding in fibroblast cultures and also modulates the production of extracellular matrix. It binds and inhibits the activity of PDGF-BB and PDGF-AB but not of PDGF-AA. Immunohistochemical staining for SPARC in atherosclerotic lesions has shown co-localisation with PDGF-B staining in macrophages as well as additional staining in macrophages and smooth muscle cells in these lesions and in underlying medial cells. Since SPARC is present in platelets, it may have a more ubiquitous role in tissue repair. Changes in the expression of SPARC may alter the bioactivity of PDGF. Growth factor binding proteins in plasma and on the cell surface or extracellular matrix may have an important role in determining growth factor availability and activity.

*Antagonists to smooth muscle mitogens*

Since several growth regulatory peptides including PDGF, TGFb, FGF and angiotensin II have been implicated in diseases involving proliferative responses, specific antagonists to these growth factors are of potential therapeutic use. Several approaches have been explored in the search for antagonists to PDGF. These include the use of antibodies and peptides with a similar structure as the extracellular domain of the PDGF receptor. The limitation of the latter approach is the relatively low affinity of binding relative to the native receptor, coupled with the high clearance of peptide necessitating a large quantity of peptide. Since binding of PDGF induces dimerisation of its receptor which is thought to be important for receptor activation, an alternative approach to the development of PDGF antagonists would be selection of ligands that prevent receptor dimerisation. As a first step towards this goal, the cysteine residues of PDGF responsible for interchain disulphide bonding have been identified and mutated [26]. The obtained monomeric PDGF was found to retain receptor binding activity but also to have agonistic activity in receptor dimerisation and phosphorylation assays [27]. Possibly monomeric PDGF induces receptor dimerisation via induction of a conformational change in the extracellular part of the receptor, or alternatively, the

"monomeric" PDGF can form non-disulphide bonded dimeric complexes. Clearly, further mutations in the PDGF molecule are needed to achieve antagonistic properties.

Angiotensin II enhances neointimal proliferation in vivo following vascular injury. Angiotensin converting enzyme (ACE) has a key role in the control of local angiotensin II production. Both angiotensin II and ACE are expressed in a variety of tissues and in the smooth muscle layer of blood vessels, and expression is increased following vascular injury. Angiotensin antagonists and inhibitors of ACE may be of use in attenuating smooth muscle proliferation after injury. Following vascular injury of the abdominal aorta in the rat with a balloon catheter, ACE activity is elevated in the smooth muscle cells of the vasculature though not in the plasma. Inhibition of tissue rather than circulating activity may therefore be important in attenuating smooth muscle cell proliferation. Animal studies have shown that inhibitors of ACE (quinapril and cilazipril) cause a dose-dependent inhibition of plasma and vascular ACE and of neointimal formation after blood vessel injury. Higher doses of inhibitor were required for inhibition of tissue activity and neointimal formation than for inhibition of plasma activity, indicating that the latter does not provide an index of inhibition of tissue activity or of the efficacy at inhibiting proliferation. This may account for the ineffectiveness of cilazipril in some human studies.

## Perspectives

The potential networking of cells, cytokines and growth regulatory molecules among the different cells in the lesions of atherosclerosis present numerous opportunities to control these interactions and to modulate lesion formation and progression. Because atherosclerosis represents the culmination of a proliferative response, understanding the factors in diabetes that induce or modify this response will be important for prevention and treatment of this disease, particularly as it relates to diabetes.

## Conclusions and recommendations

The group recommended development of models of diabetes in animals which develop vascular disease as similar to human disease as possible. Most of the data in experimental diabetic animals is derived from relatively short-term studies—hence the chronic effects of long-term diabetes remain to be assessed.

There is a need for a systematic study of lesions of atherosclerosis from diabetic humans compared to non-diabetic humans. Further study of inflammation is warranted in diabetic patients, since observations made in such studies could have relevance to the process of atherogenesis. A better understanding of the basis for deficient wound repair in diabetic patients should shed light on processes such as atherogenesis as well.

The question needs clarification whether there is a significant incidence of atherosclerosis in diabetic patients who are not hyperlipidaemic. Since oxidised lipoproteins may be increased in these patients, further study of the role of oxidised LDL in vascular disease and of the factors that cause LDL-oxidation is warranted.

In studying cellular responses and interactions in vitro, human cells from diabetic and non-diabetic individuals should be studied and compared. Further studies are needed of interactions among cells involved in atherogenesis with factors associated with diabetes such as advanced glycation end-products, insulin, insulin-like growth factors, glucose and other factors present in excess in plasma in people with diabetes. Investigation of the responsiveness of cells from diabetic patients to growth regulatory molecules and cytokines should provide insights into differences with cells from normal tissues.

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