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Inflammation and cholesterol

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Atherosclerosis develops as a result of a chronic arterial inflammation and intimal fibrosis. The disease represents in many respects a vascular repair process activated in response to injury caused by toxic breakdown products of aggregated and oxidized lipoproteins. The initial response of the artery involves expression of adhesion molecules and recruitment of leukocytes. Degenerated lipoproteins are removed from the extracellular space by macrophages. If lipoproteins continue to accumulate, the inflammatory process becomes chronic and cytokines stimulate smooth muscle to migrate into the intima. These cells proliferate and form an atherosclerotic plaque. Plaque cell death and inflammation in response to oxidized lipids and other toxic factors may cause plaques to rupture.
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Introduction

Atherosclerosis is a disease characterized by a chronic vascular inflammation and fibrotic degeneration that affects primarily the intima of large and medium-sized arteries[1,2]. It is believed that the vascular inflammation is activated in response to an injury to the vascular wall and that the major risk factors for development of cardiovascular disease, such as hypercholesterolaemia, hypertension, diabetes and smoking, are the main causes of this injury[3].

During the earliest stages of atherosclerosis, monocytes and T cells infiltrate the arterial intima[4]. Their main function is probably to remove aggregated and oxidized lipoproteins that have become trapped in the extracellular matrix of the arterial wall[5]. Should this defence mechanism become challenged over a period of many years and the removal of toxic oxidized lipoproteins fail, then the vessel wall will become subject to chronic inflammation. Because inflammation is the major signal for repair, this situation leads to activation of vascular repair responses. Evidence from several models of vascular injury suggests that the process of vascular repair is very stereotypical[6]. Irrespective of whether the artery is injured from the outside or the inside, the repair response is mediated by medial smooth muscle cells that are modulated into a fibroblast-like phenotype and migrate into the intima, where they proliferate and produce extracellular matrix. This also occurs in atherosclerosis when fatty streaks transform into raised fibromuscular lesions. In many respects the fibromuscular atherosclerotic plaque resembles the hypertrophic scar that develops when the healing of a skin wound is disturbed by a chronic inflammatory process.

Inflammation also plays an important role in more advanced stages of atherosclerosis. Atherosclerotic lesions do not usually give rise to clinical symptoms until they degenerate and rupture[7]. This process starts with accumulation of extracellular lipids and an increased rate of cell death in the core region of the plaque. Toxic oxysterols and lipid peroxides that are present in the extracellular lipid deposits are responsible for much of the cell death, but insufficient oxygen supply and apoptosis induced by inflammatory cytokines are also of importance. Disintegration of dying cells in the core of the plaque results in increased local inflammatory activity[8]. Microscopically, these lesions are characterized by a cap of fibrous tissue, containing smooth muscle cells, collagen and other extracellular matrix components, that covers a core of inflammatory cells; and accumulation of extracellular lipids, necrotic cells and tissue debris. If the fibrous cap also becomes affected by increased cell death and inflammation, then the plaque may rupture.

Inflammatory markers and risk for development of coronary heart disease

As discussed above, inflammation is a prominent aspect of all stages of atherosclerosis. Because affected arteries together represent a considerable amount of tissue,

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atherosclerosis would be likely to result in general signs of increased inflammatory activity. Indeed, during the past few years it has become clear that high levels of C-reactive protein (CRP) and other markers of inflammatory activity are associated with an increased risk for development of coronary heart disease (CHD), as well as other atherosclerosis-dependent vascular diseases[9,10]. It is likely that this association reflects the inflammatory activity of the atherosclerotic disease process and that the levels of inflammatory markers in the circulation correlate with the general severity of the disease[11]. To date 10 large prospective studies, six conducted in the U.S.A. and four in Europe, have consistently shown that CRP is a powerful predictor of future first coronary event in apparently healthy men and women (for review[12]). For example, an analysis of data from the Physicians' Health Study[13] showed that those in the highest quartile of CRP had a twofold higher risk for future stroke, a threefold higher risk for future myocardial infarction and a fourfold higher risk for peripheral vascular disease.

The increase in relative risk for development CHD caused by CRP is independent of other risk factors, and stratified analyses performed in the Women's Health Study[14] have shown that CRP is a strong predictor of future coronary events, even among women with no history of hyperlipidaemia, hypertension, smoking, diabetes, or family history of CHD. The observation that CRP is an independent predictor of risk for development of CHD is somewhat unexpected, assuming that the idea that inflammation reflects vascular injury caused by traditional risk factors is correct. This may not be entirely surprising, however, bearing in mind the complexity of the atherosclerotic process and possible individual differences in the effectiveness of defence mechanisms, and that many other factors may affect the levels of inflammatory markers.

Several studies have also suggested that individuals with increased inflammatory activity, as assessed by determination of CRP, are those who benefit most from preventive treatment. In the Cholesterol and Recurrent Events study[15], those with high CRP had a 54% reduction in coronary events as compared with 25% in those with a low CRP in response to treatment with pravastatin. Treatment with pravastatin has also been shown to reduce CRP levels, suggesting that this agent has anti-inflammatory properties[16]. Moreover, in the Physicians' Health Study[13] aspirin decreased the risk for future myocardial infarction by 60% in apparently healthy men with a high CRP (>2·1 mg . l^{-1}) but only by 14% in those with a low CRP (<0·55 mg . l^{-1}).

Lipid-induced inflammation during early lesion formation

What, then, are the causes of vascular inflammation in atherogenesis? As discussed above, it is likely that chronic injury inflicted by long-term exposure to the epidemiologically identified cardiovascular risk factors is of major importance. Several lines of experimental evidence suggest that the role of lipoprotein-derived lipids is of particular importance in this process[17]. Induction of hypercholesterolaemia in mice, rabbits, pigs and many other animals (but not all) results in activation of vascular inflammation within 6–8 weeks[18,19].

The first changes include accumulation of lipoproteins, primarily low-density lipoprotein (LDL), in the extracellular matrix of the vasculature. LDL particles tend to attach to sulphate-containing proteoglycans, where they aggregate and become oxidatively modified[20,21]. The factors that are responsible for these modifications to LDL remain to be fully elucidated, but appear to involve reactive oxygen species as well as different membrane and extracellular tissue-associated enzymes[2]. Recent gene knockout studies in mice suggest that the enzyme lipoxygenase may be of particular importance in this process[22]. Several defence mechanisms, including antioxidant vitamins and enzymes, exist to prevent oxidative damage of accumulated lipoproteins, but in situations of a continuous lipid overload these defence systems may eventually fail.

A second phase involves activation of an acute inflammatory reaction in the arterial wall. A key element of this reaction is expression and activation of adhesion molecules, including intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and E-selectin, on the endothelium[23]. The endothelial adhesion molecules serve to attach circulating monocytes and T cells that infiltrate the vascular wall. Animal experiments have also been used to study the association between vascular LDL accumulations, lipid oxidation and endothelial expression of adhesion molecules. In rats injected with human LDL, LDL particles can be observed accumulating in the aorta within 6 h, expression of epitopes specific for oxidized LDL within 6–12 h, and endothelial expression of adhesion molecules within 12–24 h[24]. Pre-incubation of LDL with antioxidants inhibits some, but not all, of the activation of endothelial adhesion molecule expression in this model, suggesting that mechanisms other than lipid oxidation also are involved. Among the other mechanisms that can explain the increased expression of endothelial cell adhesion molecules and leucocyte infiltration in atherosclerotic lesions are decreased production of nitric oxide[25], native lipoproteins (particularly triacylglycerol-rich lipoproteins)[26,27] and altered levels of certain fatty acids[28].

Do lipoprotein-derived lipids affect intracellular signal pathways in the vascular wall?

Several types of lipid and phospholipid molecules have important functions in intracellular signalling and regulation of gene transcription. Diacylglycerol, platelet-activating factor (PAF) and other PAF-like phospholipids, lysophosphatidylcholine, ceramide and lipid-derived radicals are some examples of lipid signal molecules that mediate effects of membrane-bound receptors[29]. The levels of these lipid signal substances are usually strictly controlled in the cellular environment. In the atherosclerotic

vascular wall, however, this balance may be disturbed by lipids and phospholipids released from lipoproteins that are being degraded and oxidized in extracellular matrix. Both PAF-like phospholipids and lysophosphatidylcholine are generated in significant amounts when lipoproteins are oxidized. PAF-like phospholipids act on specific PAF receptors on the cell surface, leading to activation of a number of pro-inflammatory genes[30]. Lysophosphatidylcholine activates membrane-bound protein kinase C, which is among the most important regulators of gene transcription in cells[31].

Another molecule that is believed to play an important role in lipid-induced inflammation in the vascular wall is the transcription factor nuclear factor-κB (NF-κB)[32]. This factor plays a central role in regulating inflammation and immune responses. It activates a large number of genes, including those that encode cytokines, adhesion molecules and growth factors. It is present in atherosclerotic plaques, but rarely in normal arterial tissue[32]. NF-κB is redox sensitive and is activated by radicals. Oxidation of lipoproteins is associated with generation of a large number of different radicals, suggesting that lipid-induced inflammation in the vascular wall may be mediated by activation of the pro-inflammatory transcription factor NF-κB by radicals released from oxidized lipids. Induction of hypercholesterolaemia, as well as injection of a single bolus of LDL, has been shown to activate arterial expression of NF-κB[33]. Interestingly, cell culture experiments suggest that triglyceride-rich lipoproteins, such as very-low-density lipoprotein, are more potent inducers of NF-κB in vascular cells than are LDL and oxidized LDL[28,34].

Immune responses against oxidized LDL – a possible mechanism for athero-protection

As discussed above, accumulation of modified lipoproteins in the vascular extracellular matrix results in activation of endothelial adhesion molecule expression and infiltration of mononuclear leucocytes. In the intima, monocytes differentiate into macrophages that express different forms of scavenger receptors. These receptors will effectively remove aggregated and oxidized LDL from the extracellular space, thereby limiting injury to surrounding cells. Macrophages that have taken up large amounts of lipids through this mechanism develop a characteristic foam-cell appearance. The inflammatory response to vascular lipid accumulation will in this way act as a defence mechanism, limiting further toxic effects of lipid oxidation. However, this protective response is not without risks. If lipoproteins continue to accumulate then the artery will enter a state of chronic inflammation, leading to activation of a tissue repair process and intimal fibrosis.

During recent years it has become clear that other athero-protective mechanisms exist, which involve specific immune responses against structures present in oxidized lipoproteins. Oxidized LDL that has been taken up by macrophages is processed and epitopes presented for T cells by HLA-DR receptors[35]. This leads to activation of both cell- and antibody-mediated immune responses. Up to 20% of all T cells that are present in human atherosclerotic plaques are specific for antigens present in oxidized LDL[35]. Immune responses are activated against a large number of different epitopes in oxidized LDL, including oxidized phospholipids and aldehyde-containing peptide sequences[36]. High levels of autoantibodies against oxidized LDL have been reported in persons with increased risk factors or clinically manifest atherosclerosis, including those with CHD, acute myocardial infarction, peripheral vascular disease, hypertension and pre-eclampsia[37–39].

One possible explanation for these associations could be that immune responses against oxidized LDL are atherogenic and that atherosclerosis is a lipoprotein autoimmune disease. The fact that immune responses against grafted organs are associated with development of an aggressive atherosclerosis in the transplant suggests that immune responses against vascular tissue are atherogenic. It has transpired, however, that the full picture is more complicated. The role of immune responses against oxidized LDL in atherosclerosis has been studied in hypercholesterolaemic mice and rabbits immunized with homologous oxidized LDL[40–45]. The somewhat unexpected result of these studies was that immune responses against oxidized LDL had a significant inhibitory effect on the development of atherosclerosis. These findings suggest the possibility of developing new approaches for prevention and treatment of CHD based on the selective activation of athero-protective immune responses (i.e. development of an 'atherosclerosis vaccine'). In order to achieve this goal it will be necessary to identify and characterize the exact structure of the epitopes in oxidized LDL that induce athero-protective immune responses. This work is in progress and will hopefully result in development and initial clinical studies of candidate vaccines within a few years.

The mechanism through which immune responses against epitopes in oxidized LDL protects against development of atherosclerosis is not fully understood. The recent development of highly sensitive enzyme-linked immunosorbent assays that can detect LDL with minimal oxidative damage has made it possible to measure circulating oxidized LDL and to demonstrate increased levels in CHD patients[46]. One possibility is that antibodies against oxidized LDL remove LDL particles with minimal oxidative damage from the circulation before they accumulate and injure vascular tissues.

Inflammation and intimal fibrosis

Proliferation of smooth muscle cells in intima is a key factor in the development of raised fibromuscular atherosclerotic plaques (Fig. 1). It was originally proposed that activation of smooth muscle cell proliferation occurs in response to platelet-derived growth factor released from aggregating platelets in association with a denuding endothelial injury[47]. However, it later became clear that most fibromuscular lesions develop under an intact endothelium,

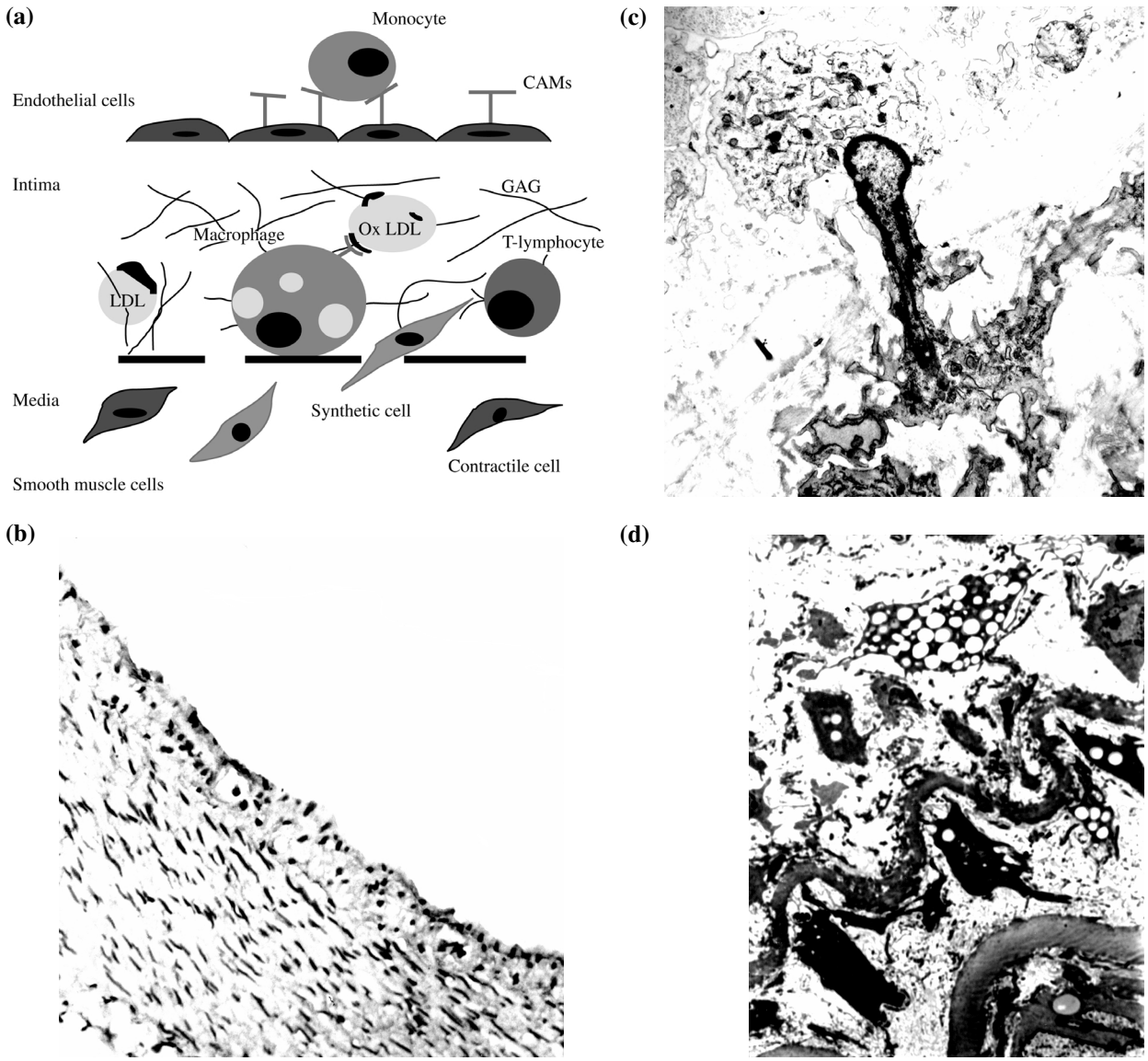

Figure 1 **(a) The early atherosclerotic lesion. Low-density lipoproteins (LDL) have accumulated in the extracellular matrix of the arterial wall, where some particles become aggregated and oxidized (Ox). Monocytes and T cells infiltrate the intima and OxLDL is taken up by macrophage scavenger receptors. Medial smooth muscle cells are activated to modulate into synthetic, fibroblast-like repair cells that migrate through small openings in the internal elastic membrane to enter the intima. (b) An early plaque from the aorta of a hypercholesterolaemic rabbit. (c) A smooth muscle cell migrating through the internal elastic membrane. (d) A macrophage foam cell surrounded by smooth muscle cells in an early lesion.**

and interest was instead focused on growth factors released as a result of an inflammatory process[48]. Activated macrophages release a number of potent growth factors for smooth muscle cells, including platelet-derived growth factor, heparin-binding epidermal growth factor, tumour necrosis factor (TNF)-α and fibroblast growth factor[1]. Smooth muscle and endothelial cells can also produce most of these growth factors in response to injury and inflammation[3]. Activated macrophages also secrete transforming growth factor-β, which inhibits smooth muscle cell growth but is a potent enhancer of extracellular matrix production in these cells. It has been difficult to identify the individual role of each growth factor in the development of fibromuscular lesions and it is likely that they partake in a complex interplay. Studies using blocking antibodies following balloon injury of rat arteries suggest that fibroblast growth factor is involved in early activation of cell proliferation[49] and that platelet-derived growth factor is important for activation of smooth muscle cell migration[50].

As discussed above, the most important role played by lipoprotein lipids in this process is probably that they cause injury and activate inflammation when they accumulate in vascular tissues. However, lipids may also directly influence

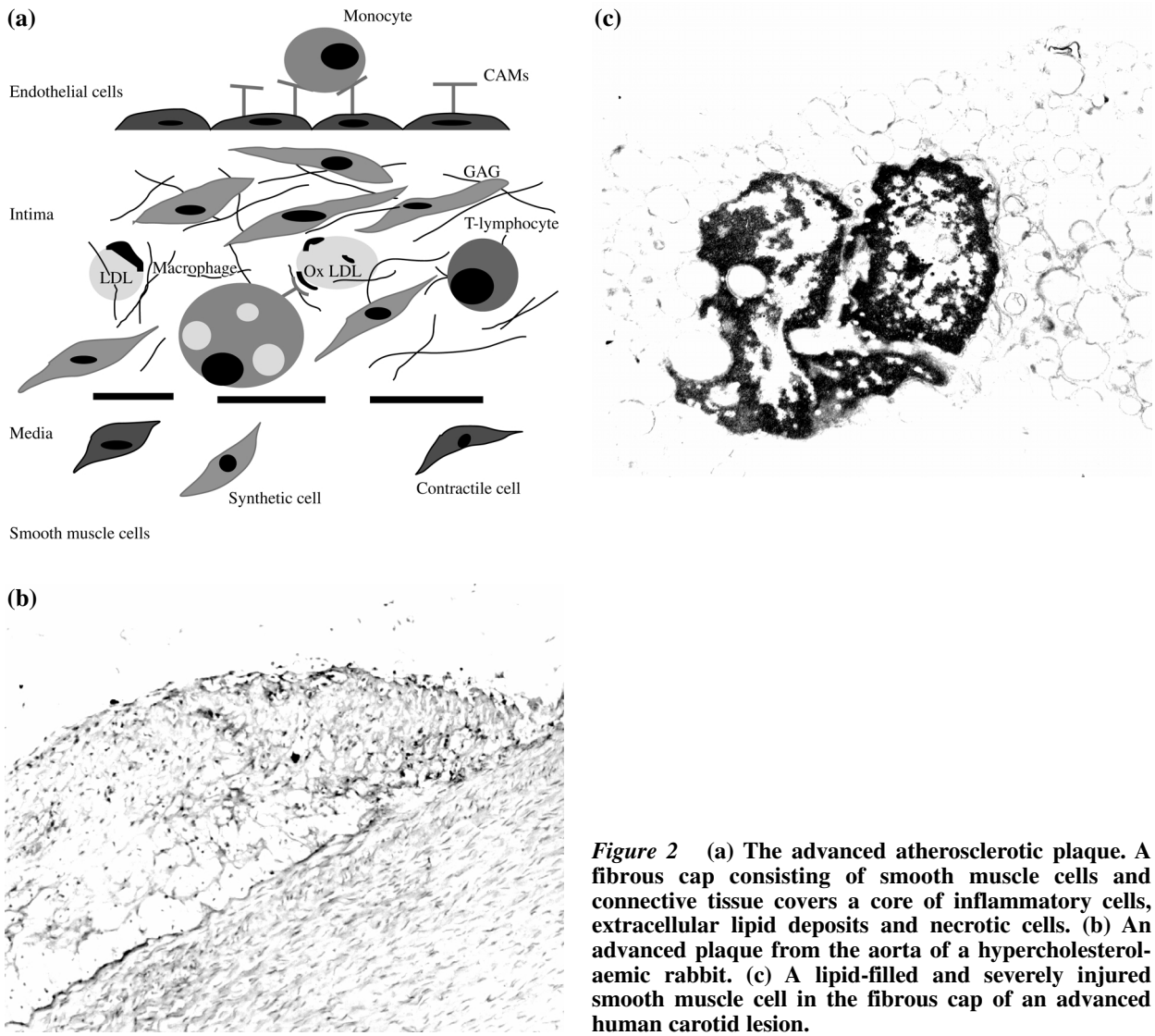

Figure 2 **(a) The advanced atherosclerotic plaque. A fibrous cap consisting of smooth muscle cells and connective tissue covers a core of inflammatory cells, extracellular lipid deposits and necrotic cells. (b) An advanced plaque from the aorta of a hypercholesterolaemic rabbit. (c) A lipid-filled and severely injured smooth muscle cell in the fibrous cap of an advanced human carotid lesion.**

the inflammatory activity. In lower concentrations many molecules generated as a result of lipoprotein oxidation stimulate macrophages and other inflammatory active cells (such as the endothelium), whereas at higher concentrations the toxic effects of these molecules becomes predominant.

TNF-α is a possible link between metabolic alterations and vascular disease in diabetes

The cytokine TNF-α is an interesting example of the complex interaction between lipoprotein lipids, inflammation and atherosclerosis. It regulates the endothelial expression of leucocyte adhesion molecules[51,52] as well as endothelial procoagulant and fibrinolytic activity[53], it activates the synthesis of growth factors and cytokines in vascular cells, and it stimulates the growth of smooth muscle cells[54]. The presence of TNF-α has been demonstrated in human atherosclerotic plaques[55,56], in proliferating smooth muscle cells in balloon-injured rabbit aorta[57], in balloon-injured rat femoral arteries[58] and in the media of coronary arteries during acute rejection of rabbit cardiac allografts[59]. It is also expressed in rat arteries within 6–12 h of a bolus injection of human LDL and in the aorta of apolipoprotein-E-null mice before and during development of atherosclerosis. Moreover, circulating TNF-α levels are significantly increased in patients with premature CHD as compared with age-matched healthy control individuals[60].

TNF-α has also been implicated in the insulin-resistance syndrome, a well characterized risk factor for CHD[61,62]. It is expressed in adipose tissue and skeletal muscle, and is believed to act locally by regulating the sensitivity of the insulin receptor as well as the activity of lipoprotein lipase[63,64]. In these tissues there is an association between increased lipid accumulation and TNF-α expression[65,66]. By inhibiting uptake of fatty acids in adipose tissue by a combined action on lipoprotein lipase and the insulin

receptor, TNF-α may induce hypertriglyceridaemia. Interestingly, TNF-α was initially discovered as the factor responsible for the hypertriglyceridaemia that may develop during septicaemia.

Several other cytokines have similar effects on lipid and glucose metabolism. The association between inflammatory cytokines and regulation of the availability of energy sources may be a way for the body to ensure sufficient energy supplies in situations of great stress, such as tissue injury or infection. It is likely that there is less need for such defence mechanisms in many societies today, but instead expression of cytokines in response to lipid overloading may cause inflammatory reactions in other tissues, such as the vasculature.

Inflammation and plaque rupture

The clinically most important role of inflammation in atherosclerosis is probably during the end stages of the disease. The development of an advanced atherosclerotic plaque is characterized by increased accumulation of extracellular lipids and cell death in the core region of the plaque (Fig. 2)[3]. As a result the plaque is kept intact only by a fibrous cap of smooth muscle cells and connective tissue. Inflammation in the plaque is further enhanced by the presence of damaged or dead cells. Many factors contribute to the increased rate of cell death in the plaques. Accumulation of toxic lipid substances is probably the most important factor, but anoxia and infectious organisms may also be involved. Finally, inflammation in itself may contribute to cell death because smooth muscle cells exposed to combinations of several cytokines may become apoptotic[67].

The plaque enters a critical stage if the fibrous cap also becomes affected by increased cell death and inflammation. The factors that cause cell damage in the cap region are probably the same as those that are responsible for necrosis in the core of the plaque. However, in the fibrous cap this process becomes more dangerous. If smooth muscle cells in the fibrous cap die and disintegrate, then the tissue will become a target for infiltrating macrophages that release collagen-degrading matrix metalloproteinases. This is part of a normal repair response after injury, and the macrophages will simultaneously release growth factors to stimulate surrounding smooth muscle cells to proliferate and produce new extracellular matrix to replace the old, degraded one. If the surrounding smooth muscle cells are themselves severely damaged or dead, however, then they will be unable to fulfil this task, leaving the fibrous cap weakened and the plaque susceptible to rupture by stress forces of the blood flow[68].

Recent studies suggest that treatment with statins increase plaque stability. Atherosclerotic plaques obtained from patients treated with pravastatin for 3 months before carotid endarterectomy contained less oxidized lipids and fewer dead and inflammatory cells than did plaques from patients given placebo[69]. Plaques from patients treated with pravastatin also had more collagen and higher expression of inhibitors of matrix-degrading enzymes. Similar studies performed in experimental animals suggest that this effect is independent of the effect of statins on the plasma LDL-cholesterol level[70].

Conclusion

In summary, inflammation plays an important role throughout the entire atherosclerotic disease process, and vascular lipid accumulation is probably the major cause of this inflammation. Treatment of traditional risk factors, and in particular hypercholesterolaemia, contributes to limit vascular injury and inflammation. In the future, new treatments may become directed more specifically toward the inflammatory process in atherosclerosis. However, it should be kept in mind that this inflammation generally occurs in response to vascular injury and aims to limit tissue damage and induce repair. Another treatment approach that may become reality in the future is the activation of atheroprotective immune responses by vaccines.

References

[1] Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 1993; 362: 801–9.

[2] Glass CK, Witztum JL. Atherosclerosis: the road ahead. Cell 2001; 104: 503–16.

[3] Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340: 115–26.

[4] Hansson GK, Jonasson L, Seifert PS, Stemme S. Immune mechanisms in atherosclerosis. Arteriosclerosis 1989; 9: 567–78.

[5] Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol. Modifications of low-density lipoprotein that increase its atherogenicity. N Engl J Med 1989; 320: 915–25.

[6] Schwartz SM, Campbell GR, Campbell JH. Replication of smooth muscle cells in vascular disease. Circ Res 1986; 58: 427–44.

[7] Falk. Why do plaques rupture? Circulation 1992; 86: III30–42.

[8] Libby P. Molecular bases of the acute coronary syndromes. Circulation 1995; 91: 2844–50.

[9] Ridker PM. Novel risk factors and markers for coronary disease. Adv Intern Med 2000; 45: 391–418.

[10] Ridker PM. Role of inflammatory biomarkers in prediction of coronary heart disease. Lancet 2001; 358: 946–8.

[11] Libby P, Ridker PM. Novel inflammatory markers of coronary risk: theory versus practice. Circulation 1999; 100: 1148–50.

[12] Rifai N, Ridker PM. High-sensitivity C-reactive protein: a novel and promising marker of coronary heart disease. Clin Chem 2001; 47: 403–11.

[13] Ridker P, Cushman M, Stampfer M, Tracy R, Hennekens C. Inflammation, asprin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997; 336: 973–9.

[14] Ridker PM, Hennekens CH, Buring JE, Rifai N. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. N Engl J Med 2000; 342: 836–43.

[15] Ridker PM, Rifai N, Pfeffer MA *et al*. Inflammation, pravastatin, and the risk of coronary events after myocardial infarction in patients with average cholesterol levels. Cholesterol and Recurrent Events (CARE) Investigators. Circulation 1998; 98: 839–44.

[16] Albert MA, Staggers J, Chew P, Ridker PM. The pravastatin inflammation CRP evaluation (PRINCE): rationale and design. Am Heart J 2001; 141: 893–8.

[17] Steinberg D, Lewis A. Oxidative modification of LDL and atherogenesis. Circulation. 1997; 95: 1062–71.

[18] Faggiotto A, Ross R, Harker L. Studies of hypercholesterolemia in the nonhuman primate. I. Changes that lead to fatty streak formation. Arteriosclerosis 1984; 4: 323–40.

[19] Li H, Cybulski MI, Gimbrone MA, Libby P. An atherogenic diet rapidly induces VCAM-1, a cytokine-regulatable mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. Arterioscler Thromb 1993; 13: 197–204.

[20] Camejo G, Olofsson S-O, Lopez F, Carlsson P, Bondjers G. Identification of apo B-100 segments mediating the interaction of low density lipoproteins with arterial proteoglycans. Arteriosclerosis 1988; 8: 368–77.

[21] Oorni K, Pentikainen MO, Ala-Korpela M, Kovanen PT. Aggregation, fusion, and vesicle formation of modified low density lipoprotein particles: molecular mechanisms and effects on matrix interactions. J Lipid Res 2000; 41: 1703–14.

[22] Cyrus T, Witztum JL, Rader DJ *et al*. Disruption of the 12/15-lipoxygenase gene diminishes atherosclerosis in apo E-deficient mice. J Clin Invest 1999; 103: 1597–604.

[23] Cybulsky M, Gimbrone M. Endothelial expression of a mononuclear adhesion molecule during atherogenesis. Science 1991; 251: 788–91.

[24] Calara F, Dimayuga P, Nieman A *et al*. An animal model to study local oxidation of LDL and its biological effects in the arterial wall. Arterioscler Thromb Vasc Biol 1998; 18: 884–93.

[25] Tsao PS, Buitrago R, Chan JR, Cooke JP. Fluid flow inhibits endothelial adhesiveness. Nitric oxide and transcriptional regulation of VCAM-1. Circulation 1996; 94: 1682–9.

[26] Alderson LM, Endemann G, Lindsay S, Pronczuk A, Hoover R, Hayes KC. LDL enhances monocyte adhesion to endothelial cells in vitro. Am J Pathol 1986; 123: 334–42.

[27] Endemann G, Pronzcuk A, Friedman G, Lindsey S, Alderson L, Hayes KC. Monocyte adherence to endothelial cells in vitro is increased by beta-VLDL. Am J Pathol 1987; 126: 1–6.

[28] Dichtl W, Nilsson L, Goncalves I *et al*. Very low-density lipoprotein activates nuclear factor-kappaB in endothelial cells. Circ Res 1999; 84: 1085–94.

[29] Spiegel S, Foster D, Kolesnick R. Signal transduction through lipid second messengers. Curr Opin Cell Biol 1996; 8: 159–67.

[30] Berliner J, Navab M, Fogelman A *et al*. Atherosclerosis: basic mechanisms. Oxidation, inflammation and genetics. J Clin Invest 1995; 91: 2488–96.

[31] Nishizuka Y. Intracellular signaling by hydrolysis of phospholipids and activation of protein kinase C. Science 1992; 258: 607–14.

[32] Collins T, Cybulsky MI. NF-kappaB: pivotal mediator or innocent bystander in atherogenesis? J Clin Invest 2001; 107: 255–64.

[33] Niemann-Jönsson A, Dimayuga P, Jovinge S *et al*. Accumulation of low density lipoprotein in rat arteries is associated with activation of tumor necrosis factor-α expression. Arterioscler Thromb Vasc Biol 2000; 20: 2205–11.

[34] Ares MPS, Kallin B, Eriksson P, Nilsson J. Oxidized low-density lipoprotein induces transcription factor AP-1 but inhibits activation of NFkB in human vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 1995; 15: 1584–90.

[35] Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci USA 1995; 92: 3893–7.

[36] Palinski W, Witztum JL. Immune responses to oxidative neoepitopes on LDL and phospholipids modulate the development of atherosclerosis. J Intern Med 2000; 247: 371–80.

[37] Maggi E, Chiesa R, Melissano G *et al*. LDL oxidation in patients with severe carotid atherosclerosis. A study of in vitro and in vivo oxidation markers. Arterioscler Thromb 1994; 14: 1892–9.

[38] Virella G, Virella I, Leman RB, Pryor MB, Lopes-Virella MF. Anti-oxidized low-density lipoprotein antibodies in patients with coronary heart disease and normal healthy volunteers. Int J Clin Lab Res 1993; 23: 95–101.

[39] Hörkkö S, Binder CJ, Shaw PX *et al*. Immunological responses to oxidized LDL. Free Radic Biol Med 2000; 28: 1771–9.

[40] Palinski W, Miller E, Witztum JL. Immunization of low density lipoprotein (LDL) receptor-deficient rabbits with homologous malondialdehyde-modified LDL reduces atherogenesis. Proc Natl Acad Sci USA 1995; 92: 821–5.

[41] Ameli S, Hultgårdh-Nilsson A, Regnström J *et al*. Effect of immunization with homologous LDL on early atherosclerosis in hypercholesterolemic rabbits. Arterioscler Thromb Vasc Biol 1996; 16: 1074–9.

[42] Nilsson J, Calara F, Regnström J *et al*. Immunization with homologous oxidized low density lipoprotein reduces neointimal formation after balloon in hypercholesterolemic rabbits. J Am Coll Cardiol 1997; 30: 1886–91.

[43] Freigang S, Horkko S, Miller E, Witztum JL, Palinski W. Immunization of LDL receptor-deficient mice with homologous malondialdehyde-modified and native LDL reduces progression of atherosclerosis by mechanisms other than induction of high titers of antibodies to oxidative neoepitopes. Arterioscler Thromb Vasc Biol 1998; 18: 1972–82.

[44] George J, Afek A, Gilburd B *et al*. Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. Atherosclerosis 1998; 138: 147–52.

[45] Zhou X, Caligiuri G, Hamsten A, Lefvert AK, Hansson GK. LDL immunization induces T-cell-dependent antibody formation and protection against atherosclerosis. Arterioscler Thromb Vasc Biol 2001; 21: 108–14.

[46] Holvoet P, Stassen JM, Van Cleemput J, Collen D, Vanhaecke J. Oxidized low density lipoproteins in patients with transplant-associated coronary artery disease. Arterioscler Thromb Vasc Biol 1998; 18: 100–7.

[47] Ross R, Glomset JA. The pathogenesis of atherosclerosis. N Engl J Med 1976; 295: 369–77, 420–5.

[48] Ross R. The pathogenesis of atherosclerosis: an update. N Engl J Med 1986; 314: 488–500.

[49] Lindner V, Lappi DA, Baird A, Majack RA, Reidy MA. Role of basic fibroblast growth factor in vascular lesion formation. Circ Res 1991; 68: 106–13.

[50] Ferns GA, Raines EW, Sprugel KH, Motani AS, Reidy MA, Ross R. Inhibition of neointimal smooth muscle cell accumulation after angioplasty by an antibody to PDGF. Science 1991; 253: 1129–32.

[51] Couffinhal T, Duplàa C, Labat L *et al*. Tumor necrosis factor-α stimulates ICAM-1 expression in human vascular smooth muscle cells. Arterioscler Thromb 1993; 13: 407–14.

[52] Roebuck K, Rahman A, Lakshminarayanan V, Janakidevi K, Malik A. H_2O_2 and tumor necrosis factor-α activate intercellular adhesion molecule 1 (ICAM-1) gene transcription through distinct cis-regulatory elements within the ICAM-1 promoter. J Biol Chem 1995; 270: 18966–74.

[53] Nawroth P, Stern D. Tumor necrosis factor/cachectin-induced modulation of endothelial cell hemostatic properties. Onkologie 1987; 10: 254–8.

[54] Jovinge S, Ares M, Kallin B, Nilsson J. Human monocytes/macrophages release TNFα in response to ox-LDL. Arterioscler Thromb Vasc Biol 1997; 16: 1573–9.

[55] Barath P, Fishbein M, Cao J, Berenson J, Helfant R, Forrester J. Detection and localization of tumour necrosis factor in human atheroma. Am J Pathol 1990; 65: 297–302.

[56] Rus H, Niculescu F, Vlaicu R. Tumor necrosis factor-alpha in human arterial wall with atherosclerosis. Atherosclerosis 1991; 89: 247–54.

[57] Tanaka H, Sukhova G, Schwartz D, Libby P. Proliferating arterial smooth muscle cells after balloon injury express TNF-α but not interleukin-1 or basic fibroblast growth factor. Arterioscler Thromb Vasc Biol 1996; 16: 12–8.

[58] Jovinge S, Hultgårdh-Nilsson A, Regnström J, Nilsson J. Smooth muscle cell migration and cytoskeletal rearrangements in response to tumor necrosis factor α. Arterioscler Thromb Vasc Biol 1997; 17: 490–7.

[59] Tanaka H, Swanson S, Sukhova G, Schoen F, Libby P. Smooth muscle cells of the coronary arterial tunica media express tumor necrosis factor-α and proliferate during acute rejection of rabbit cardiac allografts. Am J Pathol 1995; 147: 617–26.

[60] Jovinge S, Hamsten A, Tornvall P *et al*. Evidence for a role of tumor necrosis factor-α in disturbances of triglyceride and glucose metabolism predisposing to coronary heart disease. Metabolism 1998; 47: 113–8.
[61] Hotamisligil GS, Spiegelman BM. Tumor necrosis factor α: a key component of the obesity-diabetes link. Diabetes 1994; 43: 1271–8.
[62] Nilsson J, Jovinge S, Nieman A, Reneland R, Lithel H. Relation between plasma tumor necrosis factor α and insulin sensitivity in elderly men with non-insulin-dependent diabetes mellitus. Arterioscler Thromb Vasc Biol 1998; 18: 1199–202.
[63] Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 1993; 259: 87–91.
[64] Hotamisligil GS, Budavari A, Murray D, Spiegelman BM. Reduced tyrosine kinase activity of the insulin receptor in obesity-diabetes. Central role of tumor necrosis factor-α. J Clin Invest 1994; 94: 1543–9.
[65] Hotamisligil GS, Arner P, Caro JF, Atkinson RL, Spiegelman BM. Increased adipose tissue expression of tumor necrosis factor-α in human obesity and insulin resistance. J Clin Invest 1995; 95: 2409–15.
[66] Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995; 95: 2111–9.
[67] Libby P, Geng YJ, Aikawa M *et al*. Macrophages and atherosclerotic plaque stability. Curr Opin Lipidol 1996; 7: 330–5.
[68] Crisby M, Kallin B, Thyberg J *et al*. Cell death in atherosclerotic plaques involves both oncosis and apoptosis. Atherosclerosis 1997; 130: 17–27.
[69] Crisby M, Nordin-Fredriksson G, Shah PK, Yano J, Zhu J, Nilsson J. Pravastatin treatment increases collagen content and decreases lipid content, inflammation, metalloproteinases, and cell death in human carotid plaques: implications for plaque stabilization. Circulation 2001; 103: 926–33.
[70] Libby P, Aikawa M. New insights into plaque stabilisation by lipid lowering. Drugs 1998; 56: 9–13; discussion 33.