Atherosclerosis: Diet and Drugs

Editor
Arnold von Eckardstein

Springer

Handbook of Experimental Pharmacology

Volume 170

Atherosclerosis: Diet and Drugs

Contributors

J. Ahrens, M. Anthony, T. Asahara, M. Aviram, S. Bellosta, F. Bernini, C. Bode, C. Bolego, C. Bouchard, G. Chinetti, A. Cignarella, R.St. Clair, P. Cullen, A. Dendorfer, P. Dominiak, C. Fontaine, J.-C. Fruchart, B. Fuhrmann, G. Gabbiani, J. Greeve, A.K. Groen, S. Grundy, H. Hendriks, M. Hersberger, O.M. Hess, M. Kaplan, A. Kosters, K.M. Kostner, G.M. Kostner, P. Kovanen, M. Kratz, F. Kuipers, T. Lakka, T. Lüscher, F. Mach, R. Mensink, D. Müller-Wieland, R. Paoletti, K. Peters, T. Plösch, R. Robillard, M. Rosenblatt, W. Schmitz, H. Schunkert, B. Staels, R. Stocker, P. Suter, M.A.M.A. Thijssen, M. Tikkanen, A. van Tol, E. Vähäkangas, A. von Eckardstein, Q. Xu, S. Ylä-Herttuala

Editor

Arnold von Eckardstein

Springer

Professor
Dr. med. Arnold von Eckardstein
Institute of Clinical Chemistry
University Hospital of Zurich
Rämistrasse 100
8091 Zürich
Switzerland
e-mail: arnold.voneckardstein@usz.ch

With 78 Figures and 36 Tables

ISSN 0171-2004

ISBN-10 3-540-22569-2 Springer Berlin Heidelberg New York

ISBN-13 978-3-540-22569-0 Springer Berlin Heidelberg New York

Library of Congress Control Number: 2004113650

Springer is a part of Springer Science + Business Media
springeronline.com

Printed in Germany

Editor: Dr. P. Roos
Desk Editor: S. Dathe
Cover design: *design&production* GmbH, Heidelberg, Germany
Typesetting and production: LE-TEX Jelonek, Schmidt & Vöckler GbR, Leipzig, Germany
Printed on acid-free paper 27/3150-YL - 5 4 3 2 1 0

Preface

Cardiovascular diseases continue to be the leading cause of death in the majority of industrialized countries. The most frequent underlying pathology, namely atherosclerosis, and its clinical sequelae, namely coronary heart disease, cerebrovascular disease and peripheral artery disease, remain common although for a long time we have been made aware of avoidable or modifiable etiological factors such as smoking, fat-rich diet or lack of exercise, and although these adverse lifestyle factors have been extensively addressed by population-wide primary prevention programs. Cardiovascular morbidity and mortality also remain high despite successful anti-hypertensive and lipid lowering drug therapies which help to reduce cardiovascular morbidity and mortality by about 30% in both secondary and tertiary prevention settings. This can partly be explained by the increasing life expectancy and growing proportion of elderly people, especially in Europe and North America. In addition, the World Health Organization makes the alarming prediction that probably in response to the spreading of western dietary behavior and lack of exercise resulting in an increasing prevalence of diabetes, dyslipidemia and hypertension, cardiovascular diseases rather than infectious diseases will become the most frequent cause of death worldwide.

This volume of the Handbook of Experimental Pharmacology entitled "Atherosclerosis" is divided into four parts and intends to give an overview on the pathogenesis of atherosclerosis, established treatment and prevention regimen, and of perspectives for the development of new treatment modalities.

The three chapters of part I review the state-of-the-art knowledge on the pathogenesis of atherosclerosis and its underlying risk factors. Because of its increasing prevalence and corresponding public health relevance, special attention is given to the metabolic syndrome, i.e. to the clustering of risk factors within a given individual. Although the expression of single risk factors in this situation may be moderate, affected individuals are at high risk for coronary heart disease events. In addition, due to the important etiological contribution of obesity and overweight, the metabolic syndrome is an important reason why atherosclerosis continues to be a significant public health burden.

The nine chapters of part II are devoted to the role of the various major and minor components of diet in the pathogenesis of cardiovascular risk factors and atherosclerosis. This field is currently experiencing a renaissance for two

reasons: First, after fat and notably cholesterol had been accused of being "the bad guys" for a long time, novel research findings and the epidemic of obesity and diabetes produced a more differentiated view of the pathogenetic relevance of the various dietary compounds. Second, both drug and food industry have discovered diet as a therapeutic target and are currently developing drugs for the treatment and prevention of overweight and functional foods enriched by putatively cardioprotective nutrients.

The four chapters of part III give an overview of groups of drugs which in controlled intervention trials effectively prevented atherosclerotic cardiovascular disease, i.e. statins, fibrates, inhibitors of the renin-angiotensin system and antiplatelet agents. Unfortunately, beta-blockers are not covered, because the author in charge of this subject finally withdrew his commitment.

The 14 chapters of part IV present several targets and perspectives for novel pharmacological interventions. Some of these strategies led to the re-evaluation and optimization of drugs already on the market, for example nicotinic acid or agonists of peroxisome proliferating agent receptors. Other strategies helped to develop drugs which are in phase III trials and will probably be introduced into the market soon, for example inhibitors of cholesteryl ester transfer protein. Finally, some developments are still in the initial stage and must overcome methodological limitations, such as gene therapy. Especially for this part IV it is important to recall that atherosclerosis is a multifactorial disease which consequently offers many targets for treatment. Therefore, I hope that we did not leave out important developments. Some authors unfortunately withdrew their original commitment to write a chapter for this book so that, for example, important controversially discussed strategies, like hormone replacement and antibiotic therapies, are missing.

Last but not least, I wish to thank Springer Verlag and the Editorial Board for giving me the honour and chance to edit a "Handbook of Experimental Pharmacology" on atherosclerosis. I am very grateful to all authors for their excellent contributions. I also thank Mrs. Bernadette Hand (Zurich) for careful language editing and Mrs. Susanne Dathe (Springer Verlag) for her patience and help while accompanying me through this project.

Zurich, February 2005 Arnold von Eckardstein

List of Contents

List of Contributors

(Addresses stated at the beginning of respective chapters)

Part I
Background

HEP (2005) 170:3–70

The Pathogenesis of Atherosclerosis

P. Cullen[1] (✉) · J. Rauterberg[1] · S. Lorkowski[1,2]

[1]Institute of Arteriosclerosis Research, Domagkstraße 3, 48149 Münster, Germany
cullen@uni-muenster.de

[2]Institute of Biochemistry, University of Münster, Münster, Germany

Abstract Worldwide, more people die of the complications of atherosclerosis than of any other cause. It is not surprising, therefore, that enormous resources have been devoted to studying the pathogenesis of this condition. This article attempts to summarize present knowledge on the events that take place within the arterial wall during atherogenesis. Classical risk factors are not dealt with as they are the subjects of other parts of this book. First, we deal with the role of endothelial dysfunction and infection in initiating the atherosclerotic lesion. Then we describe the development of the lesion itself, with particular emphasis on the cell types involved and the interactions between them. The next section of the chap-

ter deals with the events leading to thrombotic occlusion of the atherosclerotic vessel, the cause of heart attack and stroke. Finally, we describe the advantages—and limitations—of current animal models as they contribute to our understanding of atherosclerosis and its complications.

Keywords Atherogenesis · Endothelial dysfunction · Infection · Atherosclerotic lesion · Thrombotic occlusion

1 Introduction and History

Atherosclerosis has been a companion of mankind since antiquity. Mummies from Egypt (Cockburn 1975, 1980; Magee 1998; Sandison 1962, 1981; Shattock 1909), North America (Zimmermann 1993) and China (Cockburn 1980), and dating from around 3000 B.C. to 400 A.D. showed extensive macroscopic and microscopic evidence of atherosclerosis of the aorta and of the carotid, coronary and femoral arteries (Ruffer 1911, 1920). Life expectancy even of the wealthier classes in Egypt who were subjected to mummification was in general only 25–30 years, as documented in vivid Egyptian/Roman mummy portraits dating from the first to the fourth century A.D., although some portraits of the deceased persons appear to show older individuals with wrinkles and grey hair (Egyptian Museum Cairo 1999). Even though they consumed some meat, the diet of these people was mainly vegetable and, judging from dental wear, rather coarse (Magee 1998; Ruffer 1991). Tobacco consumption was unknown although alcohol was available. It is clear therefore that atherosclerosis is an ancient process and that its pattern has always been the same regardless of race, diet and lifestyle.

It was probably Leonardo da Vinci (1452–1519) who first recognized the macroscopic changes of atherosclerosis. When he illustrated the arterial lesions in an elderly man at autopsy, he suggested that the thickening of the vessel wall was due to 'excessive nourishment' from the blood (Keele 1952; Quiney and Watts 1989). Around 1860, Félix J. Marchand (1846–1928) coined the term 'atherosclerosis' to emphasize the pathological findings of atheroma (Greek, gruel) and sclerosis (Greek, hard) seen in the intimal layer of the arteries (cited in Aschoff 1908).

From the very start, the theories concerning the pathogenesis of atherosclerosis could be divided into two broad schools, the 'cellular' and the 'humoral'. The 'cellular' school proposes that the atherosclerotic lesion mainly has its origin in changes within the artery itself. This is most commonly expressed as the 'response-to-injury' hypothesis, originally proposed in 1856 by the father of cellular pathology Rudolf Virchow (1821–1902) (Virchow 1856) and more recently championed by the late Russell Ross (1929–1999) (Ross 1993).

The 'humoral' school, by contrast, emphasizes that atherosclerosis is due to changes in the milieu within which the artery finds itself. An early proponent of such a theory was the Viennese pathologist Karl von Rokitansky (1804–1878) who in 1852 reported that fibrin plays a pivotal role in the atheromatous process (von Rokitansky 1852), a tradition that was continued by J. B. Duguid 100 years later, who also emphasized the importance of thrombosis as a factor in the pathogenesis of coronary atherosclerosis (the 'thrombogenic' hypothesis) (Duguid 1946).

Today, it is clear that aspects of both the 'cellular' and 'humoral' schools of atherogenesis are correct, in the sense that processes both outside and within the arterial wall have a profound influence on the initiation and progression of the atherosclerotic lesion. Many of the other chapters in this book deal with risk factors for atherosclerosis, with particular emphasis on diet. The present chapter will therefore confine itself to events that occur within the arterial wall during atherogenesis. Classical risk factors such as dyslipidaemia, diabetes mellitus and the metabolic syndrome, hyperhomocysteinaemia, and hypertension will not be dealt with here and we refer the reader to the relevant sections of this book for a discussion of these issues.

2
The Response-To-Injury Hypothesis of Atherosclerosis

Atherosclerosis mainly affects large and medium-sized arteries, including the aorta, the carotid arteries, the coronary arteries and the arteries of the lower extremities. The earliest lesion of atherosclerosis is called the fatty streak, which is common even in infants and young children (Napoli et al. 1997). The fatty streak is a pure inflammatory lesion, consisting only of monocyte-derived macrophages and T lymphocytes (Stary et al. 1994). In patients with hypercholesterolaemia, this influx of cells is preceded by lipid deposition (Napoli et al. 1997; Simionescu et al. 1986).

2.1
Endothelial Dysfunction

The response-to-injury hypothesis of atherosclerosis suggests that even before development of the fatty streak, damage to the endothelium lining the blood vessel sets the stage for lesion development. Originally, denudation of the endothelium was thought to be required (Ross and Glomset 1973), but more recent work emphasizes the importance of endothelial dysfunction (Bonetti et al. 2003; Widlansky et al. 2003). In fact, some workers have gone so far as to suggest that the endothelial status may be regarded as 'an integrated index of all atherogenic and atheroprotective factors present in an individual', a sort of 'threshold switch' that only when activated translates an unfavourable risk factor profile into actual atherosclerotic disease (Bonetti et al. 2003).

The endothelium is a continuous layer of cells that separates blood from the vessel wall. An active, dynamic tissue, endothelium controls many important functions such as maintenance of blood circulation and fluidity as well as regulation of vascular tone, coagulation and inflammatory responses (Gonzalez and Selwyn 2003). Under homeostatic conditions, the endothelium maintains normal vascular tone and blood fluidity and there is little or no expression of pro-inflammatory factors. The arterial endothelium responds to flow and to shear forces in the blood via a pathway that leads to phosphorylation of endothelial nitric oxide synthase (eNOS), which in turn produces the potent vasodilator nitric oxide (NO), thus leading to vasodilatation (Dimmler et al. 1999; Scotland et al. 2002). This response allows arteries to accommodate increases in flow and control changes in shear stress (Brouet et al. 2001). Regulation of eNOS occurs through its attachment to proteins such as caveolin (Fontana et al. 2002) and by means of phosphorylation reactions (Harrison 1997). In addition, the endothelium limits local thrombosis by producing tissue plasminogen activator, maintaining a negatively charged surface, and by secreting anticoagulant heparans and thrombomodulin (Behrendt and Ganz 2002).

Endothelial dysfunction is characterized first by a reduction in the bioavailability of vasodilators, in particular NO, whereas endothelium-derived vasoconstrictors such as endothelin 1 are increased (Bonetti et al. 2003; Yang et al. 1990). This leads to impairment of endothelium-derived vasodilatation, the functional hallmark of endothelial dysfunction. Second, endothelial dysfunction is characterized by a specific state of endothelial activation, which is characterized by a pro-inflammatory, proliferative and procoagulatory state that favours all stages of atherogenesis (Anderson 1999). Dysfunctional endothelium promotes the adhesion of leukocytes to the arterial wall and their migration into the subintimal space and also fails to inhibit the proliferation and migration of smooth muscle cells (Bonetti et al. 2003).

Many of the classical and 'newer' risk factors associated with atherosclerosis such as smoking, hyperlipidaemia, diabetes mellitus, hypertension (Celermajer et al. 1992; Libby et al. 2002), obesity (Steinberg et al. 1996), elevated C-reactive protein (Fichtlscherer et al. 2000), and chronic systemic infection (Prasad et al. 2002) have been found to be associated with endothelial dysfunction. The exact nature of the link is unknown, but may also involve reactive oxygen species. Thus, it has been postulated that at an early stage in the atherosclerotic process, oxidatively modified low-density lipoprotein (LDL) may activate protein kinase C and thus nuclear factor-κB (NFκB), a transcription factor that increases the transcription of genes encoding angiotensin converting enzyme, endothelial cell surface adhesion molecules and enzymes that further promote oxidative stress (Cai and Harrison 2000; Libby et al. 2002; Murohara et al. 1994). Reactive oxygen species may also react directly with NO, reducing its bioavailability and promoting cellular damage (Tomasian et al. 2000; Yura et al. 1999). In addition, binding of oxygen free radicals to NO may produce a toxic product, peroxynitrite, which destabilizes the production of

eNOS and causes uncoupling of the enzyme, leading to production of free radicals rather than NO. Increased membrane concentrations of cholesterol lead to up-regulation of caveolin, which binds eNOS and limits NO production. Cofactors in the release of NO from arginine become oxidized and may impair eNOS function (Vasquez-Vivar et al. 1998). In addition, abnormal substrates such as asymmetric dimethylarginine may compete to block the enzyme and thus also limit NO production (Cooke 2000). It is unclear which of these mechanisms predominates in human atherosclerosis, but the end result is a failure to produce sufficient amounts of NO (Murohara et al. 1994; Ohgushi et al. 1993).

However, established cardiovascular risk factors are not the only determinants of endothelial function, as evidenced by a number of studies that showed no difference in the risk factor profile between persons with normal endothelium and persons with various stages of endothelial dysfunction (Al Suwaidi et al. 2000; Gokce et al. 2002; Halcox et al. 2002; Ohgushi et al. 1993). Although local factors, in particular haemodynamic forces such as shear stress, have been recognized as important modulators of endothelial function (Gokce et al. 2002), these findings indicate a variable endothelial susceptibility to cardiovascular risk factors and indicate the presence of other, as-yet unknown factors—including genetic predisposition—both for the prevention and the promotion of endothelial dysfunction.

Finally, it is important to note that dysfunction of the arterial endothelium is important not only at the inception of the atherosclerotic lesion, but at every stage in the life of the plaque, including in particular the events surrounding plaque rupture. This will be referred to in detail below.

2.2 The Role of Infection in Atherogenesis

The suggestion that infectious agents might be involved in the causation of atherosclerosis was first proposed by Sir William Osler (1849–1919) and others at the start of the twentieth century (Frontingham 1911; Osler 1980). In more recent times, interest has focused on four organisms: the intracellular parasite *Chlamydia pneumoniae*, the herpes viruses cytomegalovirus (CMV) and herpes simplex virus (HSV) types 1 and 2, and *Helicobacter pylori*. In addition, it has been postulated that chronic low-grade infection or recurrent infections at other sites of the body—in particular of the teeth and gums in the form of periodontitis—may also increase the risk of developing atherosclerotic disease. However, the link between infection and atherosclerosis need not be limited to these organisms. In one study of 18 atherosclerotic lesions of the carotid artery, for example, three lesions were found to contain HSV type 1 DNA, and eight contained a wide range of bacterial DNA from species that belonged either to the oral, genital or faecal commensal flora or that are present in the environment (Watt et al. 2003).

Two hypotheses have been presented to explain the presence of microorganisms in the atherosclerotic plaque: (a) a microorganism may specifically cause atherosclerosis in the same way as *H. pylori* causes gastric ulcers; (b) viruses and/or bacteria may be randomly trapped by atherosclerotic tissue during viraemia or bacteraemia.

2.2.1 *Chlamydia pneumoniae*

Most attention in recent years has been devoted to the link between *C. pneumoniae* and atherosclerosis. The high motivation in relation to this organism stems mainly from the fact that is amenable to treatment with antibiotics and thus might provide a rare opportunity to causally treat atherosclerosis (Kalayoglu et al. 2002). *C. pneumoniae* was first isolated in 1965, but was not properly speciated until 1989 (Grayston et al. 1990). *C. pneumoniae* has the capacity to multiply within a wide range of host cells, including macrophages and endothelial cells (Gaydos et al. 1996; Godzik et al. 1995; Kaukoranta-Tolvanen et al. 1994). Most humans encounter *C. pneumoniae* during their lives, with seropositivity rates for anti-*C. pneumoniae* antibodies achieving about 50% at 20 years and over 70% by the age of 65 years (Grayston 1992).

Four pieces of evidence suggest a role for *C. pneumoniae* in atherosclerosis: (a) some seroepidemiological studies indicate that patients with cardiovascular disease have higher titres of anti-*C. pneumoniae* antibody than controls (Danesh et al. 1997, 2000, 2002); (b) about half of all atherosclerotic lesions contain the organism or its proteins and nucleic acids. Furthermore, the pathogen has been isolated from atheroma and propagated in vitro (Kalayoglu et al. 2002); (c) in vitro studies suggest that *C. pneumoniae* can modulate the function of atheroma-associated cell types in ways that are consistent with a contribution to atherogenesis; (d) in animal studies, *C. pneumoniae* has been found to promote lesion initiation and progression, and antibiotic treatment in animals has been shown to prevent the development of atherosclerotic lesions.

Despite the strong circumstantial evidence linking *Chlamydia* to atherogenesis, however, the results of trials investigating the anti-atherosclerotic effects of antibiotic treatment in humans have been disappointing. While an early study of azithromycin treatment in male survivors of myocardial infarction with high titres of anti-*C. pneumoniae* antibody appeared to show promising results (Gupta et al. 1997), these results were not confirmed in later larger studies (Anderson et al. 1999; Dunne 2000; Muhlestein et al. 2000). At the time of writing, results are awaited from the Azithromycin and Coronary Events study of 4,000 patients with stable coronary artery disease (Jackson 2000), and from the Pravastatin or Atorvastatin Evaluation and Infection Therapy trial, which will include 4,200 patients treated with the quinolone antibiotic gatifloxacin. It is hoped that these large trials will provide a definitive answer to the question of clinical usefulness of antibiotics in treating atherosclerosis.

At present, therefore, a causal role of *C. pneumoniae* in atherogenesis must be seen as speculative. Part of this lack of clarity is due to deficiencies in available diagnostic methods to detect and monitor acute, chronic or persistent *C. pneumoniae* infection. Seroepidemiological studies have used different criteria for the diagnosis of infection. Detection of the pathogen by polymerase chain reaction and immunohistochemistry also shows excessive variation between laboratories (Apfalter et al. 2001). It is also possible that *C. pneumoniae* interacts with classical risk factors such as an atherogenic lipid profile to modulate atheroma biology, further complicating the matter (Khovidhunkit et al. 2000).

On the balance of evidence, however, it is highly unlikely that *C. pneumoniae* is required for the initiation of atherosclerosis or alone can cause this complex disease. Hyperlipidaemic animals develop atherosclerosis in germ-free conditions, cardiovascular morbidity and mortality can be reduced by lipid-lowering treatment without antibiotics, and *C. pneumoniae* is not present in all atherosclerotic lesions. For the last reason alone, *C. pneumoniae* is unable to fulfil Robert Koch's postulates with regard to its atherogenic potential. Current clinical data therefore do not warrant the use of antibiotics for the prevention or treatment of atherosclerosis in humans (Kalayoglu et al. 2002).

2.2.2 Other Infectious Agents

2.2.2.1 Cytomegalovirus

Some workers have suggested that cytomegalovirus (CMV) may be a cofactor in atherogenesis (Bruggeman et al. 1999; Epstein et al. 1996; Levi 2001). Its mode of action has been thought to be either by local invasion of the arterial wall, by effects on the host inflammatory response, by interfering with endothelial function (Grahame-Clarke et al. 2003), or by perturbation of lipid metabolism (de Boer et al. 2000a; Fong 2000; Libby et al. 1997). CMV DNA has been detected in the walls of atherosclerotic arteries, but very little is known about its ability to replicate at this location. CMV has been shown to replicate in endothelial cells and smooth muscle cells that have been isolated from human arteries. The viral replicative process disrupts control of the cell cycle and increases the amounts or activities of procoagulant proteins, reactive oxygen species, leukocyte adhesion molecules, cholesterol uptake and esterification, cell motility, and pro-inflammatory cytokines (Nerheim et al. 2004). Thus, these in vitro findings suggest ways in which CMV might promote atherogenesis and its complications. In a recent study in human coronary artery, internal mammary artery grafts and saphenous vein grafts, infection with CMV was seen only in subpopulations of intimal and adventitial cells, and was enhanced in vessels that were affected by atherosclerosis (Nerheim et al. 2004). Smooth muscle cells were completely resistant to infection with CMV.

Overall, the evidence for a causative role of CMV in atherogenesis is less strong than that for *C. pneumoniae*. The presence of viral nucleic acid within the plaque is no proof of causality, and in vitro effects cannot be extrapolated to the in vivo situation.

2.2.2.2
Herpes simplex virus, *Helicobacter pylori*

As with *C. pneumoniae* and CMV, HSV and *H. pylori* have been found in atheromatous lesions, and increased titres of antibodies to both pathogens have been used as a predictor of adverse cardiovascular events (Espinola-Klein et al. 2000). However, there is no direct evidence that they can cause the lesions of atherosclerosis.

2.2.3
Chronic Infection and Atherogenesis

2.2.3.1
Periodontitis

Multiple cross-sectional studies have demonstrated a higher incidence of atherosclerotic complications in patients with periodontal disease (Arbes et al. 1999; Grau et al. 1997; Mattila et al. 1989, 1995; Nieminen et al. 1993; Syrjanen et al. 1989). However, a problem with cross-sectional studies is that they cannot distinguish between cause and effect. For example, it is possible that atherosclerosis might exacerbate periodontal disease by causing a systemic inflammatory response or even through subclinical ischaemia (Haynes and Stanford 2003). Prospective studies of the link between periodontal disease and atherosclerosis have been inconsistent, with some showing an increase in risk (Beck et al. 1996; Morrison et al. 1999; Wu et al. 2000), while other large studies do not (Hujoel et al. 2000; Joshipura et al. 1996). There are several possible explanations for the association between periodontal disease and atherosclerosis. First, it may reflect confounding by common risk factors that cause both conditions, such as smoking, obesity and diabetes mellitus. Second, it may reflect an individual propensity to develop an exuberant inflammatory response to intrinsic or extrinsic stimuli. Third, the presence of an inflammatory focus in the oral cavity may exacerbate atherosclerosis by stimulating humoral or cell-mediated inflammation. Fourth, the presence of periodontal infection may lead to brief episodes of bacteraemia and inoculation of the atherosclerotic plaques with such periodontal pathogens as *Porphyromonas gingivalis*, *Actinobacillus actinomycetemcomitans*, or *Bacteroides forsythus*. In one recent study, the presence of antibodies to *Porphyromonas gingivalis* was specifically linked to coronary heart disease, especially in edentulous individuals (Pussinen et al. 2003), while in another study, severe periodontal disease was associated with perturbed flow-mediated dilation of the

brachial artery, presumably as a result of endothelial dysfunction (Amar et al. 2003). Severe periodontal disease has also been linked to ischaemic stroke (Grau et al. 2004).

Overall, therefore, there is suggestive evidence of a modest link between severe periodontal disease and atherosclerosis (Scannapieco et al. 2003). To test the hypothesis of causality, it will now be necessary to show that reversal of periodontal disease will reverse or at least lessen the progression or complications of atherosclerosis. This question is currently being addressed in the Periodontitis and Vascular Events trial (PAVE) that is currently being run by the United States National Institutes of Health (http://www.cscc.unc.edu/pave); however, the results of which are not expected until 2008. Until the results of PAVE and similar trials are available, a causal role of periodontal disease in atherosclerosis must remain speculative.

2.2.3.2
Infectious Burden and Atherosclerosis

It has been suggested that the risk of developing atherosclerosis is not due to infection with a single agent but rather to the number of pathogens to which a person is exposed over his or her lifetime (Epstein et al. 2000; Zhu et al. 2000, 2001). Thus, in a number of studies, risk of atherosclerosis was associated with seropositivity to *C. pneumoniae*, CMV, Epstein–Barr virus, and HSV type 2 (Espinola-Klein et al. 2000; 2002a, 2002b; Rupprecht et al. 2001), the risk of atherosclerosis increasing with an increase in the number of agents to which the patients were seropositive. It has been suggested that this effect is due to a local or systemic inflammatory response generated by the infectious agents and/or an infection-induced autoimmune response involving molecular mimicry.

The idea that infectious burden contributes to the pathogenesis of atherosclerosis must at the present time also be regarded as speculative. It is possible, for example, that individuals with greater infectious burden may appear to be at increased vascular risk only because they have less access to care or a lower socioeconomic status.

3
Development of the Atherosclerotic Lesion

3.1
Different Cell Types in Atherosclerosis: Villains or Heroes?

3.1.1
Smooth Muscle Cells

There is no doubt that proliferation of smooth muscle cells plays a role in the development of the atherosclerotic lesion, especially during its initial phases. Intimal thickening caused by proliferation of smooth muscle cells stands at the

beginning of plaque development, although not all areas of intimal thickening will develop into full-blown atherosclerotic plaques. Adaptive thickening is a normal development at sites of high mechanical load, starting already at the time of birth or even earlier (Ikari et al. 1999).

Proliferation of smooth muscle cells was first suspected to play a role in development of atherosclerosis based on studies of experimental injury to the vascular wall, such as removal of the endothelium by balloon angioplasty (Ross and Glomset 1973). In this case, the vessel wall reacts by induction of proliferation of medial smooth muscle cells, migration of smooth muscle cells through the elastica interna and formation of a neointima. In the course of this process the smooth muscle cells change from a contractile to a synthetic, fibroblast-like phenotype showing higher proliferation rate and active synthesis of extracellular matrix components.

Several growth factors have been shown to be involved in this process. The role of platelet derived growth factor (PDGF) was demonstrated in early studies of balloon-induced injury by Ross et al. and Stephen M. Schwartz and coworkers (Murry et al. 1997; Bayes-Genis et al. 2000) showed that insulin-like growth factors are also involved. The animal model of endothelial injury may have a clinical correlate in the development of restenosis after coronary angioplasty in humans. In both cases, proliferation of intimal smooth muscle cells is decisive for the development of a neointima. On the other hand, narrowing of the lumen after injury results only partly from the growth of a neointima, since such narrowing also results from 'remodelling', a thickening of the media by contraction without enhancement of the tissue mass (Newby 1997).

In contrast to intimal thickening after injury, which occurs fairly rapidly, the formation of the atherosclerotic plaque is very slow. Replication of smooth muscle cells within the atherosclerotic plaque is also very sluggish with replication rates of less than 1% (Taylor et al. 1995). At present it is unknown if all intimal smooth muscle cells show uniformly slow rates of proliferation, if episodic bursts of proliferation occur, or if a small number of cells show high proliferation rates within a non-proliferating surrounding. In the early 1970s Earl P. Benditt produced a strong argument in favour of the latter possibility when he reported that atherosclerotic plaques contain large monoclonal cell populations (Benditt and Benditt 1973). This remarkable result was based on findings in women, each of whose X-chromosomes encoded a different electrophoretically discernible isoform of glucose-6-phosphate-dehydrogenase. Early in embryonic development one X-chromosome is inactivated so that each tissue normally contains a mosaic pattern of paternal and maternal X-chromosomes. However, if a single cell undergoes rapid proliferation, the newly formed tissue contains only cells producing a single isoform. The finding has been confirmed by other authors, and it is now clear that fairly large patches of the normal arterial media are also formed by cells of monoclonal origin (Chung et al. 1998).

3.1.2 Macrophages

In evolutionary terms, macrophages represent an ancient part of the immune system. Closely related cells are already found in the haemolymph of primitive multicellular organisms. The principal role of macrophages is the ingestion by phagocytosis, and hence neutralization, of non-self material, ranging from aged, necrotic, apoptotic or malignant cells to microbial invaders. They also have a central role in the regulation of the immune response and secrete a wide range of cytokines, chemokines (chemotactic cytokines) and other soluble mediators. Finally, they have a very important function in the presentation of foreign peptide antigens to T cells and thus in the initiation of the T cell-mediated immune response. Macrophages develop from circulating blood monocytes and only become fully developed at their final destination. Thus, in bone, macrophages are called osteoclasts, in the central nervous system microglia, in connective tissue histiocytes, in the kidney mesangial cells, and in the liver Kupffer cells. In order to become fully activated, tissue macrophages require exogenous signals and interaction with T cells. Once the danger has passed, macrophages may also be switched off, or deactivated, by cross-linking of inhibitory receptors, by anti-inflammatory cytokines and by certain compounds such as reactive oxygen intermediates (Bogdan 2001).

One of the principal characteristics of the atherosclerotic plaque is the presence of macrophages and macrophage-derived foam cells. These cells have been studied in detail for many years in humans, in various animal models and in cell culture. Huge amounts of information on their regulation and on their effects on other cells have been generated. Nevertheless, the central question remains as to whether macrophages fundamentally inhibit or promote the atherosclerotic process. The aim of the following section is to sketch out the main functions of the macrophage in atherosclerosis and to try to come to a provisional answer to this question.

3.1.2.1 Entry of Monocytes into the Subintimal Space

In addition to the endothelial dysfunction referred to above, an early event in atherogenesis is the activation of endothelial cells. The cause of this is not known, but it may be mediated by atherogenic lipoprotein remnants or by modified LDL. Activated endothelial cells express adhesion molecules on their surfaces. First, the glycoproteins P-selectin and E-selectin on the surface of endothelial cells bind P-selectin glycoprotein ligand-1 on the surface of monocytes in the circulation, causing these to adhere loosely in rolling fashion to the endothelium. Then, a firmer interaction of the monocyte with the endothelium is mediated by the integrins vascular cell-adhesion molecule 1 (VCAM-1) and intracellular cell-adhesion molecule 1, which bind to lymphocyte func-

tion antigen-1 and very late antigen-4, respectively, on the monocyte surface. VCAM-1 may be the pivotal molecule involved in monocyte recruitment into the atherosclerotic plaque: it is up-regulated in cultured endothelial cells in the presence of oxidized LDL, it is expressed at lesion-prone sites before the appearance of grossly visible lesions and it is fairly selective for monocytes. Moreover, atherosclerosis is reduced in mice lacking VCAM-1 (Li and Glass 2002).

Finally, adherent monocytes migrate into the subendothelial space by a process known as diapedesis under the influence of chemoattractant molecules, in particular the chemokine macrophage chemoattractant protein-1 (MCP-1), which is recognized by the chemokine CC motif receptor 2 (CCR2) on the monocyte. Monocytes isolated from persons with hypercholesterolaemia are more responsive to MCP-1 because they show increased expression of the CCR2. Oxidized LDL is itself a chemoattractant, and its oxidized phospholipid components induce expression of MCP-1 by endothelial cells (Cushing et al. 1990; Subbanagounder et al. 2002). In humans, other chemoattracts that may play a role in monocyte recruitment include interleukin (IL) 8 and its cognate chemokine receptor CXCR2 together with the macrophage inflammatory proteins 1α and 1β, and the protein RANTES (regulated upon activation, normal T cell expressed and secreted), all of which bind to the CC motif receptor 5 (CCR5) on the monocyte surface. In contrast to CCR2, the main function of CCRS is to recruit monocytes from the circulating blood, CCR5 and its ligands appear to act mainly on macrophages within the plaque (Østerud and Bjørklid 2003).

3.1.2.2
Proliferation of Macrophages in the Atherosclerotic Plaque

Accumulation of macrophages is an essential step in all phases of atherosclerotic plaque development. For a long time there was general agreement that this accumulation is caused by recruitment of monocytes from the blood, which then differentiate to macrophages within the tissue. This assumption was called into question by reports of histological markers of cell proliferation on plaque macrophages. In fact, Katsuda et al. reported that in early human lesions most proliferating cell nuclear antigen-positive cells were either monocytes/macrophages or lymphocytes (Katsuda et al. 1993). More recent reports describe the induction of macrophage proliferation by oxidized LDL. According to Hamilton et al. the proliferative effect of oxidized LDL is additive to that of a macrophage growth factor, colony stimulating factor 1 (Hamilton et al. 1999), which is required for cell survival.

Proliferation of macrophages in the presence of oxidized LDL is induced by cytokines secreted by antigen-activated T lymphocytes. Göran K. Hansson and coworkers (Paulsson et al. 2000) recently showed that a substantial portion of CD4+ cells [which are generally thought to be T helper (Th) lymphocytes]

isolated from atherosclerotic plaques recognize oxidized LDL as an antigen which induces them to proliferate and to secrete cytokines. This group also demonstrated oligoclonal T cell proliferation in plaques of cholesterol-fed apolipoprotein E (apoE)-deficient mice.

Thus, despite the fact that rates of cell division in the atherosclerotic plaque are very low, accumulation of cells within the lesion is caused not only by cell immigration, but also by local that local proliferation of all cell types involved.

3.1.2.3
Formation of Foam Cells: The Macrophage Dilemma—How Does the Macrophage Deal with Excess Lipid?

To be recognized by macrophage scavenger receptors, native lipoproteins must be modified to atherogenic forms. Retention of LDL within the subendothelial extracellular matrix appears to be necessary for such modifications to occur (Skalen et al. 2002). Several lines of evidence support the hypothesis that oxidation of LDL is an essential step in its conversion to an atherogenic particle (Steinberg et al. 1989). Although macrophages, endothelial cells and smooth muscle cells can all promote oxidation of LDL in vitro, we still do not know how this process occurs in vivo. Macrophages produce lipoxygenases, myeloperoxidase, inducible nitric oxide synthase (iNOS) and NADPH oxidases, all enzymes that can oxidize LDL in vitro and that are expressed within the human atherosclerotic plaque. These enzymes – in particular myeloperoxidase, iNOS and NADPH oxidase – are the means by which macrophages generate the reactive oxygen species that are essential for microbial killing and native immunity.

Unlike other cell types, macrophages express a number of scavenger receptors that are capable of taking up oxidized LDL, including scavenger receptor A, scavenger receptor B1 (SRB1), cluster of differentiation (CD) 36, CD68, and scavenger receptor for phosphatidylserine and oxidized lipoprotein (Li and Glass 2002). As a class, these proteins tend to recognize polyanionic macromolecules and may have physiological functions in the recognition and clearance of pathogens and apoptotic cells. Of the receptors present, scavenger receptor A and CD36 appear to be the most important from a quantitative point of view in terms of uptake of modified lipoprotein. In mouse models, these two receptors accounted for between 70% and 90% of degradation of LDL modified by acetylation or oxidation. This facility may also correlate directly with atherogenesis—in atherosclerosis-prone apoE knockout mice the extent of atherosclerosis is reduced when the mice also lack either scavenger receptor A or CD36. Nevertheless, the specific role that these receptors play in the development of human atheroma remains to be determined (Nicholson 2004). Uptake of oxidized LDL is mediated primarily by CD36, which recognizes the oxidized phospholipids within the particle. By contrast, scavenger receptor A recognizes the protein components of the particle.

Exposure to oxidized LDL strongly induces expression of CD36 mRNA and protein via activation of the transcription factor peroxisome proliferator-activated receptor γ (PPARγ) (Nagy et al. 1998; Tontonoz et al. 1998). PPARγ is part of the nuclear receptor superfamily that heterodimerizes with the retinoid X receptor (RXR) in order to control the transcripition of genes encoding proteins involved in adipogenesis and lipid metabolism. Two oxidized metabolites of linoleic acid present within oxidized LDL, 9-hydroxyoctadecadienoic acid (9-HODE) and 13-HODE may be responsible for this activity. Thus, macrophage expression of CD36 and foam cell formation may be driven by a cycle in which oxidized LDL drives its own uptake. Moreover, expression of CD36 increases as monocytes differentiate into macrophages. Although PPARγ is not required for macrophage differentiation, it is necessary for basal expression of CD36.

In contrast to the LDL receptor that is responsible for the physiological uptake of cholesterol-rich lipoproteins, the type A scavenger receptor and CD36 are not subject to negative feedback regulation by the intracellular cholesterol content. Thus, a central problem facing macrophages within the subintimal space is how to deal with the excess cholesterol that they ingest. Since the mammalian cell possesses no mechanisms for breaking down the sterol backbone of the cholesterol molecule, the macrophage is faced with the dilemma of how to deal with the cholesterol taken up via receptor-mediated endocytosis, a problem compounded by the fact that macrophages also ingest substantial amounts of cholesterol in the form of necrotic and apoptotic cells and cellular debris. This is not a trivial issue: as will be discussed below in more detail, excess cholesterol within the cell is toxic and can rapidly lead to cell death.

So how does the macrophage deal with the excess cholesterol? First, such cholesterol is stored in the form of cholesteryl ester droplets leading to the development of the eponymous foam cells. The cholesteryl esters present within internalized lipoproteins are first hydrolysed in lysosomes and the resulting free cholesterol is transported to other cellular sites, usually the plasma membrane. This process is disturbed in the cholesterol storage disease Niemann–Pick Type C (NPC), which is caused by mutations in the NPC1 and NPC2 proteins (Blanchette-Mackie 2000). NPC1 is a membrane spanning protein with a sterol sensing domain while NPC2 is a small cholesterol-binding protein (Carstea et al. 1997; Naureckiene 2000). On arriving at the plasma membrane, lysosome-derived free cholesterol is accessible to efflux acceptors and to the endoplasmatic reticulum where it can be re-esterified (Maxfield and Wustner 2002). The enzyme responsible for re-esterification of cholesterol is acyl-CoA:cholesterol acyltransferase (ACAT) and resides predominantly in the endoplasmatic reticulum (Chang et al. 1997). Substrate availability regulates ACAT, possibly coupled with allosteric regulation, and when a threshold level of free cholesterol is reached, ACAT activity increases dramatically (Xu and Tabas 1991). As described by us, human foam cells in vitro contain a wide variety of cholesteryl esters, principally cholesteryl eicosapentaenoate, cholesteryl docosahexaenoate, cholesteryl arachidonate, cholesteryl linoleate and cholesteryl

oleate (Cullen et al. 1997). Esterification of free cholesterol serves as a detoxification mechanism, but only free cholesterol is available for efflux to cholesterol acceptors (Rothblatt et al. 1999). The cholesteryl esters present in the foam cell must therefore first be hydrolysed before they can be removed from the cells. This process is accomplished by a neutral cholesterol ester hydrolase, which is present in the cell cytosol but which has yet to be completely characterized (Vainio and Ikonen 2003).

Macrophages are able to store about twice their content of free cholesterol in the form of cholesteryl esters. However, within the atherosclerotic plaque this capacity is soon exhausted. Thus, the second means in which the plaque macrophage deals with excess cholesterol is by exporting it via a number of pathways that include transfer to high-density lipoprotein (HDL) via SRB1, transfer to apoA1- and apoE-containing lipoprotein particles via at least one adenosine triphosphate-binding cassette (ABC) transporter, and direct transfer from the cell membrane either to apoE-containing lipoproteins or to other cholesterol acceptors (Nicholson 2004).

The regulation of cholesterol efflux in the macrophage is complex and incompletely understood. A central role is played by nuclear receptors that regulate the transcription of important genes in the process. Of particular importance are the dimer RXR/PPARγ, which regulates transcription of the CD36 scavenger receptor and the liver X receptor α (LXRα) transcription factor; and RXR/LXRα, which regulates the transcription of apoE and ABCA1 (Fig. 1). We have recently found that the RXR/LXR dimer is also responsible for controlling the transcription of other proteins that may well play a role in cholesterol efflux from macrophages, notably the ABC transporter G1 and adenosine diphosphate-ribosylation factor-like protein 7 (ARL7) (Engel et al. 2004) Lorkowski et al. 2001a, 2001b). Of the components of oxidized LDL, oxysterols act as ligands for LXRα, while oxidized fatty acids act as ligands of PPARγ. Other levels of regulation of these factors also exist. For example, after binding to its receptor SRB1, HDL activates the mitogen-activated protein kinase signalling pathway, which in turn leads to phosphorylation and hence reduction of both ligand-dependent and ligand-independent transcriptional activity of PPARγ (Han et al. 2002). There is some evidence that this effect is a result of the cholesterol efflux mediated by HDL and not the addition of lipid or lipoprotein (Nicholson 2004).

In addition to transfer to HDL, either via interaction of HDL with SRB1 or to interaction of apoA1 or apoE with ABCA1 (Fig. 1), other mechanisms for cholesterol efflux exist. We, and others, have shown that apoE is capable of mediating cholesterol efflux from macrophages even in the absence of cholesterol acceptors (Cullen et al. 1996), though the physiological importance of this process in human atherosclerosis is unknown. Supporting evidence for a potentially significant role of apoE in macrophage cholesterol efflux is provided by evidence from a mouse model in which specific expression of the apoE gene in the macrophages of apoE knockout mice rescued these animals

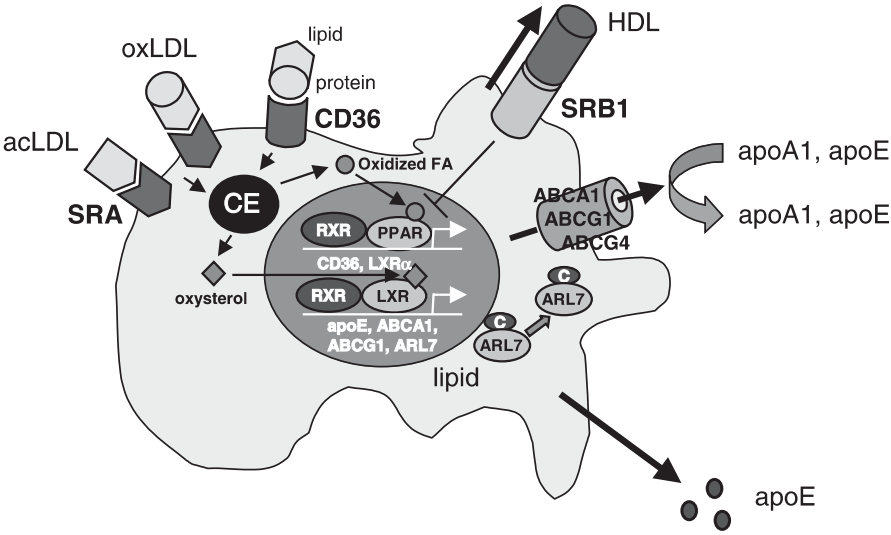

Fig. 1 Regulation of cholesterol flux in macrophages. CD36 and the ATP binding cassette transporter A1 (*ABCA1*) are regulated in response to lipid agonists derived from oxidized low-density lipoproteins (*oxLDL*), which are in turn internalized via the CD36 or the type A scavenger receptor (*SRA*). The SRA is also the principal means by which acetylated LDL is taken up into macrophages in one of the most commonly-used in vitro models of foam cell formation. CD36 and ABCA1 have major but opposite effects on macrophage lipid accumulation: increased CD36 expression increasing the intracellular content, while increased ABCA1 expression reduces cellular lipids. OxLDL increases CD36 expression because the oxidized fatty acids (*FA*) it contains act as ligands that activate the peroxisome proliferator-activated receptor γ (*PPARγ*). OxLDL also upregulates ABCA1 expression through PPARγ activation of liver X receptor α (*LXRα*). Oxysterols derived from oxLDL are ligand activators of LXRα and increase transcription of both ABCA1 and apolipoprotein (*apo*) E. The RXR/LXR dimer of transcription factors also stimulates the transcription of other genes thought to play an important role in intracellular macrophage metabolism such as the ATP binding cassette transporters G1 and G4 (*ABCG1*, *ABCG4*) and the adenosine diphosphate-ribosylation factor-like protein 7 (*ARL7*) (Engel et al. 2001, 2004; Lorkowski and Cullen 2002; Wang et al. 2004). ARL7 is induced by cholesterol loading and seems to be involved in transport of cholesterol between a perinuclear compartment and the plasma membrane, where the cholesterol is exported to high-density lipoprotein (*HDL*) via the action of ABCA1. HDL binds to its receptor scavenger receptor B1 (*SRB1*) and thus removes cholesterol from cells. Binding of HDL to SRB1 also down-regulates CD36 expression through the mitogen-activated protein kinase-mediated phosphorylation of PPARγ. The exact role of ABCG1 and of the newly-described ABC transporter ABCG4 in cholesterol efflux remains currently unknown. *CE*, Cholesteryl ester; *RXR*, retinoid X receptor. (See text for further details; adapted from Nicholson 2004)

from atherosclerosis (Bellosta et al. 1995). In the human, the relative importance of the ABCA1- and non-ABCA1-mediated pathways for apoE-dependant cholesterol efflux is unknown. A further layer of complexity is provided by the

fact that both ABCA1 and ABCG1 promote the secretion of apoE in human macrophages (von Eckardstein et al. 2001).

Despite the amount of information that already exists, many components of the cholesterol balance mechanism in macrophages remain to be discovered. For example, we found that a new member of the ABC family, ABCG4 is regulated by oxysterols and retinoids in human monocyte-derived macrophages, and may also play a role in macrophage cholesterol homeostasis (Engel et al. 2001). More recently, we discovered that ARL7, a member of a family of small regulatory guanine triphosphatases (GTPases) that control vesicle budding in the secretory and endosomal pathways of cellular vesicular transport, is also regulated by LXR/RXR and is likely to mediate transport of cholesterol between a perinuclear compartment and the plasma membrane. On arriving at the plasma membrane, this cholesterol appears to be destined for ABCA1-mediated cholesterol secretion (Engel et al. 2004).

Within recent years, a further pathway of potential cholesterol efflux in the macrophage has been discovered, namely the shedding of membranes containing so-called lipid rafts (Gargalovic and Dory 2003). Lipid rafts are tightly packed, liquid-ordered plasma membrane microdomains enriched in cholesterol, sphingomyelin and glycolipids. Their unique lipid composition may serve to compartmentalize specific membrane proteins, including caveolins. Caveolae are a subset of lipid rafts that are characterized by a high caveolin content and formation of flask-shaped invaginations of the cell membrane measuring 50–100 nm in diameter (Anderson 1998). Three isoforms of caveolin exist in mammals (caveolin 1, 2 and 3), of which caveolins 1 and 2 appear to be present in human macrophages. Because of their tightly packed liquid-ordered state, lipid rafts are an unfavourable direct source of cholesterol for efflux, and the ABCA1 transporter does not associate with them, meaning that their contribution to lipid efflux is limited to the membrane shedding mentioned above (Mendez et al. 2001; Scheiffele et al. 1999; Schroeder et al. 1994). The physiological relevance of this process in humans is unknown at the present time.

3.1.2.4
The Foam Cell: Conductor in the Cellular Orchestra of the Atherosclerotic Plaque

Macrophages and foam cells are by no means passive participants in the drama of atherosclerosis. On the contrary, they play an active role at all stages of plaque development, interacting actively with each other and with other cell types, secreting a wide range of signalling molecules, modulating the inflammatory response with the plaque, and producing a range of proteins that affect the structure of the extracellular matrix. The main biological products of macrophages are listed in Table 1. The present review will focus on just a few of these products in order to illustrate the central role of the macrophage in atherogenesis. For further detail, the reader is referred to appropriate specialist reviews.

Table 1 Biological products of monocytes and macrophages

All essential components of the complement system
All factors needed to generate fibrin: all vitamin K-dependent clotting factors: FII (prothrombin), FV, FVII and FX; fibrinogen and tissue factor
Many prostaglandins (for review see Narumiya et al. 1999)
Many leukotrienes (for review see Samuelsson 2000)
Growth factors: platelet-derived growth factor (PDGF), transforming growth factor β (TGF-β), macrophage colony-stimulating factor (M-CSF), granulocyte colony-stimulating factor (GM-CSF)
Cytokines: tumour necrosis factor (TNF) α, interleukin (IL)1-β, IL-4, IL-6, IL-10, IL-12, IL-13, IL-15, IL-18, interferon γ (IFNγ)
Platelet-activating factor, lysophosphatidylcholine
Chemotactic cytokines (chemokines): macrophage chemotactic peptide (MCP) 1, MCP-2, MCP-3, IL-8, RANTES (regulated upon activation, normal T cell expressed and secreted), Epstein–Barr virus induced molecule 1 ligand chemokine (ELC), pulmonary and activation-regulated chemokine (PARC), macrophage inhibitor peptide (MIP) 1α, MIP-1β, eotaxin (CCR-3 receptor-specific, eosinophil-selective chemokine), macrophage-derived chemokines (MDC), thymus and activation-regulated chemokines (TARC), lymphocyte-directed CC chemokines (LARC) (for review, see Baggiolini 2001)
Oxygen radicals
Proteolytic enzymes
Components of extracellular matrix: type VIII collagen, type VI collagen (unpublished), other collagens (Weitkamp et al. 1999)

One of the main ways in which the macrophage affects its surroundings is by the production of potent cytokines. Chief among these is tumour necrosis factor α (TNFα), a small (17-kDa) protein that causes the release of a whole cascade of cytokines involved in the inflammatory response. TNFα exerts its principal effects by binding as a trimer to either of two membrane receptors called TNF receptor superfamily type 1A (TNFRSF1A) and TNF receptor superfamily type 1B (TNFRSF1B). This binding leads in turn to downstream activation of the transcription factor NFκB, which is translocated into the nucleus where target genes are activated. Both cytosolic and secretory phospholipase A_2 are thought to play a role in this process.

A second important cytokine is IL-x1β. During inflammation, transcription of IL-1β is stimulated by immune complexes, coagulation and complement proteins, substance P and bacterial products, most notably lipopolysaccharide. IL-1β is also induced by cytokines of lymphocyte origin such as granulocyte-macrophage colony stimulating factor (GM-CSF) and interferon γ (IFNγ). Binding of IL-1β to its receptor also activates NFκB. Together with TNFα, IL-1β is one of the main pro-inflammatory products generated by macrophages. In fact, IL-1β may mimic activation signals typically induced by TNFα (Østerud and Bjørklid 2003). IL-1β is a chemoattractant for neutrophils, induces release of neutrophils from the bone marrow to the circulation, and enhances

leukocyte adherence to the endothelium. Like IL-6, IL-1β stimulates liver cells to secrete other acute phase proteins. It promotes endothelial cell proliferation and activates T cells by increasing IL-2 production and upregulating the IL-2 receptor (Østerud and Bjørklid 2003). Evidence that IL-1β is involved in atherogenesis derives from mouse models, in which blocking of IL-1β reduced plaque extent (Devlin et al. 2002; Elhage et al. 1998). IL-18 is a member of the IL-1 family and its receptor and signal transduction system are analogous to those of IL-1β (Akira 2000). IL-18 is a potent inducer of IFNɣ and increased lesion development in a mouse model by provoking an IFNɣ-dependent inflammatory response (Whitman et al. 2002). Moreover, IL-18 acts synergistically together with IL-12 to induce IFNɣ secretion by T cells, natural killer cells and macrophages (Munder et al. 1998). In a mouse model of atherosclerosis, IL-12 was shown to promote lesion development (Lee et al. 1999a).

IFNɣ plays a central role in inducing and modulating the immune response in humans. IFNɣ is produced by Th1 type T lymphocytes and by activated natural killer cells. It upregulates the expression of IL-1, platelet activating factor and hydrogen peroxide by macrophages. IFNɣ was shown to be atherogenic in a mouse model (Gupta et al. 1997; Nagano et al. 1997; Whitman et al. 2000).

Two further important cytokines are IL-10 and transforming growth factor β (TGF-β). IL-10 is an anti-inflammatory cytokine produced by activated macrophages and lymphocytes and has been shown to inhibit atherosclerosis formation in a mouse model (Mallat et al. 1999; Pinderski et al. 1999). TGF-β stimulates macrophage secretion of PDGF and primes macrophage chemotaxis and secretion of tissue inhibitors of matrix metalloproteinases (TIMPs). TGF-β also inhibits production of reactive oxygen and nitrogen metabolites in activated macrophages (Østerud and Bjørklid 2003).

It is important to realize that many of the cytokines produced by the macrophage have multiple and overlapping functions and that the ultimate effect also depends on the context within which the cytokine is released. The multiple and overlapping effects of some macrophage-produced cytokines are shown in Fig. 2.

A further signalling molecule that requires special mention in the context of atherogenesis is PDGF. There is much data to support the claim originally made by Russell Ross that PDGF makes a significant contribution to proliferation of smooth muscle cells in atherosclerosis (Ross et al. 1978). PDGF can be expressed by all the cells in the normal arterial wall, in particular by monocytes and macrophages. Four PDGF genes, named PDGF-A to -D exist, but only PDGF-A and PDGF-B have clearly been shown to be produced in macrophages in atherosclerosis (Evanko et al. 1998). Expression of PDGF and its receptors is increased in the atherosclerosis lesion.

3.1.2.5
Macrophage Death and Plaque Progression: Apoptosis or Necrosis?

Maintenance of a physiological ratio of free cholesterol to phospholipid in the cell membrane is essential for maintaining normal membrane fluidity (Simons and Ikonen 2000). The degree of saturation of the fatty acyl moieties of membrane phospholipids is the major determinant of lateral membrane domains, which consist of well-packed, detergent-resistant liquid-ordered rafts and more fluid, detergent-soluble liquid crystalline regions (Tabas 2002). The ability of the hydrophobic cholesterol molecule to pack tightly with the saturated fatty acyl groups of membrane phospholipids is critical for the formation of liquid-ordered rafts (Simons and Ikonen 2000), so that cholesterol depletion causes these rafts to break up. If, on the other hand, the ratio of free cholesterol to phospholipid becomes too great, then the liquid-ordered rafts become too rigid and the liquid-crystalline domains begin to lose their fluidity. These events in turn adversely affect membrane proteins that require conforma-

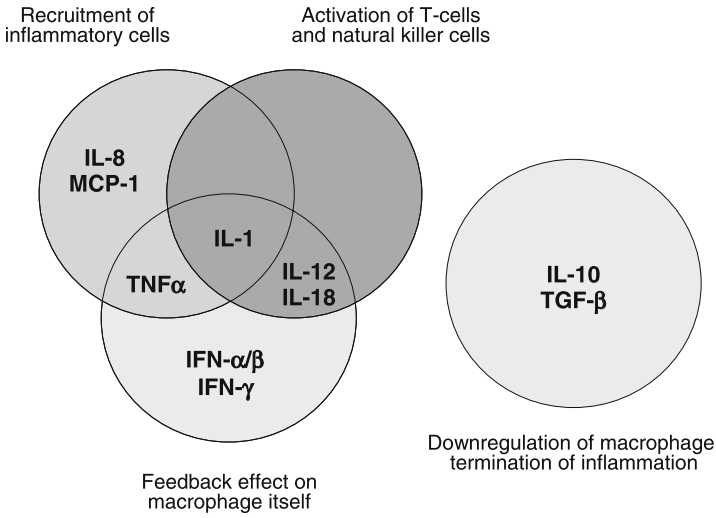

Fig. 2 Multiple and overlapping roles of macrophage-produced cytokines. Many of the cytokines produced by the macrophages within the atherosclerotic plaque have multiple and overlapping functions. Thus, interleukin 1 (*IL-1*) has functions in the recruitment of inflammatory cells and in the activation of T cells and natural killer cells, and also exerts feedback effects on the macrophage producing it. Tumour necrosis factor α (*TNF*α) helps to recruit inflammatory cells while having feedback effects on the source macrophage, while the IL-12 and IL-18 affect the source macrophage but also activate T lymphocytes and natural killer cells. By contrast, the effects of the interferons (*IFN*) α, β, and γ appears to be limited to a feedback effect on the source macrophage, while the role of IL-10 and transforming growth factor β (*TGF*-β) is limited to down-regulating the macrophage and shutting off the inflammatory response

Table 2 Potential mechanisms by which high levels of free cholesterol may kill the macrophage (from Tabas 2002)

Event	Consequence
Loss of membrane fluidity	Dysfunction of integral membrane proteins
Disruption of membrane domains	Disruption of signalling events
Induction of apoptosis	Caspase-mediated death
Intracellular cholesterol crystallization	Organelle disruption
Formation of toxic oxysterols	Oxidative damage?
Alteration of gene expression?	Change in balance of survival proteins to death proteins?

tional freedom to function properly (Yeagle 1991), such as the Na^+/K^+ ATPase, adenylate cyclase, alkaline phosphatase, rhodopsin, and transporters for glucose, organic anions, and thymidine (Tabas 2002). Thus, high free cholesterol levels may in part kill cells by inhibiting one or more vital integral membrane proteins (Table 2).

Excess membrane cholesterol may also disrupt the function of signal proteins in the membrane (Tabas 2002). Other mechanisms of toxicity include intracellular cholesterol crystallization (Kellner-Weibelo et al. 1998, 1999; Lupu et al. 1987), oxysterol formation (Brown and Jessup 1999), and triggering of apoptosis (Kellner-Weibel et al. 1998; Yao and Tabas 2000, 2001).

The response of the macrophage to excess loading with free cholesterol can be divided into two phases, an initial adaptive phase in which synthesis of phospholipids increases and a later stage when this defence is overcome and the cell dies. In the adaptive phase, an increase occurs mainly in phosphatidylcholine, synthesis of which is increased by post-translational activation of the rate-limiting enzyme in phosphatidylcholine biosynthesis, cytidine triphosphate: phosphocholine cytidylyltransferase (PCYT). How increases in free cholesterol activate PCYT is not known, but the process requires dephosphorylation of PCYT and several regulatory proteins. The up to twofold increase in cellular phosphatidylcholine leads to the appearance of whorl-like membrane structures in the cells that have been observed both in in vitro models of cholesterol loading and in lesional macrophages in a rabbit model (Shio et al. 1979).

In the face of continued exposure to rising levels of free cholesterol, the adaptive response of the macrophage will eventually fail. The basis for this adaptive failure is not known, although a decrease in PCYT activity has been seen before the onset of cellular toxicity. Morphologically, cells that are dying of free cholesterol poisoning show signs both of necrosis (e.g. disrupted cell membranes) and apoptosis (e.g. condensed nuclei) (Tabas 2002). The term apoptosis refers to the physiological process of programmed cell death that occurs in many tissues. Biochemically, apoptosis-associated caspases and their signalling pathways are activated in a portion of the cells. It is likely that

a portion of the cells becomes acutely necrotic due to direct and disruptive effects of free cholesterol toxicity on membrane proteins, while others undergo a programmed apoptotic response. Some cells that first enter an apoptotic program may become necrotic later (so-called aponecrosis), perhaps as a result of chronic ATP depletion or failure of neighbouring cells to phagocytose the apoptotic bodies.

In cell culture models of macrophages loaded with free cholesterol, about 30% show such hallmarks of apoptosis as the appearance of phosphatidylserine in the outer leaflet of the cell membrane and fragmentation of the cellular DNA. These changes can be completely prevented by inhibition of a group of enzymes called caspases that are known to play a central role in apoptosis (Yao and Tabas 2001). Partial inhibition is possible by blocking the Fas receptor or the Fas signalling pathway. Activation of the Fas receptor induces apoptosis, and loading of the cell with free cholesterol causes post-translational activation of cell-surface Fas ligand, either by inducing a conformational change in the molecule or by stimulating transport of Fas ligand from intracellular stores to the plasma membrane (Yao and Tabas 2001).

Widespread mitochondrial dysfunction, indicated by a decrease in the mitochondrial transmembrane potential, is also observed in macrophages containing excessive free cholesterol (Yao and Tabas 2001). Such cells also show evidence of release of cytochrome *c* from the mitochondria and of activation of caspase-9. Thus, in addition to the Fas pathway, a classical mitochondrial pathway of apoptosis is activated in macrophages loaded with free cholesterol. The mechanisms by which free cholesterol triggers these events are unknown, although they appear to require the ability of free cholesterol to traffic to the cell membrane.

The presence of apoptotic and necrotic macrophages in human atherosclerotic lesions is well documented (Kockx 1998; Kockx and Herman 1998; Mitchinson et al. 1996). Among the potential causes of lesional macrophage death, toxicity due to excessive free cholesterol is a good candidate because macrophages in advanced atherosclerotic lesions are known to be loaded with free cholesterol (Tabas 1997). The functional significance of cell death is unknown. On the one hand, assuming harmless disposal of apoptotic bodies by neighbouring phagocytes, macrophage apoptosis may limit the number of intimal cells in a physiological manner that avoids inducing local inflammation. On the other hand, death of macrophages by necrosis may lead to uncontrolled proteases, inflammatory cytokines, and prothrombotic molecules, which in turn may lead to plaque rupture and acute thrombotic occlusion of the artery. Necrotic areas of advanced atherosclerotic lesions are known to be associated with death of macrophages, and ruptured plaques from human lesions have been shown to be enriched in apoptotic macrophages (Mitchinson et al. 1996).

3.1.2.6 Summary—The Macrophage in the Atherosclerotic Plaque: Friend or Foe?

Based on the above, it is unclear at the present time if the net effect of the macrophage in the atherosclerotic plaque is beneficial or harmful. Evidence exists from some mouse models that macrophages are necessary for development of the atherosclerotic plaque, and it is likely that generation of the foam cell, and in particular the overwhelming of the macrophage's capacity to deal with excess cholesterol, lie at the heart of macrophage death in the lesion. Macrophages are perhaps the central cell governing the inflammatory response within the plaque, but it is unclear if this response is physiological in that it indicates an attempt by the body to heal the atherosclerotic lesion, or if it is pathological in that it leads to growth and destabilization of the plaque. Finally, macrophages produce a very wide range of enzymes that degrade various components of the extracellular matrix. This may be one of the main mechanisms underlying plaque rupture, a complication that is compounded by macrophage expression of tissue factor and other components of the clotting cascade. On the other hand, more recent research from our own laboratory indicates that macrophages within the atherosclerotic lesion also produce a range of collagens—including several involved specifically in wound healing—and may therefore be active agents of plaque stabilization. The Janus-like nature of the macrophage within the atherosclerotic plaque is indicated in Fig. 3.

Perhaps the answer to this paradox is that net effect of the macrophage within the atherosclerotic plaque may be either beneficial or harmful depending on the stage of the lesion, its cellular composition and other compounding factors such as intercurrent illness in the host. It is in any case premature to conclude that simply because macrophage-derived foam cells are present in the advanced atherosclerotic plaque then they must be harmful, and that therefore prevention of foam cell formation must be beneficial. This is not a purely theoretical consideration. At the time of writing, ACAT inhibitors are undergoing clinical trials in humans based on just this logic (Brown 2001). Such inhibitors have been shown to prevent atherosclerosis in animal models, but the results may not apply to humans, particularly in view of the known toxic effects of raised free cholesterol levels in human macrophages (Tabas 2002). The site of action of these drugs may be the key to explaining the beneficial effects. First, even for ACAT1 inhibitors, which suppress macrophage-associated ACAT activity, the drug's ability to enter the lesion may be limited and moderate suppression of ACAT activity within the cells may be offset by increased cholesterol efflux. ACAT2 inhibitors, on the other hand, should have no direct effect on lesional macrophages and may turn out to be beneficial because of their ability to suppress production of atherogenic lipoproteins in the intestine (Buhman et al. 2000).

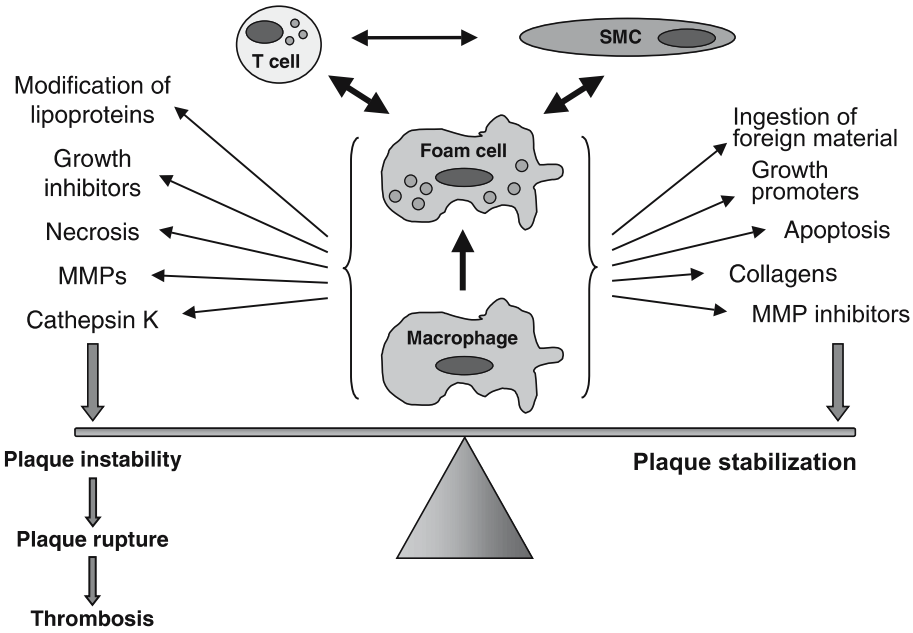

Fig. 3 The Janus-like nature of the macrophage within the atherosclerotic plaque. The macrophage of the arterial wall plays a central role in the development of the atherosclerotic plaque. The macrophage accumulates cholesterol and other lipids by uptake of modified lipoproteins and it is likely that the subsequent formation of foam cells lies at the heart of macrophage death and generation of a lipid core-containing lesion. In addition, macrophages are part of a complex network of interactions between different cell types that contribute to the pathology of the atherosclerotic artery such as smooth muscle cells (*SMCs*) and T cells. Macrophages produce an enormous range of compounds, which impact on the progression of atherosclerotic plaque formation and plaque rupture. For example, macrophages secrete several proteases such as cathepsins and matrix metalloproteinases (*MMPs*) that degrade for example collagenous components of the extracellular matrix. This may be one of the main mechanisms underlying plaque rupture. On the other hand, more recent research indicates that macrophages within the atherosclerotic lesion also produce MMP inhibitors and a range of collagens and may therefore be active agents of plaque stabilization. In addition, one of the main functions of the macrophage is to ingest—and thus to neutralize—toxic substances such as modified lipoproteins and cell detritus that would otherwise accumulate in the subintimal space. It is therefore not clear if the macrophage has a net beneficial or harmful effect on the progression of atherosclerotic plaques. The answer to this paradox may be that net effect of the macrophage within the atherosclerotic plaque may be either beneficial or harmful depending on the stage of the lesion, its cellular composition and other compounding factors such as intercurrent illness in the host

3.1.3 Mast Cells

Mast cells were first characterized in the late nineteenth century by the German physiologist Paul Ehrlich, who observed cells with metachromically staining granules in connective tissue. Ehrlich believed that the granules resulted from overfeeding of cells, and named the cells after the German word 'Mästung', to 'stuff with food' (Ehrlich 1879). His ideas regarding granule origin proved wrong, but the somewhat misleading name remained. Since then, mast cells have been shown to participate in various physiological and pathological processes, notably in allergic reactions, in the defence against parasites and bacteria, in gastric acid secretion, in lipoprotein metabolism and in autoimmune diseases (Benoist and Mathis 2002; Kovanen 1995; Metcalfe et al. 1981; Wedemeyer et al. 2000; Williams and Galli 2000).

Mast cells derive from haematopoietic stem cells in the bone marrow. The undifferentiated progenitor cells circulate in blood and in the lymphatic system before migrating to target tissues (Li and Krilis 1999; Rodewald et al. 1996), where they proliferate and differentiate into T- and TC-type mature mast cells, varying in content of tryptase, chymase and a cathepsin G-like protease as well as in immunobiology (Schechter et al. 1990; Wasserman 1990). The migration and differentiation is influenced by several cytokines such as IL-3, IL-4, and IL-9, nerve growth factor and stem cell factor (Galli et al. 1993; Madden et al. 1991; Mekori and Metcalfe 2000). The most prominent functional feature of mast cells is their ability, upon activation, to exocytose preformed mediators that are vasoactive, that regulate inflammation and cellular growth, or that have immune-modulatory effects. These mediators include the neutral proteases chymase, tryptase and carboxypeptidase A, heparin proteoglycans and histamine, prostaglandin D2, the leukotrienes B4 and C4, TNFα, TGF-β, and IL-4, IL-5, IL-6, and IL-13 (Bachert 2002; Metcalfe et al. 1997; Ra et al. 1994; Repka-Ramirez and Baraniuk 2002; Schwartz and Austen 1984; Young et al. 1987).

Mast cells are present both in normal blood vessels and in atherosclerotic lesions, where they form part of the inflammatory cell infiltrate (Kaartinen et al. 1994; Stary 1990). Increased numbers of activated mast cells are seen in the culprit lesions of patients with unstable coronary syndromes (Kaartinen et al. 1998), an observation that has led to the suggestion that mast cells participate in the pathogenesis of atherosclerosis. Indeed, there is increasing evidence that mast cells play a role in (a) recruitment of inflammatory cells; (b) foam cell formation; and (c) destabilization of atherosclerotic plaques (Kovanen 1995; Kelley et al. 2000).

3.1.3.1
Role of Mast Cells in Recruitment of Inflammatory Cells

Adhesion of circulating monocytes to the endothelium is one of the earliest steps in atherosclerosis (Li et al. 1993). Their entry into the arterial intima depends on the interaction with adhesion molecules on the surface of the endothelium. Activated mast cells secrete a variety of pro-inflammatory substances (Bradding 1996), many of which, such as TNFα, tryptase and histamine (Burns et al. 1999; Compton et al. 2000; Pober et al. 1986), cause endothelial cells to express adhesion molecules such as P-selectin and VCAM-1, which are responsible for the recruitment of monocytes and lymphocytes. Mast cells also stimulate production of macrophage chemotactic peptide 1 in fibroblasts by means of the action of TNFα and TGF-β (Gordon 2000). This in turn increases monocyte penetration into the intima. Thus, mast cells probably participate in the initiation of atherosclerosis by recruiting monocytes and lymphocytes into the vascular intima. Neutrophil infiltration has recently been shown to occur in culprit lesions in acute coronary syndromes (Naruko et al. 2002), but the triggers of this phenomenon are unknown. Both human mast cell tryptase and chymase have been shown to lead to enhanced recruitment of neutrophils into the skin of guinea pigs (He et al. 1997, 1998), but although the relevance of these findings in humans is unknown.

3.1.3.2
Role of Mast Cells in Foam Cell Formation

In atherosclerotic lesions, mast cells often reside in close association with macrophages and extracellular lipids, as well as sites of foam cell formation (Kaartinen et al. 1994b; Jeziorska et al. 1997). The 'balance theory' of atherogenesis proposes that cholesterol, carried into the arterial intima by plasma LDL, is re-circulated back to the circulation by plasma HDL. Thus, cholesterol accumulation and foam cell formation result from an imbalance between these two processes (Kovanen 1990). Increasing evidence shows that mast cells contribute to the transformation of macrophages and smooth muscle cells to foam cells in vitro by disturbing the balance between cholesterol uptake and efflux.

In order to enter the intima, LDL particles must cross the barrier of the arterial endothelium (Stender and Zilversmit 1981). Histamine from mast cells enhances vascular permeability to macromolecules (Wu and Baldwin 1992), suggesting that activated mast cells lower the endothelial barrier and increase the intimal concentration of LDL. In an animal model of passive cutaneous anaphylaxis, local activation of skin mast cells resulted in acute accumulation of LDL in areas in which mast cells were activated to secrete vasoactive components such as histamine (Ma and Kovanen 1997). Mast cells also increase the uptake of LDL by macrophages and smooth muscle cells (Kokkonen and

Kovanen 1987, 1989; Piha et al. 1995; Wang et al. 1995). The heparin proteoglycans of mast cell granule remnants bind LDLs, facilitating chymase-mediated degradation of the apoB within the particles. This results in fusion of the LDL particles and accumulation of fused LDL on granule remnants. Granule remnants coated with fused LDL particles are then phagocytosed by macrophages and smooth muscle cells, thus increasing formation of foam cells. Moreover, soluble heparin proteoglycans released from activated mast cells stimulate scavenger receptor-mediated uptake of LDL (Lindstedt et al. 1992).

Efflux of cellular cholesterol is promoted by extracellular cholesterol acceptors, most notably small discoidal lipid-poor preβ-migrating (preβ-) HDL (Lee et al. 1992). Mast cell chymase can proteolyse the apoA1 of preβ-HDL. This leads to reduced efflux of cholesterol from foam cells, thus increasing cholesterol deposition in the macrophages (Lee et al. 1992, 1999b; Lindstedt et al. 1996). Moreover, mast cell tryptase degrades apolipoproteins of HDL and blocks its function as an acceptor of cholesterol (Lee et al. 2002a, 2002b), although the clinical significance of this is unknown.

3.1.3.3 Role of Mast Cells in Destabilization of the Atherosclerotic Plaque

As described in detail elsewhere in this chapter, the most important mechanism of sudden onset of coronary syndromes such as unstable angina, acute myocardial infarction and sudden cardiac death, is erosion or rupture of an atheroma (Falk 1992; Fuster et al. 1992a, 1992b; Virmani et al. 2000). In addition to macrophages, increased numbers of activated mast cells are found at sites of plaque rupture in patients who have died of acute myocardial infarction (Kovanen et al. 1995). The stability of plaques depends on the thickness and quality of the fibrous cap overlaying the lipid-rich core. The cap consists of smooth muscle cells and extracellular matrix, mostly collagen that is produced and maintained by smooth muscle cells (Lee and Libby 1997). Processes that reduce the number of smooth muscle cells, that inhibit collagen synthesis by these cells, or that increase degradation of the extracellular matrix tend to destabilize the atherosclerotic plaque.

A decrease in the number of smooth muscle cells can be caused by a lower proliferation rate or increased elimination. Mast cell-derived heparin proteoglycans have been shown to inhibit the proliferation of smooth muscle cells in vitro (Wang and Kovanen 1999), suggesting that mast cells may participate in the regulation of smooth muscle cell growth. Since the rate of proliferation of smooth muscle cells in atherosclerotic lesions is rather low (Pickering et al. 1993), the clinical significance of such a mechanism is likely to be small. Under conditions of low proliferation, numbers of smooth muscle cells are largely controlled by cell death, either through necrosis or apoptosis. Some of the mediators released by mast cells are pro-apoptotic, such as chymase which induces cardiomyocyte apoptosis (Hara et al. 1999) and TNFα which triggers

apoptosis of endothelial cells (Slowik et al. 1997). This raises the possibility that mast cells might induce apoptosis of smooth muscle cells and thus reduce plaque stability (Leskinen et al. 2001, 2003a, 2003b).

Matrix metalloproteinases (MMPs) are thought to play a prominent role in degradation of the components of the extracellular matrix of atherosclerotic plaques and to contribute to cap rupture and erosion (Galis et al. 1994; Lijnen 2002). By releasing TNFα, a potent pro-inflammatory cytokine (Kaartinen et al. 1996), mast cells induce synthesis and release of MMP9, both from adjacent macrophages (Saren et al. 1996) and from the TNFα-containing mast cells themselves (Baram et al. 2001). Moreover, TNFα has been shown to increase the expression of the MMP3, MMP8 and MMP9 in endothelial cells (Nelimarkka et al. 1998). Mast cells also synthesize and release MMP1 (Di Girolamo and Wakefield 2000), which has been found in atherosclerotic lesions (Nikkari et al. 1995).

MMPs are synthesized and secreted as zymogens, i.e. as inactive proenzymes (pro-MMPs), and must be activated after secretion (Birkedal-Hansen et al. 1993). Chymase and tryptase are both capable of activating MMPs in vitro, chymase activating pro-MMP1 and tryptase activating pro-MMP3 (Gruber et al. 1989; Saarinen et al. 1994). MMP3, in addition to being a powerful matrix-degrading enzyme, can activate other pro-MMPs, thus triggering a more extensive degradation of the surrounding extracellular matrix. In addition, chymase and tryptase can directly degrade components of the matrix such as fibronectin and vitronectin (Lohi et al. 1992; Vartio et al. 1981).

In addition to the potentially harmful effects outline above, mast cells may also have beneficial effects in atherosclerosis. Heparin proteoglycans released from activated mast cells strongly prevent collagen-induced platelet aggregation (Kauhanen et al. 2000; Lassila et al. 1997), and may thus attenuate the thrombogenicity of the exposed matrix collagen. Mast cell tryptase can interfere with coagulation by degrading fibrinogen and procoagulative kininogen (Maier et al. 1983; Schwartz et al. 1985), which could slow thrombus formation at the sites of plaque rupture. Moreover, serosal mast cells have been shown to block oxidation of LDL in vitro (Lindstedt 1993). Thus, mast cells are also anti-thrombotic and anti-oxidative cells.

3.1.4 T Lymphocytes

Atherosclerosis bears many similarities to autoimmune inflammatory diseases such as rheumatoid arthritis and multiple sclerosis (Hansson 2001; Ross 1999). As noted above, the notion that atherosclerosis has an inflammatory component was already proposed in the nineteenth century by Rudolf Virchow on the basis of light microscopic analysis of human atherosclerotic plaques. The hypothesis was later supported by electron microscopic studies and was confirmed when immunohistochemical analysis revealed that

the CD14+ macrophage indeed was the major cell type in the plaque (Gown et al. 1986; Jonasson et al. 1986). More surprising was the finding that T lymphocytes were also present in substantial numbers in human atherosclerotic plaques (Jonasson et al. 1986). Recent studies demonstrated that presence of T lymphocytes has functional consequences in atherogenesis, because their complete absence reduces lesion formation during moderate hypercholesterolaemia (Dansky et al. 1997; Daugherty et al. 1997; Song et al. 2001).

T lymphocytes are cellular representatives of the specific, adaptive immune system and are designed to perform effector functions after activation by a specific antigen via the T-cell receptor. An obvious question is therefore what antigen these cells might be reactive to. In addition, is there a limited number of atherosclerosis-related antigens taking part in atherogenesis to which T cells show reactivity? The cloning of T cells specific for atherosclerosis-related antigens, such as modified LDLs (Stemme et al. 1995), heat shock proteins (Xu et al. 1993), and *C. pneumoniae* (de Boer et al. 2000b; Curry et al. 2000; Mosorin et al. 2000), from atherosclerotic lesions suggests that a cell-mediated immune reaction is taking place. Initially it was thought that atherosclerotic lesions show a monotypic or oligotypic complementarity-determining spectrum with a restricted heterogeneity of T cells (Paulsson et al. 2000). However, more recent work shows that advanced human plaques demonstrate a polyclonal T-cell composition. This does not constitute evidence that T cells are 'non-specific' (i.e. are carrying reactivities not related to atherosclerosis), but it does suggest that no single antigen reactivity dominates the T-cell population. This result in itself is not surprising, because it is known from other inflammatory conditions with known eliciting antigens that antigen-specific cells constitute a minority of infiltrating T cells. Furthermore, there is little data to support the concept of antigen-specific T-cell recruitment, suggesting instead that T-cell infiltrates arise by predominantly non-antigen specific recruitment, which may be followed by local, clonal, antigen-driven proliferation (Stemme 2001).

Many studies performed in recent years have shown pronounced effects of immunization or different approaches to immunosuppresion (Ameli et al. 1996; Fredrikson et al. 2003; Freigang et al. 1998; George et al. 1998; Maron et al. 2002; Nicoletti et al. 1998; Palinski et al. 1995; Xu et al. 1996; Zhou et al. 2001; Zhou and Hansson 2004). This is in line with the working hypothesis stating that antigen-specific T-cell activation is an important component of the atherosclerotic process. However, although interesting trials of vaccination against atherosclerosis have been performed in animals, it is unclear if a vaccination strategy would be helpful to treat or prevent atherosclerosis in humans.

The major class of T lymphocytes present in atherosclerotic lesions is CD4+. In response to the local milieu of cytokines, CD4+ cells differentiate into the Th1 or Th2 lineage (Mosmann and Sad 1996). Among the principal inducers of the Th1 and Th2 cells are IL-12 and IL-10, respectively. Activated T lymphocytes are functionally defined by the cytokines produced with IFNγ secreted from the Th1 cells and IL-4 from the Th2 cells (Daugherty and Rateri 2002). Th1 induces

macrophage activation and promotes inflammation. Th1 cells accomplish this largely by secreting IFNγ, an important pro-inflammatory cytokine, which is produced in the human atherosclerotic lesion and accelerates atherosclerosis in mice (Hansson 2001). Counteracting this subset, the Th2 cell suppresses inflammation and dampens macrophage activity. Several different cytokines may be responsible for these effects, including IL-4, IL-10, and TGF-β (Hansson 2002; Hansson et al. 2002).

Thus, in summary, the presence of activated T lymphocytes in all stages of human atherosclerotic lesion implies that they are involved in the disease, although their specific role is unclear at the present time.

3.2 The Role of the Extracellular Matrix

A short look at a cross-section of a typical fibrous plaque, especially after collagen-specific staining, will immediately reveal the importance of formation of extracellular matrix in development of the atherosclerotic plaque. Large sections of the sub-intima consist of tissue that is rich in collagen but poor in cells. This exaggerated matrix deposition contributes significantly to narrowing of the arterial lumen. On the other hand, weakening of extracellular matrix in certain areas of the plaque plays a central role in plaque rupture, the most dangerous complication of atherosclerosis. 'Too much and not enough'—a description coined by Mark D. Rekhter (Rekhter 1999) aptly describes the ambivalent role of extracellular matrix formation in atherosclerosis.

Although extracellular matrix normally represents only a small part of the arterial media, its contribution to the function of the arterial wall cannot be overestimated. Extracellular matrix is the main component responsible for the elasticity and tensile strength of the arterial wall. Tensile strength is provided mainly by collagen fibres, including type I, III, and V collagens and fibril-associated components such as type XII and XIV collagens; and small proteoglycans, especially decorin and lumican. Due to their water-binding capacities, other proteoglycans, in particular the high-molecular weight versican, fill the extrafibrillar space within the extracellular matrix and contribute essentially to the regulation of water content and of the viscoelastic properties of the arterial wall. Elastic membranes providing elasticity are complex structures in which a number of microfibrillar proteins, among them fibrillin 1, are tightly associated with the rubber-like elastin.

As noted above, migration of smooth muscle cells from the media into the intima is connected with a change of phenotype from a contractile to a fibroblast-like synthetic phenotype (Owens et al. 1996). These synthetic smooth muscle cells secrete proteins of the extracellular matrix, in particular the fibril-forming collagens type I and III. This seems to be a normal physiological process at sites of high mechanical load. At some high-stress sites such as arterial bifurcations, these processes start as early as

the first weeks of life and even before birth (Velican and Velican 1980). Thus, in infants, enhanced expression of type I and III collagen was localized to smooth muscle cells at a site of pressure-induced intimal thickening on the proximal site of inborn coarctation of the aorta (Jaeger et al. 1990). The formation of a neointima by recruitment of smooth muscle cells from the media is of clinical relevance in the process of restenosis after lumen widening by coronary angioplasty or atherectomy. Growth of a neointima is in this case much faster than in physiological or atherosclerotic neointma formation, leading to complete stenosis within weeks. Enhanced proliferation of smooth muscle cells stands at the beginning of this process. However, the decisive contribution to intimal thickening leading to restenosis comes from enhanced synthesis of components of the extracellular matrix (Fuster et al. 1995).

The role of enhanced formation of extracellular matrix in the development of atherosclerotic plaque is much more complicated than its role in restenosis and far from being understood. Recruitment of monocytes from the circulation and accumulation of subintimal macrophages to form a 'fatty streak' or 'xanthoma' may mark the start of atherogenesis, but most such fatty streaks/xanthomas regress and to do not develop into atherosclerotic lesions. As noted elsewhere, the distribution of fatty streaks and intimal thickenings in children differs from that in adults (Velican and Velican 1980; Virmani et al. 2000). Nevertheless, D. N. Kim observed formation of plaques in coronary arteries of pigs on a hyperlipidaemic diet preferably at locations of pre-existing intimal thickening (Kim et al. 1987). In hypercholesterolaemia in humans, lipids tend to be deposited in the intima in the vicinity of proteoglycans (Kovanen and Pentikainen 1999). Interaction with invading monocytes/macrophages leads to oxidation of LDL which provokes foam cell formation and accumulation and, via interaction with T lymphocytes, induction of an inflammatory process (Hansson 1997). Enhanced cytokine expression induces proliferation of smooth muscle cells, which in turn secrete enhanced amounts of extracellular matrix. Not only oxidized lipoproteins but also chemical modification of structural proteins of the extracellular matrix can initiate inflammation. Thus, non-enzymatic glycosylation (glycation) of collagen as it occurs in persons with diabetes mellitus increases the risk of plaque formation. Final products of glycosylation (advanced glycation end products, AGEs) activate macrophages via a specific receptor for AGEs called RAGE. They also enhance permeability of the endothelium and proliferation of smooth muscle cells and play a role in T-cell activation (for review see Vlassara 1996).

The final consequence of excessive formation of extracellular matrix is the formation of the typical atherosclerotic lesion, the fibrous cap atheroma, in which a core of accumulated and partially necrotic foam cells is surrounded and separated from the lumen by smooth muscle cell-derived fibrotic tissue. The smooth muscle cell-derived extracellular matrix plays and unclear role in this process. On the one hand accumulation of fibrotic tissue contributes to

formation of the necrotic core by hindering nutrition of the deeper layers of the arterial wall; on the other hand the fibrous cap prevents the contact between the bloodstream and the thrombogenic content of the necrotic core.

The morphology of the intimal plaque extracellular matrix shows characteristic differences from the medial extracellular matrix. Extracellular matrix in the intima makes up a bigger proportion of total tissue and varies considerably in the degree of cellularity even within an atherosclerotic plaque. While in the cap region cell density is relatively high, the remainder of the intimal plaque contains very few cells. Compared to medial extracellular matrix, matrix in the intima contains more collagen and less elastin. In addition, the proportion of type III collagen is smaller and there is more type I, V and VI collagen (Barnes and Farndale 1999; Ooshima 1981; Rauterberg et al. 1993). Immunohistology shows the dominance of type I collagen in the fibrotic masses, but staining for basement membrane components reveals surprisingly strong occurrence of typical smooth muscle cell-associated basement membrane proteins such as type IV collagen, mostly in form of empty envelopes of former cells.

It is generally accepted that intimal smooth muscle cells are mainly involved in building up the fibrous cap and in synthesizing the collagenous matrix that provides its tensile strength. Invasion of macrophages is believed to weaken the cap by secretion of matrix-degrading enzymes such as MMP3 and MMP9 and cathepsins (Galis et al. 1994). Recent observations, however, suggest that macrophages may also be able to synthesize components of the extracellular matrix. Active collagen type I expression can be demonstrated by in situ hybridization only in smooth muscle cells in the vicinity of non-foamy macrophages (Jaeger et al. 1990). In the fibrous plaque atheroma is restricted to the cap and shoulders of the lesion and to the plaque base, there mostly in connection with vasa vasora. This suggests that macrophages may stimulate collagen synthesis in cells in their vicinity, probably by synthesis and secretion of TGF-β. It has been known for some time that macrophages themselves are producers of components of the extracellular matrix such as fibronectin, osteopontin, and proteoglycans. Recently, we showed that they are also able to synthesize and secrete at least one collagen (Weitkamp et al. 1999). Synthesis of type VIII collagen was found in human blood-derived macrophages at different stages of differentiation, and its expression was demonstrated by in situ hybridization in macrophages in the cap and shoulder regions of atherosclerotic plaques. The Janus-like nature of monocytes/macrophages in the atherosclerotic plaque can be understood if we bear in mind the main biologic function of this cell type as a wound healer. Beyond its main task of removing debris, the macrophage should have the ability to form a provisional matrix that allows and supports immigration of new tissue-forming cells.

3.3 The Role of Thrombus Formation

Thrombus formation plays an important role in atherogenesis (Burke et al. 2002; Libby 2000). Though there is little evidence that the formation of a blood clot is an early feature of lesion formation as was originally thought by Karl von Rokitansky (Schwartz et al. 1988; von Rokitansky 1852), thrombosis affects the growth and outcome of the pathologic process in several ways:

1. Thrombus formation at the site of an atherosclerotic lesion is the commonest cause of myocardial infarction and stroke; the thrombus may occlude the artery at the site of formation or may detach and block the blood vessel downstream.
2. In most cases, the thrombus does not occlude the artery but is organized and incorporated into the vessel wall, thus contributing to the growth of the atherosclerotic plaque.

According to Renu Virmani and her colleagues (Virmani et al. 2000), thrombus may form at the site of atherosclerosis for three reasons:

1. Rupture of the cap or shoulder of a thin fibrous cap may lead to direct contact of the highly thrombogenic core with the blood stream.
2. Erosion of the endothelial layer exposes the subendothelial collagenous matrix of the intima to the bloodstream. In autopsy studies of victims of sudden coronary death erosion was the cause of thrombus formation in about 40% of cases (Arbustini et al. 1999). Erosion is more common in women than in men.
3. Rarely, thrombus may form at the site of 'calcified nodules', small regions of mineralization that protrude from the intima into the bloodstream.

Thrombi arising due to plaque rupture often fill large areas within the plaque and may be surrounded or infiltrated by areas of haemorrhage. Haemorrhagic events occur frequently in advanced atherosclerotic lesions either by infiltration of blood from the lumen through fissures or by rupture or by degradation of vasa vasora which frequently grow at the plaque base (Kolodgie et al. 2003). Due to the high thrombogenicity of the plaque base, intra-plaque haemorrhages are usually subject to clotting and undergo essentially the same fate as lumenal thrombi.

Thrombus formation is an important part of the normal process of wound healing. In injured vessels, thrombosis is the main mechanism by which blood loss is prevented. The thrombus also serves as a provisional matrix for tissue remodelling. The thrombus initially consists of a fibrin network containing degranulated thrombocytes and other blood cells. This is followed by invasion from the blood, both by polymorphonuclear leucocytes, monocytes and lym-

phocytes and by mesenchymal cells of the adjacent tissue. The latter consist of endothelial cells, which lead to formation of new blood vessels, and smooth muscle cells of a migrating, proliferating and synthetic phenotype. Thrombus organization is an early phase of wound healing and tissue repair. In wound healing four distinct, overlapping phases can be defined: haemostasis, inflammation, proliferation and remodelling. The process of thrombus organization in plaques reflects these phases. The phase of thrombus formation is followed by an inflammatory phase characterized by leukocyte immigration and then by a proliferative phase, which is characterized by immigration and proliferation of smooth muscle cells and endothelium and by synthesis of extracellular matrix. In the remodelling phase, which corresponds to wound contraction, the newly formed collagenous 'scar' tissue contracts, narrowing the lumen of the vessel (Yee and Schwartz 1999). The final stage in the process is not, however, the healed wound but the enlarged plaque.

Both monocytes and polymorphonuclear leucocytes adhere to and invade thrombi, although the rate of adhesion of monocytes is greater (Kirchofer et al. 1997). Young mural thrombi often show clustering of monocytes/macrophages beneath their lumenal surface. Recently, it was shown that invading monocytes not only degrade and phagocytose tissue debris but also contribute to building of a new matrix. This is achieved not only by release of chemotactic factors that induce invasion of matrix-producing smooth muscle cells, but also by expression of matrix proteins such as type VIII collagen (Weitkamp et al. 1999).

Since the middle of the twentieth century, a debate has raged concerning the origin of the mesenchymal vascular cells contributing to thrombus organization. Some have suggested that mesenchymal endothelial or smooth muscle cells may derive from blood monocytes (Leu et al. 1988). However, no in vitro conditions have yet been described in which blood-derived monocytes differentiate into endothelial or smooth muscle cells. By contrast, monocytes in culture differentiate first into macrophages and finally into polynuclear giant cells (Zuckerman et al. 1979). The discussion recently received impetus from the detection in the circulation of stem cells, especially endothelial progenitor cells with the capacity to differentiate to mesenchymal vascular cells after invasion into thrombi (Moldovan 2003).

Another important parallel between wound repair and thrombus-driven plaque growth is that both processes are driven by almost the same panel of chemokines, cytokines and growth factors. The most important factor initiating platelet activation leading to thrombus formation in both cases is tissue factor (Tremoli et al. 1999), which is present at high concentration in plaque tissue (Asada et al. 1998; Fernandez-Ortiz et al. 1994). Invasion of monocytes is stimulated by MCP-1 and invasion and proliferation of smooth muscle cells is driven by PDGF and by thrombin. Thrombin also activates smooth muscle cells via protease-activated receptors (PARs) and stimulates synthesis of type 1 collagen by a PAR-1 mediated mechanism (Dabbagh et al. 1998). The fibrin matrix of the thrombus also supports migration of smooth muscle cells. Production

of components of the extracellular matrix by smooth muscle cells is stimulated by TGF-β, which is released both by platelets and by monocyte-derived macrophages.

Finally, degradation and solubilization of thrombi is inhibited by specific anti-fibrinolytic properties of atherosclerotic vessels. Christ et al. showed that smooth muscle cells from atherosclerotic vessels produce less tissue plasminogen activator and more plasminogen activator inhibitor than smooth muscle cells from normal vessels (Christ et al. 1997).

Why did evolution allow development of an apparently self-destructive mechanism whereby thrombus formation leads to growth of the atherosclerotic plaque? Russell Ross once called atherosclerosis 'a defence mechanism gone awry' (Ross 1981). This idea fits very well to the thrombotic process in atherosclerosis. Thus, our question may be answered by another one. Why should evolution care about atherosclerosis at all? In the vast majority of cases, atherosclerosis occurs at an age that is of minor relevance for reproduction. Efficient wound healing mechanisms, however, are essential for survival at any period of life.

3.4 The Role of Calcification

Calcification is a common and early feature of atheroma. Indeed, calcification within a coronary artery is almost always an indication of the presence of an atherosclerotic plaque (Detrano et al. 2000; Sangiorgi et al. 1998; Stary 2000).

Three types of calcification are recognized in vascular tissue: cardiac valve calcification, calcification of the intimal layer associated with atherosclerosis and calcification of the tunica media (Mönckeberg calcification), which is associated with electrolyte disturbances or with metabolic disorders such as vitamin D poisoning, end-stage renal failure and diabetes mellitus. Medial calcification tends to affect arteries such as those of the abdominal viscera or the arms that are less prone to develop atherosclerosis and has never been reported in coronary arteries. It is unclear at present if medial calcification is associated with increased risk of cardiovascular events, although this may be the case in patients with diabetes mellitus (Doherty et al. 2004).

In contrast to medial calcification, calcification of the intima is seen in the distinct setting of the atherosclerotic plaque. At least two distinct patterns are seen, a punctate distribution of mineralization in the basal regions of the intima adjacent to the media, and a diffuse pattern in all areas of the intima. The latter pattern is often missed because of routine decalcification of histological specimens and is also less likely to be picked up by imaging methods (Fitzpatrick et al. 1994). The former pattern may even be accompanied by features of bone formation such as the presence of haematopoietic marrow, chondrocyte-like cells, osteoblast-like cells and osteoclast-like cells.

Several parallels exist between arterial calcification in atherosclerosis and bone formation. Three general models have been advanced. First, numerous bone-related proteins are expressed in atherosclerotic plaques at sites of calcification (Dhore et al. 2001). For this reason, it has been proposed that the mechanism of intimal arterial calcification is the same as that of bone formation (Parhami et al. 2001). Second, Cees Vermeer and colleagues have proposed a physiochemical model (Gijsbers et al. 1990; Spronk et al. 2001), whereby calcification results from a disturbance of the normal mechanism by which calcium precipitation is prevented by the presence of proteins containing γ-carboxyglutamic amino acid residues. In this model calcification occurs when matrix γ-carboxyglutamic amino acid proteins such as osteopontin and possibly other calcium chelators are no longer able to prevent the ionic calcium concentration in the extracellular fluid of the plaque from reaching sufficiently high levels to allow precipitation to occur. The third model of calcification involves the presence of osteoclast-like cells that actively inhibit calcification (Doherty et al. 2002). Many aspects of all three hypotheses are based on in vitro data and it is not known if any or all are operative in life.

Thus, overall, the role of calcification in lesion progression and in the complications of atherosclerosis is unclear at present. The main importance of calcification of the coronary arteries at the present time is therefore its usefulness as a tool to predict risk of coronary events. A range of very accurate non-invasive imaging methods exist, and many studies suggest that the coronary calcium score is a reliable and independent indicator of risk of myocardial infarction. In particular, the importance of calcification lies in the stratification of risk in asymptomatic patients at intermediate risk of coronary heart disease, in whom the calcium score appears to provide information over and above that provided by conventional risk factors.

4
From Lesion to Infarction: The Vulnerable Plaque

Until quite recently, it was assumed that the risk of myocardial infarction, stroke or sudden coronary death was related simply to the total burden of atherosclerotic disease: the greater the extent of atherosclerosis, the higher the event risk. About 10 years ago, a paradigm shift occurred when it was realized that the severe and sometimes fatal complications of atherosclerosis do not necessarily take place in those with the heaviest burden of disease. Rather, acute blockage of an artery is often caused by a clot that forms at the site of rupture of a so-called vulnerable plaque. Such vulnerable plaques consist of a lipid-rich thrombogenic core that is separated from the arterial bloodstream only by a slender and fragile layer of connective tissue, the fibrous cap. These lesions need not be large, nor need they be particularly old. No longer is the final event seen as the ‘straw that breaks the camel’s back’, the last link in

an inexorable process taking place over a very long time, but as a catastrophe resulting from an acute imbalance of stabilizing and destabilizing forces within the lesion. Such ruptures recur over many years, but do not usually cause complete occlusion of the vessel, resulting instead in mural thrombi that are incorporated into the lesion. Accordingly, rupture of the atherosclerotic plaque is often clinically silent. In addition, it is important to note that thrombosis may occur at the site of an eroded atherosclerotic plaque even without a tear in the fibrous cap of the lesion (Virmani et al. 1999, 2000).

There are therefore three main points that we need to remember:

1. The likelihood of thrombosis of an atherosclerotic vessel is not necessarily related to the volume of atherosclerotic tissue within the vessel. Rather, the likelihood of thrombosis is increased by the presence of metabolically active vulnerable plaques, which may be relatively young and small in size.
2. Thrombosis often occurs at the site of plaque rupture, but most of these thromboses are clinically silent and are incorporated into the lesion (Burke et al. 2001; Farb et al. 1996). Rupture and repair of vulnerable atherosclerotic plaques probably occur on an ongoing basis over many years.
3. Thromboses, including some leading to myocardial infarction, stroke or sudden coronary death, often occur at the site of a vulnerable atherosclerotic plaque that shows only erosion but no rupture (van der Wal et al. 1994; Virmani et al. 1999, 2000).

4.1 The Vulnerable Plaque—Rupture and Erosion

About 15 years ago, based on autopsy findings Michael J. Davies and colleagues proposed that fissuring and rupture of advanced atherosclerotic plaques are the main cause of acute myocardial infarction and sudden coronary death (Davies 1992; Davies and Thomas 1985). More recent studies, carried out in particular by Renu Virmani and colleagues at the Armed Forces Institute of Pathology in Washington DC, indicate that this picture is only partially correct (Virmani et al. 2000).

The concept of plaque rupture supposes that fracture of the fibrous cap exposes thrombogenic material, initiating platelet aggregation and coagulation in the infiltrating and overlying blood. These thrombotic changes result from activation of the clotting cascade by tissue factor, and further propagation of the thrombus through interaction of platelets with the active thrombogenic matrix. Platelet activation and thrombin formation, combined with the evulsion of thrombogenic plaque contents into the lumen of the vessel results in its sudden occlusion. This concept is based on morphological data from autopsies as well as clinical angiographic studies in which the presence of surface irregularities has been identified as evidence of plaque rupture (Ambrose et al.

1986; Giroud et al. 1992; Nobuyoshi et al. 1991). In addition, the studies by Davies and colleagues had found evidence of plaque rupture associated with thrombosis in 73% of cases (Davies 1992). This combined evidence led to the long-held and mechanistically satisfying assumption that plaque rupture is the critical event leading to coronary artery death (Ross 1999).

The major limitation of this paradigm is the lack of direct experimental test in a prospective model in humans or animals. For a variety of reasons that will be discussed in more detail below, it is unlikely that a good animal model of plaque rupture will be available in the near future (Cullen et al. 2003). Lesions in most animal models consist of masses of lipid-laden intimal macrophages without a well-developed fibrous cap, a situation that is quite atypical of human disease.

A further assumption that is unlikely to be correct in every case is that inflammation in the atherosclerotic plaque is a necessary event leading to thrombotic occlusion (Arbustini et al. 1991; Ross 1999).

Based on her findings, Renu Virmani has proposed the following classification of coronary atherosclerosis based on morphology alone as shown in Table 3 (Virmani et al. 2000). Based on this classification, the scheme for the development of the atherosclerotic plaque shown in Fig. 4 has been proposed. Examples of different stages of non-atherosclerotic arteries and atherosclerotic lesions classified according to the Virmani classification are shown in Figs. 5 and 6.

The key features defining the seven categories of lesion in the Virmani classification (initial xanthoma, intimal thickening, fibrous cap atheroma, calcified nodule, thin fibrous cap atheroma, pathological intimal thickening, fibrocalcific plaque) are the accretion of lipid in relation to the formation of the fibrous cap, changes over time in the lipid to form a necrotic core, thickening or thinning of the fibrous cap, and thrombosis. Remaining issues such as the culprit lesion associated with the thrombosis and specific plaque features representing processes critical to changes in the lesion such as angiogenesis, intraplaque haemorrhage, inflammation, calcification, cell death and proteolysis are listed as descriptive terms (Virmani et al. 2000).

Renu Virmani and colleagues propose adopting the term 'intimal xanthoma' in place of 'fatty streak', since xanthoma is a general pathological term that describes focal accumulations of fat-laden macrophages. In humans most fatty streaks/intimal xanthomas regress, as their distribution in adults is very different from that seen in children. In contrast with a widely held assumption, Renu Virmani assumes that most atherosclerotic lesions do not develop from fatty streaks/intimal xanthomas, but rather from more intimal cell masses, based mainly on the finding that the distribution of normal developmental intimal cell masses in children can be correlated with the distribution of atheroma in adults (Schwartz et al. 1995; Velican and Velican 1980).

The 'fibrous cap' of the plaque is a distinct layer of connective tissue completely covering the lipid core. It consists purely of smooth muscle cells in a collage-

Table 3 Classification of coronary atherosclerosis based on morphology according to Virmani et al. 2000

	Description	Thrombosis
Nonatherosclerotic intimal lesions		
Intimal thickening	Normal accumulation of smooth muscle cells in the intima, absence of lipid or macrophage foam cells	Thrombus absent
Intimal xanthoma, 'fatty streak'	Luminal accumulation of smooth muscle cells, no necrotic core, no fibrous cap; such lesions usually regress	Thrombus absent
Progressive atherosclerotic lesions		
Pathological intimal thickening	Smooth muscle cells in proteoglycan-rich matrix, extracellular lipid accumulation, no necrosis	Thrombus absent
Erosion	Plaque as above, luminal thrombosis	Thrombus mostly mural, occlusion rare
Fibrous cap atheroma	Well-formed necrotic core with overlying fibrous cap	Thrombus absent
Erosion	Plaque as above, luminal thrombosis, no communication of thrombus with necrotic core	Thrombus mostly mural, occlusion rare
Thin fibrous cap atheroma	Thin fibrous cap infiltrated by macrophages and lymphocytes with rare smooth muscle cells and necrotic core	Thrombus absent, may contain intraplaque haemorrhage, fibrin
Plaque rupture	Fibroatheroma with cap disruption; luminal thrombus communicates with necrotic core	Thrombus usually occlusive
Calcified nodule	Eruptive nodular calcification with underlying fibrocalcific plaque	Thrombus usually nonocclusive
Fibrocalcific plaque	Collagen-rich plaque with significant stenosis, usually contains large areas of calcification with few inflammatory cells, necrotic core may be present	Thrombus absent

nous proteoglycan matrix, with varying degrees of infiltration by macrophages and lymphocytes. Renu Virmani and colleagues define a 'thin' fibrous cap as one that is less than 65 μm thick. Fibrous caps are in fact often much thinner when they rupture—in one series of ruptured plaques they had a mean thickness of only 23 μm (Burke et al. 1997). In a series of 200 cases of sudden death, about 60% of acute thrombi resulted from rupture of a thin fibrous cap, while most of the remaining 40% of thrombi were seen at an area of plaque erosion, characterized by an area of intima denuded of endothelium where smooth muscle cells and proteoglycans are exposed to the circulating blood (Farb et al. 1995).

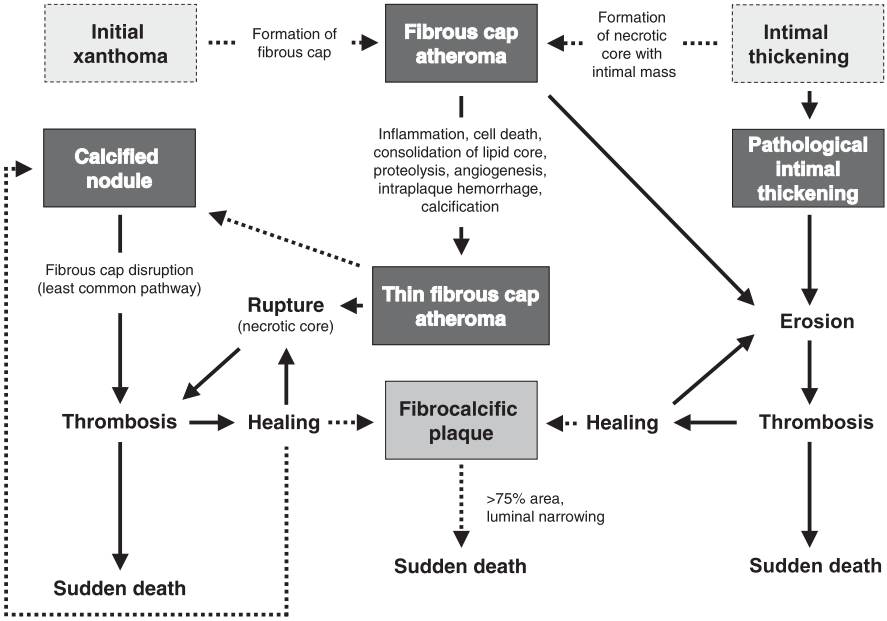

Fig. 4 Simplified scheme for classifying atherosclerotic lesions. The scheme is a modification of the current recommendations of the American Heart Association (AHA) as proposed by Renu Virmani and colleagues (Virmani et al. 2000). The boxed areas represent the seven categories of lesion. Dashed lines have been used for two categories (intimal xanthoma, intimal thickening) because there is controversy over the role that these categories play in the initial phase of lesion formation and both categories can exist without progressing to a fibrous cap atheroma (AHA type IV lesion). The processes leading to lesion progression are listed between categories. *Lines* (*solid* and *dotted*, the latter representing the least-established processes) depict current concepts of how one category may progress to another with the *thickness of the line* representing the strength of the evidence for the step depicted

A rare cause of thrombotic occlusion without rupture is the 'calcified nodule', a lesion characterized by fibrous cap disruption and thrombi in the presence of eruptive dense calcific nodules. The origin of the calcified nodule is unknown, but it may be associated with healed plaques (Virmani et al. 2000). Calcified nodules are found primarily in the right coronary artery where coronary torsion stress is maximal.

Calcified nodules should not be confused with fibrocalcific lesions that are not associated with thrombi. Fibrocalcific lesions are characterized by thick fibrous caps overlying extensive accumulations of calcium in the intima close to the media (Kragel et al. 1989). It is possible that fibrocalcific lesions are the end stage of a process of atheromatous plaque rupture and/or erosion with healing and calcification.

Despite much intensive research, we know surprisingly little about how the atherosclerotic lesion progresses and how the clinically relevant complications of stenosis, plaque erosion and plaque rupture occur.

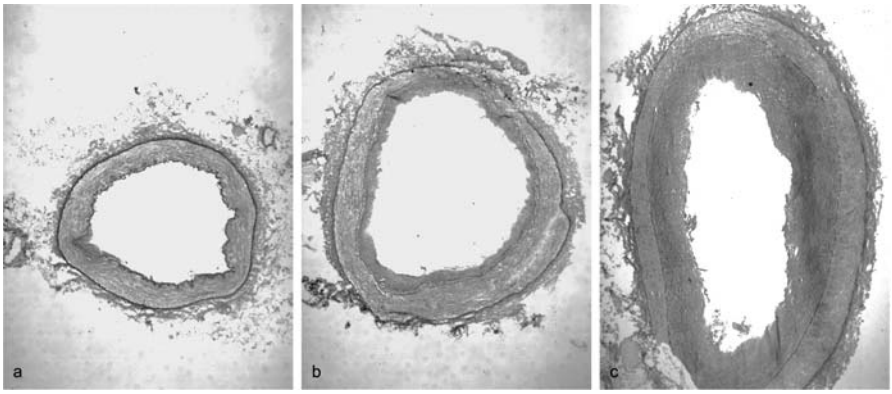

Fig. 5a–c Examples of different stages of non-atherosclerotic arteries. Samples were taken from the MAFAPS arterial tissue database and classified using the Virmani classification (Virmani et al. 2000). Human coronary arteries were obtained from hearts explanted during heart transplantation for advanced coronary heart disease as part of a tissue bank of human coronary arteries established by the MAFAPS consortium (Bellosta et al. 2002; Brinck et al. 2003). The arteries were cut into approximately 1-cm sections and snap-frozen in liquid nitrogen-cooled isopentane within minutes of explantation. Thereafter, coronary arteries were embedded and stored at −80°C until use. The grade of atherosclerosis of each sample was characterized and classified using histochemistry and immunohistology. The sections shown are stained using a van Gieson staining of the lamina elastica. **a** No intimal thickening. **b** Intimal thickening without xanthoma. **c** Intimal thickening with xanthoma

Stenosis of atherosclerotic vessels is the most common therapeutic target. However, this is the change that is least understood from a histological point of view. In an important paper published in 1987, Seymour Glagov and colleagues reported that human coronary arteries affected by atherosclerosis undergo compensatory enlargement, so that plaque mass does not correlate with the size of the lumen (Glagov et al. 1987; Virmani et al. 2000). Thus, the origin of stenosis of the lumen of atherosclerotic coronary arteries in humans is unknown, though it may be related to an attempt by the artery to heal the atherosclerotic lesion.

The origin of erosion of the coronary plaque is a complete mystery. The mechanism of fibrous cap thinning is also unknown, although we have some pointers as to how this might arise. One possibility is by means of apoptosis, yet another feature of atherosclerosis that was presciently described by Rudolf Virchow: ‘thus we have here an active process which really produces new tissues but then hurries on to destruction in consequence of its own development’ (Virchow 1858), cited in (Virmani et al. 2000). Many markers of apoptosis of smooth muscle cells have been found in the atherosclerotic plaque, and plaque smooth muscle cells show elevated levels of apoptosis in vitro and in vivo.

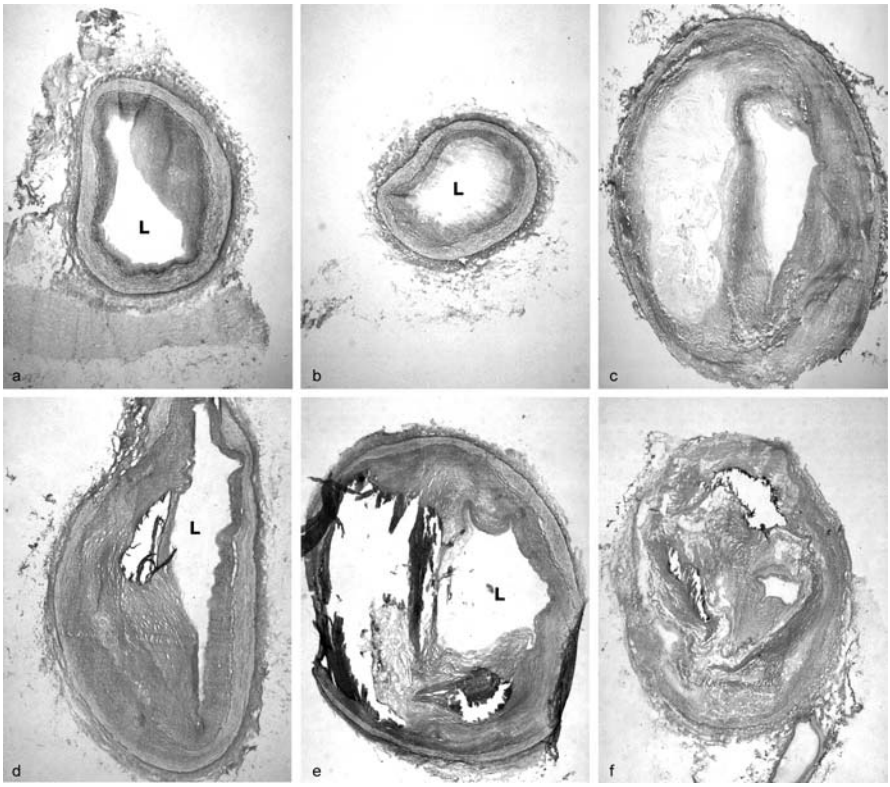

Fig. 6a–f Examples of different stages of atherosclerotic lesions. Samples were chosen from the MAFAPS arterial tissue database (Bellosta et al. 2002; Brinck et al. 2003) and classified using the Virmani classification (Virmani et al. 2000). Artery samples were obtained and processed as mentioned in the legend to Fig. 5. **a** Pathological intimal thickening. **b** Pathological intimal thickening with erosion. **c** Fibrous cap atheroma. **d** Thin fibrous cap atheroma. **e** Plaque rupture. **f** Fibrocalcific plaque. Examples for a fibrous cap atheroma with erosion and a plaque rupture by calcified nodule are not shown. *L*, Lumen

5
Animal Models of Atherosclerosis

Animals have been used for nearly a century to study atherosclerosis and have yielded very important insights into pathogenesis and therapy. However, these successes have sometimes led to uncritical transfer of results of findings in animal models to the situation in humans. In the following we will therefore focus on some of the limitations of existing models as they apply to pathology in humans. This issue has been reviewed in more detail by us elsewhere (Cullen et al. 2003).

5.1 Non-mouse Animal Models of Atherosclerosis

Rabbits develop lipid-rich arterial lesions with some of the features of atherosclerosis only if they are fed large amounts of cholesterol and fat—components that are usually lacking in their vegetarian diet. Indeed, it was in cholesterol-fed rabbits that aortic cholesterol accumulation was first noted by Nikolai Anitschkow in St. Petersburg 90 years ago (Anitschkow 1913). Such diets result in cholesterol levels many times greater than those seen in humans. The lesions that rabbits develop bear only a superficial resemblance to human atheroma, being more fatty and macrophage rich (Badimon 2001).

White Carneau pigeons develop lesions that are morphologically and ultrastructurally more similar to human atherosclerosis (Clarkson et al. 1959; Jerome and Lewis 1985, 1997; Santerre et al. 1972). However, in contrast with humans, susceptibility to atherosclerosis in these birds lies entirely at the level of the arterial wall. Cholesterol levels are normal and other risk factors are absent (Clarkson et al. 1959), the lesions in the pigeons being thought to be entirely due to an inherited (Smith et al. 2001) defect in cholesterol efflux from macrophages (Yancey and St. Clair 1992, 1994).

On a high-cholesterol diet, primates including chimpanzees, squirrel monkeys, howler monkeys, rhesus monkeys and cynomolgous monkeys develop a form of atherosclerosis that is very similar to that of humans (Malinow and Maruffo 1965, 1966; Maruffo and Malinow 1966; Stary and Malinow 1982). However, the cost of primates is prohibitive and many of these species are protected. Thus, work on atherosclerosis in primates is today generally confined to the study of complex issues such as the effects of psychological stress (Rozanski et al. 1999).

The pig is one of the most useful currently available animal models of atherosclerosis. In time, pigs develop atherosclerosis even on a normal porcine diet (Badimon et al. 1985; Fuster et al. 1985; Poeyo Palazón et al. 1998; Royo et al. 2000; Steele et al. 1985). When fed with cholesterol, they develop plasma cholesterol levels and atherosclerotic lesions that are similar to those seen in humans. The white Belgian pig variety also exhibits sudden coronary death when under stress (Badimon 2001). However, maintenance of pigs is expensive and difficult, requiring special facilities that are beyond the capabilities of most laboratories.

Dogs and rats are generally very resistant to atherosclerosis, and develop it only when their diets are very extensively modified (Badimon 2001). In recent years, however, some transgenic rat models have been produced that develop lesions resembling atherosclerosis (Herrera et al. 1999; Richardson et al. 1998; Russel et al. 1998a, 1998b).

5.2 Of Mice and Men, or Why Small Is Not Always Beautiful

Because of ease in handling, the wide knowledge base concerning mouse physiology, and the large amount of mouse genetic information available, most researchers in recent years have focused on mouse models for the study of atherosclerosis (Braun et al. 2002; Calara et al. 2001; Caligiuri et al. 1999; der Thüsen et al. 2002; Ishibashi et al. 1994; Johnson and Jackson 2001; Nakashima et al. 1994; Plump et al. 1992; Rosenfeld et al. 2000; Williams et al. 2002; Zhang et al. 1992).

Before proceeding to a description of the individual models, it is important first to recall the fundamental limitations of the mouse model. Mice do not develop atherosclerosis without genetic manipulation. They have a lipid physiology that is radically different from that of humans, most of the cholesterol being transported in HDL-like particles. Furthermore, mice weigh about 25 g, some 3,000 times less than the average human. Since mouse cells are about the same size as human cells, this means that a section of coronary artery in the mouse contains about 3,000 times fewer cells than an equivalent section of human coronary artery. This is reflected in the histology of mouse arteries, in which the endothelial layer lies directly on the internal elastic lamina and the media consists of only a few layers of smooth muscle cells. In contrast with their counterparts in humans, atherosclerotic lesions in the mouse coronary artery often extend beyond the elastic lamina (Calara et al. 2001). Remodelling of the media and aneurysms are also common in mice (Carmeliet et al. 1997; Daugherty et al. 2000; Heymans et al. 1999; Tangirala et al. 1995). Furthermore, it is difficult in mice to make a distinction between plaque erosion—as defined by endothelial denudation—and complete rupture of the fibrous cap (Calara et al. 2001). Although classical eccentric atheromas with a single fibrous cap exist in lesion-prone mouse models, multiple necrotic core areas with or without separate fibrous caps are the norm (Nakashima et al. 1994; Palinski et al. 1994; Reddick et al. 1994). As pointed out by Federico Calara and colleagues, disruption of these lesions may not mimic plaque rupture in humans, placing a fundamental limit on the applicability of mouse models for investigation of rupture mechanisms (Calara et al. 2001).

In addition to these difficulties arising from the differences between mouse and human biology, there are important issues that need to be remembered in interpreting the results obtained in mouse models that have been derived by genetic manipulation. Problems may occur, for example, when two different genetic models of a particular illness are used to investigate the effect of a third genetic manipulation. An important example in the field of atherosclerosis research concerns studies investigating knocking out the gene for the type A scavenger receptor in different genetic models of atherosclerosis. Hiroshi Suzuki and colleagues reported that deleting this scavenger receptor in apoE

knockout mice reduced atherosclerotic lesion size by 60% (Suzuki et al. 1997). However, Menno de Winther and colleagues found that in the apoE3 Leiden mouse model of atherosclerosis, inactivation of the scavenger receptor actually increases lesion size (de Winther et al. 1999). A possible explanation for this difference relates to the role of apoE in the vessel wall. ApoE has been shown to mediate efflux of cholesterol from macrophages, and it is therefore possible that deficiency in apoE predisposes to macrophage foam cell formation. This process of foam cell formation might be expected to be inhibited by deletion of the scavenger receptor, the main route by which cholesterol-loading of macrophages occurs. By contrast, macrophages from mice bearing the *apoE3 Leiden* gene show normal apoE-mediated cholesterol efflux, so that scavenger receptor-mediated cholesterol uptake does not lead to enhanced foam cell formation, allowing other presumably anti-atherogenic functions of the scavenger receptor to come to the fore.

As indicated by Curt D. Sigmund, a second major problem is the genetic heterogeneity that exists among the strains used to generate transgenic and knockout mice (Sigmund 2000). This may lead to a situation where animals containing exactly the same genetic manipulation exhibit profoundly different phenotypes when present on diverse genetic backgrounds. For example, the extent of atherosclerosis among apoE knockout mice on a standard atherosclerosis-prone C57BL/6 background was found to be seven times greater than apoE knockout mice with an atherosclerosis-resistant FVB genetic background (Dansky et al. 1999). The ideal solution to this problem is to use inbred isogenic strains in which the experimental and control mice differ only at the target locus. The next best alternative is to develop a program of inbreeding to a common, congenic strain, that is, one that is genetically identical to the control strain except for the single region of the chromosome containing the target gene. This is time consuming and expensive. Six generations, or 2 years, of backcross breeding are required before the genetic backgrounds are more than 99% homogenous, with rapidly diminishing returns thereafter. For example, four more generations are needed to increase genetic homogeneity from 99.2% to 99.9% (Sigmund 2000). These problems make it imperative that a detailed description of the genetic background of all mouse models used in transgenic experiments be published, and remind us that the genetic background should always be taken into account when assessing experimental results.

5.3 Animal Models of Plaque Instability and Rupture

Despite the drawbacks mentioned above, several models have been reported recently that plausibly reproduce many of the salient features of plaque ruptures in humans. The only non-mouse model of plaque rupture was presented by Mark D. Rekhter and colleagues in 1998 (Rekhter et al. 1998). The aim of

this model was not to investigate the pathophysiological mechanisms underlying the development of the vulnerable lesion but rather to design a model 'to evaluate plaque mechanical strength/vulnerability characteristics'. In this model, two balloon catheters were used to mechanically injure and thus produce a lesion in the thoracic aorta of a cholesterol and fat-fed rabbit. A third indwelling balloon catheter was then inflated and deflated to produce rupture of the lesion. From this description, it is clear that this animal model may be suitable for measuring the mechanical strength of a plaque, and, perhaps, for investigating thrombotic sequelae, but cannot be expected to provide much information about the pathophysiology of plaque rupture in humans.

The first indirect evidence of plaque rupture in the apoE knockout mouse model of atherosclerosis appeared in 1998, when Robert L. Reddick and colleagues reported thrombus formation in the aortas of mice that were injured by squeezing with a forceps (Reddick et al. 1998). This rather unphysiological model was followed up by a report by Michael E. Rosenfeld that elderly apoE knockout mice (60 weeks old) develop lesions in the brachiocephalic artery that are characterized by the presence of collagen-rich fibrofatty nodules and xanthomas (Rosenfeld et al. 2000). These nodules contained necrotic cores and displayed evidence of intramural bleeding. This bleeding was interpreted as possibly being due to plaque rupture. Moreover, from 42 weeks onwards, mice exhibited layered lesions, implying, the authors suggested, multiple events. In a more recent report, Rosenfeld's group reported that in 30-week-old apoE deficient mice, administration of a large dose of simvastatin (50 mg/kg/day) reduced the frequency of bleeding and calcification within lesions in the brachiocephalic artery, which was interpreted as evidence for 'stabilizing effects [of simvastatin] on advanced atherosclerotic lesions' (Bea et al. 2002). Federico Calara and colleagues followed 82 cholesterol-fed apoE and LDL receptor knockout mice for up to 12 months and 33 chow-fed apoE knockout mice for up to 20 months (Calara et al. 2001). Of the 82 cholesterol-fed animals, three showed aortic plaque rupture and/or thrombi, while of the 33 chow-fed mice, 18 showed atherosclerosis of the coronary arteries. In 3 of these 18 animals, blood-filled channels were seen within the coronary lesions. This was taken to indicate the presence of previous plaque disruption and thrombosis, followed by recanalization. These three mice also showed deep ruptures and thrombosis of the aortic origin.

Finally, much interest was generated by two recently reported models of plaque rupture in apoE knockout mice. In the first of these, from Bristol in the United Kingdom, apoE knockout mice with an unusual mixed C57BL6/129SvJ genetic background were fed a diet containing 21% lard and 0.15% cholesterol for up to 14 months (Johnson and Jackson 2001; Williams et al. 2002). Most of these mice developed atherosclerotic plaque rupture associated with luminal thrombus at the point where the brachiocephalic artery branches into the right common carotid artery. The ruptures were characterized by fragmentation and loss of elastin and smooth muscle cells in the fibrous caps of relatively

small and lipid-rich plaques overlying large complex lesions. Of 98 such mice, 51 had an acutely ruptured plaque in the brachiocephalic artery and 64 died suddenly. However, the incidence of sudden death did not differ between those with brachiocephalic rupture and those without. An undisclosed number of mice in this study also suffered myocardial infarction. In the second study, lesions were induced in apoE knockout mice by placement of a silastic collar around the carotid artery (der Thüsen et al. 2002). The resultant plaques were then incubated transluminally with adenovirus bearing a p53 transgene. Over-expression of p53, a tumour suppressor gene that promotes apoptosis, reduced the cellular and extracellular content of the cap of the lesion, with a reduced cap/intima ratio. When these mice were made hypertensive by treatment with phenylephrine, 40% developed rupture of the p53-treated plaques. Several papers have also appeared in recent years of myocardial infarction in apoE knockout mice without definite evidence of plaque rupture (Braun et al. 2002; Caligiuri et al. 1999; Kuhlencordt et al. 2001). For the sake of brevity, therefore, these models will not be discussed further here, even though some have enthused that their existence should 'finally put to rest the notion that mice cannot be models of plaque rupture' (Palinski and Napoli 2002).

5.4 Usefulness of Current Animal Models of Plaque Instability and Rupture

Of the models of plaque rupture presented thus far, none can be regarded as ideal. Both the rabbit model presented by Rekhter (Rekhter et al. 1998) and the apoE knockout mouse p53/silastic cuff model (der Thüsen et al. 2002) required such heroic measures to produce evidence of plaque rupture that they can tell us little about the pathophysiology of this condition. The usefulness of these models is thus more or less confined to studies of the mechanical process of rupture itself. More interesting from the aetiological and therapeutic points of view are the apoE mouse models in which plaque rupture was seen in elderly fat- and cholesterol-fed mice (Calara et al. 2001; Johnson and Jackson 2001; Rosenfeld et al. 2000; Williams et al. 2002). However, these models too are surrounded by caveats. In the report of Calara and colleagues (Calara et al. 2001), evidence of rupture was indirect and was seen much less frequently (about 5% of the animals) than occurs in human atherosclerosis. In the Rosenfeld model, evidence of rupture was also indirect and was seen in the brachiocephalic artery in particular (Rosenfeld et al. 2000). Finally, in the Bristol model (Johnson and Jackson 2001; Williams et al. 2002), plaque rupture was again focused on the brachiocephalic artery, and was seen only in older mice after prolonged feeding with a very-high-fat diet. The Bristol group has speculated that the predilection for plaque rupture in the brachiocephalic artery may reflect the high degree of tension in the wall of this artery in the mouse. A more general drawback of both the Rosenfeld and Bristol models is that neither shows convincing evidence of the formation of platelet- and fibrin-rich thrombus at

the site of presumed rupture. This is a very important limitation, as infarction of the heart or brain in humans is not caused by rupture of the artery per se, but by the formation of an occlusive blood clot that is rich in platelets and fibrin. Perhaps as a reflection of this lack of thrombosis, death of the mice in Bristol was not related to plaque rupture. Furthermore, in the absence of thrombosis, intra-plaque haemorrhage in these models has been presumed to reflect prior plaque rupture, but this may not necessarily be the case (Majesky 2002). The Rosenfeld and Bristol models also have the disadvantages of the expense required to maintain the mice for more than a year and the variable incidence of plaque rupture.

6 Conclusions

Atherosclerosis in humans is a multi-factorial condition that develops over many years, and we are far from completely understanding its pathogenesis. Of the early lesions that form, most will regress, and some will go on to form atherosclerosis, although we do not know why a particular lesion takes one path or the other. In particular, we are in the dark about the features of atherosclerosis that lead to its clinical impact: stenosis, thrombosis and occlusion. Human coronary arteries affected by atherosclerosis undergo compensatory enlargement, and plaque mass does not correlate with the size of the lumen, so that the origin of stenosis of the lumen is unknown. Occlusive thrombosis often occurs at the site of plaque rupture, but many, perhaps even most plaque ruptures do not cause occlusive thrombosis. Equally, occlusive thrombosis may occur in the absence of plaque rupture at the site of superficial erosion of the endothelium. Perhaps the most that can be said is that occlusive thrombosis of a coronary artery requires some degree of atherosclerosis and will not occur if the vessels are normal. And although we know much of the risk factors leading to myocardial infarction, we do not know in the individual case why an occlusive clot occurs at a particular location at a particular time. Nevertheless, much knowledge of a pragmatic nature exists on how to prevent and treat atherosclerosis. This will form the subject of the remainder of this book.

References

Akira S (2000) The role of IL-18 in innate immunity. Curr Op Lipidol 12:59–63

Al Suwaidi J, Hamasaki S, Higano ST, Nishimura RA, Holmes DR Jr, Lerman A (2000) Long-term follow-up of patients with mild coronary artery disease and endothelial dysfunction. Circulation 101:948–954

Amar S, Gokce N, Morgan S, Loukideli M, Van Dyke TE, Vita JA (2003) Periodontal disease is associated with endothelial dysfunction and systemic inflammation. Arterioscler Thromb Vasc Biol 23:1245–1249

Ambrose JA, Winters SL, Arora RR, Eng A, Riccio A, Gorlin R et al. (1986) Angiographic evolution of coronary artery morphology in unstable angina. J Am Coll Cardiol 7:472–478

Ameli S, Hultgardh-Nilsson A, Regnstrom J, Calara F, Yano J, Cercek B et al. (1996) Effect of immunization with homologous LDL and oxidized LDL on early atherosclerosis in hypercholesterolemic rabbits. Arterioscler Thromb Vasc Biol 16:1074–1079

Anderson JL, Muhlestein JB, Carlquist J, Allen A, Trehan S, Nielson C et al. (1999) Randomized secondary prevention trial of azithromycin in patients with coronary artery disease and serological evidence for Chlamydia pneumoniae infection: The Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia (ACADEMIC) study. Circulation 99:1540–1547

Anderson RG (1998) The caveolae membrane system. Annu Rev Biochem 67:199–225

Anderson TJ (1999) Assessment and treatment of endothelial dysfunction in coronary artery disease and implications for therapy. J Am Coll Cardiol 34:631–638

Anitschkow N (1913) Über die Veränderungen der Kaninchenaorta bei experimenteller Cholesterinsteatose. Beiträge zur pathologischen Anatomie und zur allgemeinen Pathologie 56:379–404

Apfalter P, Blasi F, Boman J, Gaydos CA, Kundi M, Maass M et al. (2001) Multicenter comparison trial of DNA extraction methods and PCR assays for detection of Chlamydia pneumoniae in endarterectomy specimens. J Clin Microbiol 39:519–524

Arbes SJ Jr., Slade GD, Beck JD (1999) Association between extent of periodontal attachment loss and self-reported history of heart attack: an analysis of NHANES III data. J Dent Res 78:1777–1782

Arbustini E, Grasso M, Diegoli M, Pucci A, Bramerio M, Ardissino D et al. (1991) Coronary atherosclerotic plaques with and without thrombus in ischemic heart syndromes: a morphologic, immunohistochemical, and biochemical study. Am J Cardiol 68:36B–50B

Arbustini E, Dal Bello B, Morbini P, Burke AP, Bocciarelli M, Specchia G et al. (1999) Plaque erosion is a major substrate for coronary thrombosis in acute myocardial infarction. Heart 82:269–272

Asada Y, Marutsuka K, Hatakeyama K, Sato Y, Hara S, Kisanuki A et al. (1998) The role of tissue factor in the pathogenesis of thrombosis and atherosclerosis. J Atheroscler Thromb 4:135–139

Aschoff KAL (1908) Über Atherosklerose und andere Sklerosen des Gafässystems. Urban and Schwarzenberg, Berlin

Bachert C (2002) The role of histamine in allergic disease: re-appraisal of its inflammatory potential. Allergy 57:287–296

Badimon L, Steele P, Badimon JJ, Bowie EJ, Fuster V (1985) Aortic atherosclerosis in pigs with heterozygous von Willebrand disease. Comparison with homozygous von Willebrand and normal pigs. Arteriosclerosis 5:366–370

Badimon L (2001) Atherosclerosis and thrombosis: lessons from animal models. Thromb Haemost 86:356–365

Baggiolini M (2001) Chemokines in pathology and medicine. J Intern Med 250:91–104

Baram D, Vaday GG, Salamon P, Drucker I, Hershkoviz R, Mekori YA (2001) Human mast cells release metalloproteinase-9 on contact with activated T cells: juxtacrine regulation by TNF-alpha. J Immunol 167:4008–4016

Barnes MJ, Farndale RW (1999) Collagens and atherosclerosis. Exp Gerontol 34:513–525

Bayes-Genis A, Conover CA, Schwartz RS (2000) The insulin-like growth factor axis: A review of atherosclerosis and restenosis. Circ Res 86:125–130

Bea F, Blessing E, Bennett B, Levitz M, Wallace EP, Rosenfeld ME (2002) Simvastatin promotes atherosclerotic plaque stability in apoE-deficient mice independently of lipid lowering. Arterioscler Thromb Vasc Biol 22:1832–1837

Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S (1996) Periodontal disease and cardiovascular disease. J Periodontol 67:1123–1137

Behrendt D, Ganz P (2002) Endothelial function: from vascular biology to clinical applications. Am J Cardiol 90:40L–80L

Bellosta S, Mahley RW, Sanan DA, Murata J, Newland DL, Taylor JM et al. (1995) Macrophage specific expression of human apolipoprotein E reduces atherosclerosis in hypercholesterolemic apolipoprotein E-null mice. J Clin Invest 96:2170–2179

Bellosta S, Bernini F, Chinetti G, Cignarella A, Cullen P, von Eckardstein A, et al. (2002) Macrophage Function and Stability of Atherosclerotic Plaque Consortium. Macrophage function and stability of the atherosclerotic plaque: progress report of a European project. Nutr Metab Cardiovasc Dis 12:3–11

Benditt EP, Benditt JM (1973) Evidence for a monoclonal origin of human atherosclerotic plaques. Proc Natl Acad Sci USA 70:1753–1756

Benoist C, Mathis D (2002) Mast cells in autoimmune disease. Nature 420:875–878

Birkedal-Hansen H, Moore WG, Bodden MK, Windsor LJ, Birkedal-Hansen B, DeCarlo A et al. (1993) Matrix metalloproteinases: a review. Crit Rev Oral Biol Med 4:197–250

Blanchette-Mackie EJ (2000) Intracellular cholesterol trafficking: role of the NPC1 protein. Biochim Biophys Acta 1486:171–183

Bogdan C (2001) Macrophages. Nature Encyclopedia of Life Sciences; http://www.els.net/. Nature Publishing Group, London

Bonetti PO, Lerman LO, Lerman A (2003) Endothelial dysfunction—a marker of atherosclerotic risk. Arterioscler Thromb Vasc Biol 23:168–175

Bradding P (1996) Human mast cell cytokines. Clin Exp Allergy 26:13–19

Braun A, Trigatti BL, Post MJ, Sato K, Simons M, Edelberg JM et al. (2002) Loss of SR-BI expression leads to the early onset of occlusive atherosclerotic coronary artery disease, spontaneous myocardial infarctions, severe cardiac dysfunction, and premature death in apolipoprotein E-deficient mice. Circ Res 90:270–276

Brinck H, Cullen P, Exley A, Goddard MJ, Kummer S, Lorkowski S, et al. (2003) Internet-based image database for atherosclerosis research. Proceedings of the 17th International Congress and Exhibition Computer Assisted Radiology and Surgery (CARS) Elsevier, Amsterdam, p 301

Brouet A, Sonveaux P, Dessy C, Balligand JL, Feron O (2001) Hsp90 ensures the transition from the early Ca2+-dependent to the late phosphorylation-dependent activation of the endothelial nitric-oxide synthase in vascular endothelial growth factor-exposed endothelial cells. J Biol Chem 276:32663–32669

Brown AJ, Jessup W (1999) Oxysterols and atherosclerosis. Atherosclerosis 142:1–28

Brown WV (2001) Therapies on the horizon for cholesterol reduction. Clin Cardiol 24:III24–III27

Bruggeman CA, Marjorie HJ, Nelissen-Vrancken G (1999) Cytomegalovirus and atherosclerosis. Antiviral Res 43:191–200

Buhman KK, Accad M, Novak S, Choi RS, Wong JS, Hamilton RL et al. (2000) Resistance to diet-induced hypercholesterolemia and gallstone formation in ACAT2-deficient mice. Nat Med 6:1341–1347

Burke AP, Farb A, Malcolm GT, Liang YH, Smialek J, Virmani R (1997) Coronary risk factors and plaque morphology in men with coronary disease who died suddenly. N Engl J Med 336:1276–1282

Burke AP, Kolodgie FD, Farb A, Weber DK, Malcom GT, Smialek J et al. (2001) Healed plaque ruptures and sudden coronary death: evidence that subclinical rupture has a role in plaque progression. Circulation 103:934–940

Burke AP, Farb A, Kolodgie FD, Narula J, Virmani R (2002) Atherosclerotic plaque morphology and coronary thrombi. J Nucl Cardiol 9:95–103

Burns AR, Bowden RA, Abe Y, Walker DC, Simon SI, Entman ML et al. (1999) P-selectin mediates neutrophil adhesion to endothelial cell borders. J Leukoc Biol 65:299–306

Cai H, Harrison DG (2000) Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. Circ Res 87:840–844

Calara F, Silvestre M, Casanada F, Yuan N, Napoli C, Palinski W (2001) Spontaneous plaque rupture and secondary thrombosis in apolipoprotein E-deficient and LDL receptor-deficient mice. J Pathol 195:257–263

Caligiuri G, Levy B, Pernow J, Thoren P, Hansson GK (1999) Myocardial infarction mediated by endothelin receptor signaling in hypercholesterolemic mice. Proc Natl Acad Sci USA 96:6920–6924

Carmeliet P, Moons L, Lijnen R, Baes M, Lemaitre V, Tipping P et al. (1997) Urokinase-generated plasmin activates matrix metalloproteinases during aneurysm formation. Nat Genet 17:439–444

Carstea ED, Morris JA, Coleman KG, Loftus SK, Zhang D, Cummings C et al. (1997) Niemann-Pick C1 disease gene: homology to mediators of cholesterol homeostasis. Science 277:228–231

Celermajer DS, Sorensen KE, Gooch VM, Spiegelhalter DJ, Miller OI, Sullivan ID et al. (1992) Non-invasive detection of endothelial dysfunction in children and adults at risk of atherosclerosis. Lancet 340:1111–1115

Chang TY, Chang CCY, Cheng D (1997) Acyl-coenzyme A: cholesterol acyltransferase. Annu Rev Biochem 66:613–638

Christ G, Hufnagl P, Kaun C, Mundigler G, Laufer G, Huber K et al. (1997) Antifibrinolytic properties of the vascular wall. Dependence on the history of smooth muscle cell doublings in vitro and in vivo. Arterioscler Thromb Vasc Biol 17:723–730

Chung IM, Schwartz SM, Murry CE (1998) Clonal architecture of normal and atherosclerotic aorta: implications for atherogenesis and vascular development. Am J Pathol 152:913–923

Clarkson TB, Pritchard RW, Netsky MG, Lofland HB (1959) Atherosclerosis in pigeons: its spontaneous occurrence and resemblance to human atherosclerosis. AMA Arch Pathol 68:143–147

Cockburn A, Barraco RA, Reyman TA, Peck WH (1975) Autopsy of an Egyptian mummy. Science 187:1155–1160

Cockburn A (1980) Miscellaneous mummies. In: Cockburn A, Cockburn E, (eds). Mummies, disease and ancient cultures. Cambridge University Press, Cambridge

Compton SJ, Cairns JA, Holgate ST, Walls AF (2000) Human mast cell tryptase stimulates the release of an IL-8-dependent neutrophil chemotactic activity from human umbilical vein endothelial cells (HUVEC). Clin Exp Immunol 121:31–36

Cooke JP (2000) Does ADMA cause endothelial dysfunction? Arterioscler Thromb Vasc Biol 20:2032–2037

Cullen P, Fobker M, Tegelkamp K, Meyer K, Kannenberg F, Cignarella A et al. (1997) An improved method for quantification of cholesterol and cholesteryl esters in human monocyte-derived macrophages by high performance liquid chromatography with identification of unassigned cholesteryl ester species by means of secondary ion mass spectrometry. J Lipid Res 38:401–409

Cullen P, Cignarella A, von Eckardstein A, Mohr S, Assmann G (1996) Phenotype dependent differences in apolipoprotein E gene expression and protein secretion in human monocyte-derived macrophages. Circulation 101:1670–1677

Cullen P, Baetta R, Bellosta S, Bernini F, Chinetti G, Cignarella A et al. (2003) Rupture of the atherosclerotic plaque. Does a good animal model exist? Arterioscler Thromb Vasc Biol 23:529–534

Curry AJ, Portig I, Goodall JC, Kirkpatrick PJ, Gaston JS (2000) T lymphocyte lines isolated from atheromatous plaque contain cells capable of responding to Chlamydia antigens. Clin Exp Immunol 121:261–269

Cushing SD, Berliner JA, Valente AJ, Territo MC, Navab M, Parhami F et al. (1990) Minimally modified low density lipoprotein induces monocyte chemotactic protein 1 in human endothelial cells and smooth muscle cells. Proc Natl Acad Sci USA 87:5134–5138

Dabbagh K, Laurent GJ, McAnulty RJ, Chambers RC (1998) Thrombin stimulates smooth muscle cell procollagen synthesis and mRNA levels via a PAR-1 mediated mechanism. Thromb Haemost 79:405–409

Danesh J, Collins R, Peto R (1997) Chronic infections and coronary heart disease: is there a link? Lancet 350:430–436

Danesh J, Whincup P, Walker M, Lennon L, Thomson A, Appleby P et al. (2000) Chlamydia pneumoniae IgG titres and coronary heart disease: prospective study and meta-analysis. BMJ 321:208–213

Danesh J, Whincup P, Lewington S, Walker M, Lennon L, Thomson A et al. (2002) Chlamydia pneumoniae IgA titres and coronary heart disease; prospective study and meta-analysis. Eur Heart J 23:371–375

Dansky HM, Charlton SA, Harper MM, Smith JD (1997) T and B lymphocytes play a minor role in atherosclerotic plaque formation in the apolipoprotein E-deficient mouse. Proc Natl Acad Sci USA 94:4642–4646

Dansky HM, Charlton SA, Sikes JL, Heath SC, Simantov R, Levin LF et al. (1999) Genetic background determines the extent of atherosclerosis in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 19:1960–1968

Daugherty A, Pure E, Delfel-Butteiger D, Chen S, Leferovich J, Roselaar SE et al. (1997) The effects of total lymphocyte deficiency on the extent of atherosclerosis in apolipoprotein E-/- mice. J Clin Invest 100:1575–1580

Daugherty A, Manning MW, Cassis LA (2000) Angiotensin II promotes atherosclerotic lesions and aneurysms in apolipoprotein E-deficient mice. J Clin Invest 105:1605–1612

Daugherty A, Rateri DL (2002) T lymphocytes in atherosclerosis: the yin-yang of Th1 and Th2 influence on lesion formation. Circ Res 90:1039–1040

Davies MJ, Thomas AC (1985) Plaque fissuring: the cause of acute myocardial infarction, sudden ischaemic death, and crescendo angina. Br Heart J 53:363–373

Davies MJ (1992) Anatomic features in victims of sudden coronary death. Circulation 85:119–124

de Boer OJ, van der Wal AC, Becker AE (2000a) Atherosclerosis, inflammation, and infection. J Pathol 190:237–243

de Boer OJ, van der Wal AC, Houtkamp MA, Ossewaarde JM, Teeling P, Becker AE (2000b) Unstable atherosclerotic plaques contain T-cells that respond to Chlamydia pneumoniae. Cardiovasc Res 48:402–408

de Winther MP, Gijbels MJ, van Dijk KW, van Gorp PJ, Suzuki H, Kodama T et al. (1999) Scavenger receptor deficiency leads to more complex atherosclerotic lesions in APOE3Leiden transgenic mice. Atherosclerosis 144:315–321

der Thüsen JH, van Vlijmen BJ, Hoeben RC, Kockx MM, Havekes LM, van Berkel TJ et al. (2002) Induction of atherosclerotic plaque rupture in apolipoprotein E-/- mice after adenovirus-mediated transfer of p53. Circulation 105:2064–2070

Detrano RC, Doherty TM, Davies MJ, Stary HC (2000) Predicting coronary events with coronary calcium: pathophysiologic and clinical problems. Curr Probl Cardiol 25:374–402

Devlin CM, Kuriakose G, Hirsch E, Tabas I (2002) Genetic alterations of IL-1 receptor antagonists in mice affect plasma cholesterol level and foam cell lesion size. Proc Natl Acad Sci USA 99:6280–6285

Dhore CR, Cleutjens JP, Lutgens E, Cleutjens KB, Geusens PP, Kitslaar PJ et al. (2001) Differential expression of bone matrix regulatory proteins in human atherosclerotic plaques. Arterioscler Thromb Vasc Biol 21:1998–2003

Di Girolamo N, Wakefield D (2000) In vitro and in vivo expression of interstitial collagenase/MMP-1 by human mast cells. Dev Immunol 7:131–142

Dimmeler S, Fleming I, Fisslthaler B, Hermann C, Busse R, Zeiher AM (1999) Activation of nitric oxide synthase in endothelial cells by Akt-dependent phosphorylation. Nature 399:601–605

Doherty TM, Uzui H, Fitzpatrick LA, Tripathi PV, Dunstan CR, Asotra K et al. (2002) Rationale for the role of osteoclast-like cells in arterial calcification. FASEB J 16:577–582

Doherty TM, Fitzpatrick LA, Shaheen A, Rajavashisth TB, Detrano RC (2004) Genetic determinants of arterial calcification associated with atherosclerosis. Mayo Clin Proc 79:197–210

Duguid JB (1946) Thrombosis as a factor in the pathogenesis of coronary atherosclerosis. J Pathol Bacteriol 58:207–212

Dunne MW (2000) Rationale and design of a secondary prevention trial of antibiotic use in patients after myocardial infarction: the WIZARD (Weekly Intervention with Zithromax (azithromycin) for Atherosclerosis and its Related Disorders. J Infect Dis 181:S572–S578

Egyptian Museum Cairo. Portraits from the Desert. A temporary exhibition organised by the Kunsthistorisches Museum Vienna in conjunction with the Egyptian Museum Cairo 1999

Ehrlich P (1879) Beiträge zur Kenntniss der granulirten Bindegewebszellen und der eosinophilen Leukocythen. Arch Anat Physiol 3:166–169

Elhage R, Maret A, Pieraggi MT, Thiers JC, Arnal JF, Bayard F (1998) Differential effects of interleukin-1 receptor antagonist and tumor necrosis factor binding protein on fatty-streak formation in apolipoprotein E-deficient mice. Circulation 97:242–244

Engel T, Lorkowski S, Lueken A, Rust S, Schluter B, Berger G et al. (2001) The human ABCG4 gene is regulated by oxysterols and retinoids in monocyte-derived macrophages. Biochem Biophys Res Commun 288:483–488

Engel T, Lueken A, Bode G, Hobohm U, Lorkowski S, Schlueter B et al. (2004) ADP-ribosylation factor (ARF)-like 7 (ARL7) is induced by cholesterol loading and participates in apolipoprotein AI-dependent cholesterol export. FEBS Lett 566:241–246

Epstein SE, Speir E, Zhou YF, Guetta E, Leon M, Finkel T (1996) The role of infection in restenosis and atherosclerosois: focus on cytomegalovirus. Lancet 348:13–17

Epstein SE, Zhu J, Burnett MS, Zhou YF, Vercellotti G, Hajjar D (2000) Infection and atherosclerosis: potential roles of pathogen burden and molecular mimicry. Arterioscler Thromb Vasc Biol 20:1417–1420

Espinola-Klein C, Rupprecht HJ, Blankenberg S, Bickel C, Kopp H, Rippin G et al. (2000) Are morphological or functional changes in the carotid artery wall associated with Chlamydia pneumoniae, Helicobacter pylori, cytomegalovirus, or herpes simplex virus infection? Stroke 31:2127–2133

Espinola-Klein C, Rupprecht HJ, Blankenberg S, Bickel C, Kopp H, Rippin G et al. (2002a) Impact of infectious burden on extent and long-term prognosis of atherosclerosis. Circulation 105:15–21

Espinola-Klein C, Rupprecht HJ, Blankenberg S, Bickel C, Kopp H, Victor A et al. (2002b) Impact of infectious burden on progression of carotid atherosclerosis. Stroke 33:2581–2586

Evanko SP, Raines EW, Ross R, Gold LI, Wight TN (1998) Proteoglycan distribution in lesions of atherosclerosis depends on lesion severity, structural characteristics, and the proximity of platelet-derived growth factor and transforming growth factor-beta. Am J Pathol 152:533–546

Falk E (1992) Why do plaques rupture? Circulation 86:III30–III42

Farb A, Tang AL, Burke AP, Sessums L, Liang Y, Virmani R (1995) Sudden coronary death. Frequency of active coronary lesions, inactive coronary lesions, and myocardial infarction. Circulation 92:1701–1709

Farb A, Burke AP, Tang AL, Liang TY, Mannan P, Smialek J et al. (1996) Coronary plaque erosion without rupture into a lipid core. A frequent cause of coronary thrombosis in sudden coronary death. Circulation 93:1354–1363

Fernandez-Ortiz A, Badimon JJ, Falk E, Fuster V, Meyer B, Mailhac A et al. (1994) Characterization of the relative thrombogenicity of atherosclerotic plaque components: implications for consequences of plaque rupture. J Am Coll Cardiol 23:1562–1569

Fichtlscherer S, Rosenberger G, Walter DH, Breuer S, Dimmeler S, Zeiher AM. Elevated C-reactive protein levels and impaired endothelial vasoreactivity in patients with coronary artery disease. Circulation (2000) 102:1000–1006

Fitzpatrick LA, Severson A, Edwards WD, Ingram RT (1994) Diffuse calcification in human coronary arteries. Association of osteopontin with atherosclerosis. J Clin Invest 94:1597–1604

Fong IW (2000) Emerging relations between infectious diseases and coronary artery disease and atherosclerosis. CMAJ 163:49–56

Fontana J, Fulton D, Chen Y, Fairchild TA, McCabe TJ, Fujita N et al. (2002) Domain mapping studies reveal that the M domain of hsp90 serves as a molecular scaffold to regulate Akt-dependent phosphorylation of endothelial nitric oxide synthase and NO release. Circ Res 90:866–873

Fredrikson GN, Soderberg I, Lindholm M, Dimayuga P, Chyu KY, Shah PK et al. (2003) Inhibition of atherosclerosis in apoE-null mice by immunization with apoB-100 peptide sequences. Arterioscler Thromb Vasc Biol 23:879–884

Freigang S, Horkko S, Miller E, Witztum JL, Palinski W (1998) 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 18:1972–1982

Frothingham C (1911) The relationship between acute infectious diseases and arterial lesions. Arch Intern Med 8:153–162

Fuster V, Badimon L, Badimon JJ, Chesebro JH (1992a) The pathogenesis of coronary artery disease and the acute coronary syndromes (1). N Engl J Med 326:242–250

Fuster V, Badimon L, Badimon JJ, Chesebro JH (1992b) The pathogenesis of coronary artery disease and the acute coronary syndromes (2). N Engl J Med 326:310–318

Fuster V, Lie JT, Badimon L, Rosemark JA, Badimon JJ, Bowie EJ (1985) Spontaneous and diet-induced coronary atherosclerosis in normal swine and swine with von Willebrand disease. Arteriosclerosis 5:67–73

Fuster V, Falk E, Fallon JT, Badimon L, Chesebro JH, Badimon JJ (1995) The three processes leading to post PTCA restenosis: dependence on the lesion substrate. Thromb Haemost 74:552–559

Galis ZS, Sukhova GK, Lark MW, Libby P (1994) Increased expression of matrix metalloproteinases and matrix degrading activity in vulnerable regions of human atherosclerotic plaques. J Clin Invest 94:2493–2503

Galli SJ, Iemura A, Garlick DS, Gamba-Vitalo C, Zsebo KM, Andrews RG (1993) Reversible expansion of primate mast cell populations in vivo by stem cell factor. J Clin Invest 91:148–152

Gargalovic P, Dory L (2003) Caveolins and macrophage lipid metabolism. J Lipid Res 44:11–21

Gaydos CA, Summersgill JT, Sahney NN, Ramirez JA, Quinn TC (1996) Replication of chlamydia-pneumoniae in-vitro in human macrophages, endothelial-cells, and aortic artery smooth-muscle cells. Infect Immun 64:1614–1620

George J, Afek A, Gilburd B, Levkovitz H, Shaish A, Goldberg I et al. (1998) Hyperimmunization of apo-E-deficient mice with homologous malondialdehyde low-density lipoprotein suppresses early atherogenesis. Atherosclerosis 138:147–152

Gijsbers BL, van Haarlem LJ, Soute BA, Ebberink RH, Vermeer C (1990) Characterization of a Gla-containing protein from calcified human atherosclerotic plaques. Arteriosclerosis 10:991–995

Giroud D, Li JM, Urban P, Meier B, Rutishauser W (1992) Relation of the site of acute myocardial-infarction to the most severe coronary arterial-stenosis at prior angiography. Am J Cardiol 69:729–732

Glagov S, Weisenberg E, Zarins CK, Stankunavicius R, Kolettis GJ (1987) Compensatory enlargement of human atherosclerotic coronary arteries. N Engl J Med 316:1371–1375

Godzik KL, Obrien ER, Wang SK, Kuo CC (1995) In vitro susceptibility of human vascular wall cells to infection with Chlamydia pneumoniae. J Clin Microbiol 33:2411–2414

Gokce N, Keaney JF, Hunter LM, Watkins MT, Menzoian JO, Vita JA (2002) Risk stratification for postoperative cardiovascular events via noninvasive assessment of endothelial function: a prospective study. Circulation 105:1567–1572

Gonzalez MA, Selwyn AP (2003) Endothelial function, inflammation, and prognosis in cardiovascular disease. Am J Med 115:99S–106S

Gordon JR (2000) TGFbeta1 and TNFalpha secreted by mast cells stimulated via the FcepsilonRI activate fibroblasts for high-level production of monocyte chemoattractant protein-1 (MCP-1). Cell Immunol 201:42–49

Gown AM, Tsukada T, Ross R (1986) Human atherosclerosis. II. Immunocytochemical analysis of the cellular composition of human atherosclerotic lesions. Am J Pathol 125:191–207

Grahame-Clarke C, Chan NN, Andrew D, Ridgway GL, Betteridge DJ, Emery V et al. (2003) Human cytomegalovirus seropositivity is associated with impaired vascular function. Circulation 108:678–683

Grau AJ, Buggle F, Ziegler C, Schwarz W, Meuser J, Tasman AJ et al. (1997) Association between acute cerebrovascular ischemia and chronic and recurrent infection. Stroke 28:1724–1729

Grau AJ, Becher H, Ziegler CM, Lichy C, Buggle F, Kaiser C et al. (2004) Periodontal disease as a risk factor for ischemic stroke. Stroke 35:496–501

Grayston JT, Campbell LA, Kuo CC, Mordhorst CH, Saikku P, Thom DH et al. (1990) A new respiratory tract pathogen: Chlamydia pneumoniae strain TWAR. J Infect Dis 161:618–625

Grayston JT (1992) Infections caused by Chlamydia pneumoniae strain TWAR. Clin Infect Dis 15:757–761

Gruber BL, Marchese MJ, Suzuki K, Schwartz LB, Okada Y, Nagase H et al. (1989) Synovial procollagenase activation by human mast cell tryptase dependence upon matrix metalloproteinase 3 activation. J Clin Invest 84:1657–1662

Gupta S, Leatham EW, Carrington D, Mendall MA, Kaski JC, Camm AJ. (1997a) Elevated Chlamydia pneumoniae antibodies, cardiovascular events, and azithromycin in male survivors of myocardial infarction. Circulation 96:404–407

Gupta S, Pablo AM, Jiang X, Wang N, Tall AR, Schindler C (1997b) IFN-gamma potentiates atherosclerosis in ApoE knock-out mice. J Clin Invest 99:2752–2761

Halcox JPJ, Schenke WH, Zalos G, Mincemoyer R, Prasad A, Waclawiw MA et al. (2002) Prognostic value of coronary vascular endothelial dysfunction. Circulation 106:653–658

Hamilton TA, Ohmori Y, Tebo JM, Kishore R (1999) Regulation of macrophage gene expression by pro- and anti-inflammatory cytokines. Pathobiology 67:241–244

Han J, Hajjar DP, Zhou X, Gotto AM, Jr., Nicholson AC (2002) Regulation of peroxisome proliferator-activated receptor-gamma-mediated gene expression. A new mechanism of action for high density lipoprotein. J Biol Chem 277:23582–23586

Hansson GK (1997) Cell-mediated immunity in atherosclerosis. Curr Op Lipidol 8:301–311

Hansson GK (2001) Immune mechanisms in atherosclerosis. Arterioscler Thromb Vasc Biol 21:1876–1890

Hansson GK (2002) Vaccination against atherosclerosis: science or fiction? Circulation 106:1599–1601

Hansson GK, Libby P, Schonbeck U, Yan ZQ (2002) Innate and adaptive immunity in the pathogenesis of atherosclerosis. Circ Res 91:281–291

Hara M, Matsumori A, Ono K, Kido H, Hwang MW, Miyamoto T et al. (1999) Mast cells cause apoptosis of cardiomyocytes and proliferation of other intramyocardial cells in vitro. Circulation 100:1443–1449

Harrison DG (1997) Cellular and molecular mechanisms of endothelial cell dysfunction. J Clin Invest 100:2153–2157

Haynes WG, Stanford C (2003) Periodontal disease and atherosclerosis. From dental to arterial plaque. Arterioscler Thromb Vasc Biol 23:1309–1311

He S, Peng Q, Walls AF (1997) Potent induction of a neutrophil and eosinophil-rich infiltrate in vivo by human mast cell tryptase: selective enhancement of eosinophil recruitment by histamine. J Immunol 159:6216–6225

He S, Walls AF (1998) Human mast cell chymase induces the accumulation of neutrophils, eosinophils and other inflammatory cells in vivo. Br J Pharmacol 125:1491–1500

Herrera VL, Makrides SC, Xie HX, Adari H, Krauss RM, Ryan US et al. (1999) Spontaneous combined hyperlipidemia, coronary heart disease and decreased survival in Dahl salt-sensitive hypertensive rats transgenic for human cholesteryl ester transfer protein. Nat Med 5:1383–1389.

Heymans S, Luttun A, Nuyens D, Theilmeier G, Creemers E, Moons L et al. (1999) Inhibition of plasminogen activators or matrix metalloproteinases prevents cardiac rupture but impairs therapeutic angiogenesis and causes cardiac failure. Nat Med 5:1135–1142

Hujoel PP, Drangsholt M, Spiekerman C, DeRouen TA (2000) Periodontal disease and coronary heart disease risk. JAMA 248:1406–1410

Ikari Y, Mcmanus BM, Kenyon J, Schwartz SM (1999) Neonatal intima formation in the human coronary artery. Arterioscler Thromb Vasc Biol 19:2036–2040

Ishibashi S, Goldstein JL, Brown MS, Herz J, Burns DK (1994) Massive xanthomatosis and atherosclerosis in cholesterol-fed low density lipoprotein receptor-negative mice. J Clin Invest 93:1885–1893

Jackson LA (2000) Description and status of the Azithromycin and Coronary Events Study (ACES). J Infect Dis 181:S579–S581

Jaeger E, Rust S, Scharffetter K, Roessner A, Winter J, Buchholz B et al. (1990) Localization of cytoplasmic collagen mRNA in human aortic coarctation: mRNA enhancement in high blood pressure-induced intimal and medial thickening. J Histochem Cytochem 38:1365–1375

Jerome WG, Lewis JC (1985) Early atherogenesis in White Carneau pigeons. II. Ultrastructural and cytochemical observations. Am J Pathol 1985; 119:210–222

Jerome WG, Lewis JC (1997) Cellular dynamics in early atherosclerotic lesion progression in white carneau pigeons—Spatial and temporal analysis of monocyte and smooth muscle invasion of the intima. Arterioscler Thromb Vasc Biol 17:654–664

Jeziorska M, McCollum C, Woolley DE (1997) Mast cell distribution, activation, and phenotype in atherosclerotic lesions of human carotid arteries. J Pathol 182:115–122

Johnson JL, Jackson CL (2001) Atherosclerotic plaque rupture in the apolipoprotein E knockout mouse. Atherosclerosis 154:399–406

Jonasson L, Holm J, Skalli O, Bondjers G, Hansson GK (1986) Regional accumulations of T cells, macrophages, and smooth muscle cells in the human atherosclerotic plaque. Arteriosclerosis 6:131–138

Joshipura KJ, Rimm EB, Douglass CW, Trichopoulos D, Ascherio A, Willett WC (1996) Poor oral health and coronary heart disease. J Dent Res 75:1631–1636

Kaartinen M, Penttila A, Kovanen PT (1994a) Accumulation of activated mast cells in the shoulder region of human coronary atheroma, the predilection site of atheromatous rupture. Circulation 90:1669–1678

Kaartinen M, Penttila A, Kovanen PT (1994b) Mast cells of two types differing in neutral protease composition in the human aortic intima. Demonstration of tryptase- and tryptase/chymase-containing mast cells in normal intimas, fatty streaks, and the shoulder region of atheromas. Arterioscler Thromb 14:966–972

Kaartinen M, Penttila A, Kovanen PT (1996) Mast cells in rupture-prone areas of human coronary atheromas produce and store TNF-alpha. Circulation 94:2787–2792

Kaartinen M, van der Wal AC, Van der Loos CM, Piek JJ, Koch KT, Becker AE et al. (1998) Mast cell infiltration in acute coronary syndromes: implications for plaque rupture. J Am Coll Cardiol 32:606–612

Kalayoglu MV, Libby P, Byrne GI (2002) Chlamydia pneumoniae as an emerging risk factor in cardiovascular disease. JAMA 288:2724–2731

Katsuda S, Coltrera MD, Ross R, Gown AM (1993) Human atherosclerosis. IV. Immunocytochemical analysis of cell activation and proliferation in lesions of young adults. Am J Pathol 142:1787–1793

Kauhanen P, Kovanen PT, Lassila R (2000) Coimmobilized native macromolecular heparin proteoglycans strongly inhibit platelet-collagen interactions in flowing blood. Arterioscler Thromb Vasc Biol 20:E113–E119

Kaukoranta-Tolvanen SS, Laitinen K, Saikku P, Leinonen M (1994) Chlamydia pneumoniae multiplies in human endothelial cells in vitro. Microbial Pathogenesis 16:313–319

Keele KD (1952) Leonardo da Vinci on movement of the heart and blood. Harvey and Blythe, London

Kelley JL, Chi DS, Abou-Auda W, Smith JK, Krishnaswamy G (2000) The molecular role of mast cells in atherosclerotic cardiovascular disease. Mol Med Today 6:304–308

Kellner-Weibel GL, Jerome WG, Small DM, Warner GJ, Stoltenborg JK, Kearney MA et al. (1998) Effects of intracellular free cholesterol accumulation on macrophage viability—A model for foam cell death. Arterioscler Thromb Vasc Biol 18:423–431

Kellner-Weibel G, Yancey PG, Jerome WG, Walser T, Mason RP, Phillips MC et al. (1999) Crystallization of free cholesterol in model macrophage foam cells. Arterioscler Thromb Vasc Biol 19:1891–1898

Khovidhunkit W, Memon RA, Feingold KR (2000) Infection and inflammation-induced proatherogenic changes of lipoproteins. J Infect Dis 181:S462–S472

Kim DN, Schmee J, Lee KT, Thomas WA (1987) Atherosclerotic lesions in the coronary arteries of hyperlipidemic swine. Part 1. Cell increases, divisions, losses and cells of origin in first 90 days on diet. Atherosclerosis 64:231–242

Kirchhofer D, Riederer MA, Baumgartner HR (1997) Specific accumulation of circulating monocytes and polymorphonuclear leukocytes on platelet thrombi in a vascular injury model. Blood 89:1270–1278

Kockx MM (1998) Apoptosis in the atherosclerotic plaque—Quantitative and qualitative aspects. Arterioscler Thromb Vasc Biol 18:1519–1522

Kockx MM, Herman AG (1998) Apoptosis in atherogenesis: implications for plaque destabilization. Eur Heart J 19:G23–G28

Kokkonen JO, Kovanen PT (1987) Stimulation of mast cells leads to cholesterol accumulation in macrophages in vitro by a mast cell granule-mediated uptake of low density lipoprotein. Proc Natl Acad Sci USA 84:2287–2291

Kokkonen JO, Kovanen PT (1989) Proteolytic enzymes of mast cell granules degrade low density lipoproteins and promote their granule-mediated uptake by macrophages in vitro. J Biol Chem 264:10749–10755

Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK et al. (2003) Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med 349:2316–2325

Kovanen PT (1990) Atheroma formation: defective control in the intimal round-trip of cholesterol. Eur Heart J 11:238–246

Kovanen PT (1995) Role of mast cells in atherosclerosis. Chem Immunol 62:132–170

Kovanen PT, Kaartinen M, Paavonen T (1995) Infiltrates of activated mast cells at the site of coronary atheromatous erosion or rupture in myocardial infarction. Circulation 92:1084–1088

Kovanen PT, Pentikainen MO (1999) Decorin links low-density lipoproteins (LDL) to collagen: a novel mechanism for retention of LDL in the atherosclerotic plaque. Trends Cardiovasc Med 9:86–91

Kragel AH, Reddy SG, Wittes JT, Roberts WC (1989) Morphometric analysis of the composition of atherosclerotic plaques in the four major epicardial coronary arteries in acute myocardial infarction and in sudden coronary death. Circulation 80:1747–1756

Kuhlencordt PJ, Gyurko R, Han F, Scherrer-Crosbie M, Aretz TH, Hajjar R et al. (2001) Accelerated atherosclerosis, aortic aneurysm formation, and ischemic heart disease in apolipoprotein E/endothelial nitric oxide synthase double-knockout mice. Circulation 104:448–454

Lassila R, Lindstedt K, Kovanen PT (1997) Native macromolecular heparin proteoglycans exocytosed from stimulated rat serosal mast cells strongly inhibit platelet-collagen interactions. Arterioscler Thromb Vasc Biol 17:3578–3587

Lee M, Lindstedt LK, Kovanen PT (1992) Mast cell-mediated inhibition of reverse cholesterol transport. Arterioscler Thromb 12:1329–1335

Lee RT, Libby P (1997) The unstable atheroma. Arterioscler Thromb Vasc Biol 17:1859–1867

Lee M, von Eckardstein A, Lindstedt L, Assmann G, Kovanen PT (1999) Depletion of pre beta 1LpA1 and LpA4 particles by mast cell chymase reduces cholesterol efflux from macrophage foam cells induced by plasma. Arterioscler Thromb Vasc Biol 19:1066–1074

Lee TS, Yen HC, Pan CC, Chau LY (1999) The role of interleukin 12 in the development of atherosclerosis in ApoE-deficient mice. Arterioscler Thromb Vasc Biol 19:734–742

Lee M, Calabresi L, Chiesa G, Franceschini G, Kovanen PT (2002a) Mast cell chymase degrades apoE and apoA-II in apoA-I-knockout mouse plasma and reduces its ability to promote cellular cholesterol efflux. Arterioscler Thromb Vasc Biol 22:1475–1481

Lee M, Sommerhoff CP, von Eckardstein A, Zettl F, Fritz H, Kovanen PT (2002b) Mast cell tryptase degrades HDL and blocks its function as an acceptor of cellular cholesterol. Arterioscler Thromb Vasc Biol 22:2086–2091

Leskinen M, Wang Y, Leszczynski D, Lindstedt KA, Kovanen PT (2001) Mast cell chymase induces apoptosis of vascular smooth muscle cells. Arterioscler Thromb Vasc Biol 21:516–522

Leskinen MJ, Lindstedt KA, Wang Y, Kovanen PT (2003a) Mast cell chymase induces smooth muscle cell apoptosis by a mechanism involving fibronectin degradation and disruption of focal adhesions. Arterioscler Thromb Vasc Biol 23:238–243

Leskinen MJ, Kovanen PT, Lindstedt KA (2003b) Regulation of smooth muscle cell growth, function and death in vitro by activated mast cells—a potential mechanism for the weakening and rupture of atherosclerotic plaques. Biochem Pharmacol 66:1493–1498

Leu HJ, Feigl W, Susani M, Odermatt B (1988) Differentiation of mononuclear blood cells into macrophages, fibroblasts and endothelial cells in thrombus organization. Exp Cell Biol 56:201–210

Levi M (2001) CMV endothelitis as a factor in the pathogenesis of atherosclerosis. Cardiovasc Res 50:432–433

Li H, Cybulsky MI, Gimbrone MA, Jr., Libby P (1993) Inducible expression of vascular cell adhesion molecule-1 by vascular smooth muscle cells in vitro and within rabbit atheroma. Am J Pathol 143:1551–159

Li L, Krilis SA (1999) Mast-cell growth and differentiation. Allergy 54:306–312

Li AC, Glass CK (2002) The macrophage foam cell as a target for therapeutic intervention. Nat Med 8:1235–1242

Libby P, Egan D, Skarlatos S (1997) Roles of infectious agents in atherosclerosis and restenosis—An assessment of the evidence and need for future research. Circulation 96:4095–4103

Libby P (2000) Multiple mechanisms of thrombosis complicating atherosclerotic plaques. Clin Cardiol 23:3–7

Libby P, Ridker PM, Maseri A (2002) Inflammation and atherosclerosis. Circulation 105:1135–1143

Lijnen HR (2002) Extracellular proteolysis in the development and progression of atherosclerosis. Biochem Soc Trans 30:163–167

Lindstedt KA, Kokkonen JO, Kovanen PT (1992) Soluble heparin proteoglycans released from stimulated mast cells induce uptake of low density lipoproteins by macrophages via scavenger receptor-mediated phagocytosis. J Lipid Res 33:65–75

Lindstedt KA (1993) Inhibition of macrophage-mediated low density lipoprotein oxidation by stimulated rat serosal mast cells. J Biol Chem 268:7741–7746

Lindstedt L, Lee M, Castro GR, Fruchart JC, Kovanen PT (1996) Chymase in exocytosed rat mast cell granules effectively proteolyzes apolipoprotein AI-containing lipoproteins, so reducing the cholesterol efflux-inducing ability of serum and aortic intimal fluid. J Clin Invest 97:2174–2182

Lohi J, Harvima I, Keski-Oja J (1992) Pericellular substrates of human mast cell tryptase: 72,000 dalton gelatinase and fibronectin. J Cell Biochem 50:337–349

Lorkowski S, Kratz M, Wenner C, Schmidt R, Weitkamp B, Fobker M et al. (2001) Expression of the ATP-binding cassette transporter gene ABCG1 (ABC8) in Tangier disease. Biochem Biophys Res Commun 283:821–830

Lorkowski S, Rust S, Engel T, Jung E, Tegelkamp K, Galinski EA et al. (2001) Genomic sequence and structure of the human ABCG1 (ABC8) gene. Biochem Biophys Res Commun 280:121–131

Lorkowski S, Cullen P (2002) Subfamily G of the ATP-binding cassette transporter protein family. Pure Appl Chem 74:2057–2081
Lupu F, Danaricu I, Simionescu N (1987) Development of intracellular lipid deposits in the lipid-laden cells of atherosclerotic lesions. A cytochemical and ultrastructural study. Atherosclerosis 67:127–142
Ma H, Kovanen PT (1997) Degranulation of cutaneous mast cells induces transendothelial transport and local accumulation of plasma LDL in rat skin in vivo. J Lipid Res 38:1877–1887
Madden KB, Urban JF, Jr., Ziltener HJ, Schrader JW, Finkelman FD, Katona IM (1991) Antibodies to IL-3 and IL-4 suppress helminth-induced intestinal mastocytosis. J Immunol 147:1387–1391
Magee R (1998) Arterial disease in antiquity. Med J Australia 169:663–666
Maier M, Spragg J, Schwartz LB (1983) Inactivation of human high molecular weight kininogen by human mast cell tryptase. J Immunol 130:2352–2356
Majesky MW (2002) Mouse model for atherosclerotic plaque rupture. Circulation 105:2010–2011
Malinow MR, Maruffo CA (1965) Aortic atherosclerosis in free-ranging howler monkeys (Alouatta caraya). Nature 206:948–949
Malinow MR, Maruffo CA (1966) Naturally occurring atherosclerosis in howler monkeys (Alouatta caraya). J Atheroscler Res 6:368–380
Mallat Z, Besnard S, Duriez M, Deleuze V, Emmanuel F, Bureau MF et al. Protective role of interleukin-10 in atherosclerosis. Circ Res (1999) 85:e17–e24
Maron R, Sukhova G, Faria AM, Hoffmann E, Mach F, Libby P et al. (2002) Mucosal administration of heat shock protein-65 decreases atherosclerosis and inflammation in aortic arch of low-density lipoprotein receptor-deficient mice. Circulation 106:1708–1715
Maruffo CA, Malinow MR (1966) Dissecting aneurysm of the aorta in a howler monkey (Alouatta caraya). J Pathol Bacteriol 92:567–570
Mattila KJ, Nieminen MS, Valtonen VV, Rasi VP, Kesaniemi YA, Syrjala SL et al. (1989) Association between dental health and acute myocardial infarction. BMJ 298:779–781.
Mattila KJ, Valtonen VV, Nieminen M, Huttunen JK (1995) Dental infection and the risk of new coronary events—prospective study of patients with documented coronary-artery disease. Clin Infect Dis 20:588–592
Maxfield FR, Wustner D (2002) Intracellular cholesterol transport. J Clin Invest 110:891–898
Mekori YA, Metcalfe DD (2000) Mast cells in innate immunity. Immunol Rev 173:131–140
Mendez AJ, Lin G, Wade DP, Lawn RM, Oram JF (2001) Membrane lipid domains distinct from cholesterol/sphingomyelin-rich rafts are involved in the ABCA1-mediated lipid secretory pathway. J Biol Chem 276:3158–3166
Metcalfe DD, Kaliner M, Donlon MA (1981) The mast cell. Crit Rev Immunol 3:23–74
Metcalfe DD, Baram D, Mekori YA (1997) Mast cells. Physiol Rev 77:1033–1079
Mitchinson MJ, Hardwick SJ, Bennett MR (1996) Cell-death in atherosclerotic plaques. Curr Op Lipidol 7:324–329
Moldovan NI (2003) Current priorities in the research of circulating pre-endothelial cells. Adv Exp Med Biol 522:1–8
Morrison HI, Ellison LF, Taylor GW (1999) Periodontal disease and risk of fatal coronary heart and cerebrovascular diseases. J Cardiovasc Risk 6:7–11
Mosmann TR, Sad S (1996) The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 17:138–146
Mosorin M, Surcel HM, Laurila A, Lehtinen M, Karttunen R, Juvonen J et al. (2000) Detection of Chlamydia pneumoniae-reactive T lymphocytes in human atherosclerotic plaques of carotid artery. Arterioscler Thromb Vasc Biol 20:1061–1067

Muhlestein JB, Anderson JL, Carlquist JF, Salunkhe K, Horne BD, Pearson RR et al. (2000) Randomized secondary prevention trial of azithromycin in patients with coronary artery disease: primary clinical results of the ACADEMIC study. Circulation 102:1755–1760

Munder M, Mallo M, Eichmann K, Modolell M (1998) Murine macrophages secrete interferon gamma upon cobined stimulation with interleukin (IL)-12 and IL-18: a novel pathway of autocrine macrophage activation. J Exp Med 187:2103–2108

Murohara T, Kugiyama K, Ohgushi M, Sugiyama S, Ohta Y, Yasue H (1994) LPC in oxidized LDL elicits vasocontraction and inhibits endothelium-dependent relaxation. Am J Physiol 36:H2441–H2449

Murry CE, Gipaya CT, Bartosek T, Benditt EP, Schwartz SM (1997) Monoclonality of smooth muscle cells in human atherosclerosis. Am J Pathol 151:697–705

Nagano H, Mitchell RN, Taylor MK, Hasegawa S, Tilney NL, Libby P (1997) Interferon-gamma deficiency prevents coronary arteriosclerosis but not myocardial rejection in transplanted mouse hearts. J Clin Invest 100:550–557

Nagy L, Tontonoz P, Alvarez JA, Chen HW, Evans RM (1998) Oxidized LDL regulates macrophage gene expression through ligand activation of PPAR gamma. Cell 93:229–240

Nakashima Y, Plump AS, Raines EW, Breslow JL, Ross R (1994) ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. Arterioscler Thromb 14:133–140

Napoli C, DArmiento FP, Mancini FP, Postiglione A, Witztum JL, Palumbo G et al. (1997) Fatty streak formation occurs in human fetal aortas and is greatly enhanced by maternal hypercholesterolemia—Intimal accumulation of low density lipoprotein and its oxidation precede monocyte recruitment into early atherosclerotic lesions. J Clin Invest 100:2680–2690

Naruko T, Ueda M, Haze K, van der Wal AC, Van der Loos CM, Itoh A et al. (2002) Neutrophil infiltration of culprit lesions in acute coronary syndromes. Circulation 106:2894–2900

Narumiya S, Sugimoto Y, Ushikubi F (1999) Prostanoid receptors: structures, properties, and functions. Physiol Rev 79:1193–1226

Naureckiene S, Sleat DE, Lackland H, Fensom A, Vanier MT, Wattiaux R et al. (2000) Identification of HE1 as the second gene of Niemann-Pick C disease. Science 290:2298–2301

Nelimarkka LO, Nikkari ST, Ravanti LS, Kahari VM, Jarvelainen HT (1998) Collagenase-1, stromelysin-1 and 92 kDa gelatinase are associated with tumor necrosis factor-alpha induced morphological change of human endothelial cells in vitro. Matrix Biol 17:293–304

Nerheim PL, Meier JL, Vasef MA, Li.W.-G., Hu L, Rice JB et al. (2004) Enhanced cytomegalovirus infection in atherosclerotic human blood vessels. Am J Pathol 164:589–600

Newby AC (1997) Molecular and cell biology of native coronary and vein-graft atherosclerosis: regulation of plaque stability and vessel-wall remodelling by growth factors and cell-extracellular matrix interactions. Coron Artery Dis 8:213–224

Nicholson AC (2004) Expression of CD36 in macrophages and atherosclerosis. The role of lipid regulation and PPARg signalling. Trends Cardiovasc Med 14:8–12

Nicoletti A, Kaveri S, Caligiuri G, Bariety J, Hansson GK (1998) Immunoglobulin treatment reduces atherosclerosis in apo E knockout mice. J Clin Invest 102:910–918

Nieminen MS, Mattila K, Valtonen V (1993) Infection and inflammation as risk-factors for myocardial-infarction. Eur Heart J 14:12–16

Nikkari ST, Obrien KD, Ferguson M, Hatsukami T, Welgus HG, Alpers CE et al. (1995) Interstitial collagenase (MMP 1) expression in human carotid atherosclerosis. Circulation 92:1393–1398

Nobuyoshi M, Tanaka M, Nosaka H, Kimura T, Yokoi H, Hamasaki N et al. (1991) Progression of coronary atherosclerosis: is coronary spasm related to progression? J Am Coll Cardiol 18:904–910

Ohgushi M, Kugiyama K, Fukunaga K, Murohara T, Sugiyama S, Miyamoto E et al. (1993) Protein kinase C inhibitors prevent impairment of endothelium-dependent relaxation by oxidatively modified LDL. Arterioscler Thromb 13:1525–1532

Ooshima A (1981) Collagen alpha B chain: increased proportion in human atherosclerosis. Science 213:666–668

Osler W (1980) Modern medicine: its theory and practice. Lea and Febiger, Philadelphia

Østerud B, Bjørklid E (2003) Role of monocytes in atherogenesis. Physiol Rev 83:1069–1112

Owens GK, Vernon SM, Madsen CS (1996) Molecular regulation of smooth muscle cell differentiation. J Hypertens Suppl 14:S55–S64

Palinski W, Ord VA, Plump AS, Breslow JL, Steinberg D, Witztum JL (1994) ApoE-deficient mice are a model of lipoprotein oxidation in atherogenesis. Demonstration of oxidation-specific epitopes in lesions and high titers of autoantibodies to malondialdehyde- lysine in serum. Arterioscler Thromb 14:605–616

Palinski W, Miller E, Witztum JL (1995) Immunization of low density lipoprotein (ldl) receptor-deficient rabbits with homologous malondialdehyde-modified ldl reduces atherogenesis. Proc Natl Acad Sci USA 92:821–825

Palinski W, Napoli C (2002) Unraveling pleiotropic effects of statins on plaque rupture. Arterioscler Thromb Vasc Biol 22:1745–1750

Parhami F, Tintut Y, Patel JK, Mody N, Hemmat A, Demer LL (2001) Regulation of vascular calcification in atherosclerosis. Z Kardiol 90:27–30

Paulsson G, Zhou X, Tornquist E, Hansson GK (2000) Oligoclonal T cell expansions in atherosclerotic lesions of apolipoprotein E-deficient mice. Arterioscler Thromb Vasc Biol 20:10–17

Pickering JG, Weir L, Jekanowski J, Kearney MA, Isner JM (1993) Proliferative activity in peripheral and coronary atherosclerotic plaque among patients undergoing percutaneous revascularization. J Clin Invest 91:1469–1480

Piha M, Lindstedt L, Kovanen PT (1995) Fusion of proteolyzed low-density lipoprotein in the fluid phase: a novel mechanism generating atherogenic lipoprotein particles. Biochemistry 34:10120–10129

Pinderski Oslund LJ, Hedrick CC, Olvera T, Hagenbaugh A, Territo M, Berliner JA et al. (1999) Interleukin-10 blocks atherosclerotic events in vitro and in vivo. Arterioscler Thromb Vasc Biol 19:2847–2853

Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG et al. (1992) Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. Cell 71:343–353

Pober JS, Gimborne MA, Jr., Lapierre DL, Mendrick W, Fiers W, Rothlein R et al. (1986) Overlapping patterns of activation of human endothelial cells by interleukin I, tumor necrosis factor, and immune interferon. J Immunol 137:1893

Prasad A, Zhu J, Halcox JP, Waclawiw MA, Epstein SE, Quyyumi AA (2002) Predisposition to atherosclerosis by infections: role of endothelial dysfunction. Circulation 106:184–190

Pueyo Palazón P, Alfón J, Gaffney P, Berrozpe M, Royo T, Badimon L (1998) Effects of reducing LDL and increasing HDL with gemfibrozil in experimental coronary lesion development and thrombotic risk. Atherosclerosis 136:333–345

Pussinen PJ, Jousilahti P, Alfthan G, Palosuo T, Asikainen S, Salomaa V (2003) Antibodies to periodontal pathogens are associated with coronary heart disease. Arterioscler Thromb Vasc Biol 24:1250–1254

Quiney JR, Watts GB. (1989) Introduction. In: Quiney JR, Watts GB (eds) Classic papers in hyperlipidemia. Science Press Limited, London
Ra C, Yasuda M, Yagita H, Okumura K. Fibronectin receptor integrins are involved in mast cell activation. J Allergy Clin Immunol 1994; 94:625–628
Rauterberg J, Jaeger E, Althaus M (1993) Collagens in atherosclerotic vessel wall lesions. Curr Top Pathol 87:163–192
Reddick RL, Zhang SH, Maeda N (1994) Atherosclerosis in mice lacking Apo E—evaluation of lesional development and progression. Arterioscler Thromb 14:141–147
Reddick RL, Zhang SH, Maeda N (1998) Aortic atherosclerotic plaque injury in apolipoprotein E deficient mice. Atherosclerosis 140:297–305
Rekhter MD, Hicks GW, Brammer DW, Work CW, Kim JS, Gordon D et al. (1998) Animal model that mimics atherosclerotic plaque rupture. Circ Res 83:705–713
Rekhter MD (1999) Collagen synthesis in atherosclerosis: too much and not enough. Cardiovasc Res 41:376–384
Repka-Ramirez MS, Baraniuk JN (2002) Histamine in health and disease. Clin Allergy Immunol 17:1–25
Richardson M, Schmidt AM, Graham SE, Achen B, DeReske M, Russell JC (1998) Vasculopathy and insulin resistance in the JCR:LA-cp rat. Atherosclerosis 138:135–146
Rodewald HR, Dessing M, Dvorak AM, Galli SJ (1996) Identification of a committed precursor for the mast cell lineage. Science 271:818–822
Rosenfeld ME, Polinsky P, Virmani R, Kauser K, Rubanyi G, Schwartz SM (2000) Advanced atherosclerotic lesions in the innominate artery of the ApoE knockout mouse. Arterioscler Thromb Vasc Biol 20:2587–2592
Ross R, Glomset JA (1973) Atherosclerosis and the arterial smooth muscle cell: proliferation of smooth muscle is a key event in the genesis of the lesions of atherosclerosis. Science 180:1332–1339
Ross R, Glomset J, Kariya B, Raines E (1978) Role of platelet factors in the growth of cells in culture. Natl Cancer Inst Monogr 48:103–108
Ross R (1981) The Gordon Wilson Lecture: atherosclerosis—a response to injury gone awry. Trans Am Clin Climatol Assoc 93:78–86
Ross R (1993) The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature 362:801–809
Ross R (1999) Atherosclerosis, an inflammatory disease. N Engl J Med 340:115–126
Rothblat GH, Llera-Moya M, Atger V, Kellner-Weibel G, Williams DL, Phillips MC (1999) Cell cholesterol efflux: integration of old and new observations provides new insights. J Lipid Res 40:781–796
Royo T, Alfon J, Berrozpe M, Badimon L (2000) Effect of gemfibrozil on peripheral atherosclerosis and platelet activation in a pig model of hyperlipidemia. Eur J Clin Invest 30:843–852
Rozanski A, Blumenthal JA, Kaplan J (1999) Impact of psychological factors on the pathogenesis of cardiovascular disease and implications for therapy. Circulation 99:2192–2217
Ruffer MA (1911) On arterial lesions found in Egyptian mummies. J Pathol Bacteriol 15:453–462
Ruffer MA (1920) Remarks on the histology and pathological anatomy of Egyptian mummies. Cairo Scientific J 4:3–7
Rupprecht HJ, Blankenberg S, Bickel C, Rippin G, Hafner G, Prellwitz W et al. (2001) Impact of viral and bacterial infectious burden on long-term prognosis in patients with coronary artery disease. Circulation 104:25–31
Russell JC, Graham SE, Richardson M (1998a) Cardiovascular disease in the JCR:LA-cp rat. Mol Cell Biochem 188:113–126

Russell JC, Graham SE, Amy RM, Dolphin PJ (1998b) Cardioprotective effect of probucol in the atherosclerosis-prone JCR:LA-cp rat. Eur J Pharmacol 350:203–210
Saarinen J, Kalkkinen N, Welgus HG, Kovanen PT (1994) Activation of human interstitial procollagenase through direct cleavage of the leu(83)-thrbond by mast cell chymase. J Biol Chem 269:18134–18140
Samuelsson B (2000) The discovery of the leukotrienes. Am J Respir Crit Care Med 161:S2–S6
Sandison AT (1962) Degenerative diseases in the Egyptian mummy. Med Hist 6:77–81
Sandison AT (1981) Diseases of the ancient world. In: Anthony PP, MacSween RNM (eds) Recent advances in pathology, Vol 11. Churchill Livingstone, Edinburgh, pp 1–18
Sangiorgi G, Rumberger JA, Severson A, Edwards WD, Gregoire J, Fitzpatrick LA et al. (1998) Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. J Am Coll Cardiol 31:126–133
Santerre RF, Wight TN, Smith SC, Brannigan D (1972) Spontaneous atherosclerosis in pigeons. A model system for studying metabolic parameters associated with atherogenesis. Am J Pathol 67:1–22
Saren P, Welgus HG, Kovanen PT (1996) TNF-a and IL-1b selectively induce expression of 92-kDa gelatinase by human macrophages. J Immunol 157:4159–4165
Scannapieco FA, Bush RB, Paju S (2003) Associations between periodontal disease and riks for atherosclerosis, cardiovascular disease, and stroke. A systematic review. Ann Periodontol 8:38–53
Schechter NM, Irani AM, Sprows JL, Abernethy J, Wintroub B, Schwartz LB (1990) Identification of a cathepsin G-like proteinase in the MCTC type of human mast cell. J Immunol 145:2652–2661
Scheiffele P, Rietveld A, Wilk T, Simons K (1999) Influenza viruses select ordered lipid domains during budding from the plasma membrane. J Biol Chem 274:2038–2044
Schroeder R, London E, Brown D (1994) Interactions between saturated acyl chains confer detergent resistance on lipids and glycosylphosphatidylinositol (GPI)-anchored proteins: GPI-anchored proteins in liposomes and cells show similar behavior. Proc Natl Acad Sci USA 91:12130–12134
Schwartz LB, Austen KF (1984) Structure and function of the chemical mediators of mast cells. In: Ishizaka K (ed) Mast cell activation and mediator release. Karger, Basel, pp 271–321
Schwartz LB, Bradford TR, Littman BH, Wintroub BU (1985) The fibrinogenolytic activity of purified tryptase from human lung mast cells. J Immunol 135:2762–2767
Schwartz CJ, Valente AJ, Kelley JL, Sprague EA, Edwards EH (1988) Thrombosis and the development of atherosclerosis: Rokitansky revisited. Semin Thromb Hemost 14:189–195
Schwartz SM, de Blois D, O'Brien ER (1995) The intima: soil for atherosclerosis and restenosis. Circ Res 77:445–465
Scotland RS, Morales-Ruiz M, Chen Y, Yu J, Rudic RD, Fulton D et al. (2002) Functional reconstitution of endothelial nitric oxide synthase reveals the importance of serine 1179 in endothelium-dependent vasomotion. Circ Res 90:904–910
Shattock SG (1909) A report upon the pathological condition of the aorta of King Menephthah, traditionally regarded as the Pharoah of the Exodus. Proc Roy Soc Med Path 2:122–127
Shio H, Haley NJ, Fowler S (1979) Characterization of lipid-laden aortic cells from cholesterol-fed rabbits. III. Intracellular localization of cholesterol and cholesteryl esters. Lab Invest 41:160–167

Sigmund CD (2000) Viewpoint: are studies in genetically altered mice out of control? Arterioscler Thromb Vasc Biol 20:1425–1429

Simionescu N, Vasile E, Lupu F, Popescu G, Simionescu M (1986) Prelesional events in atherogenesis. Accumulation of extracellular cholesterol-rich liposomes in the arterial intima and cardiac valves of the hyperlipidemic rabbit. Am J Pathol 123:109–125

Simons K, Ikonen E (2000) How cell handle cholesterol. Science 290:1721–1726

Skalen K, Gustafsson M, Rydberg EK, Hulten LM, Wiklund O, Innerarity TL et al. (2002) Subendothelial retention of atherogenic lipoproteins in early atherosclerosis. Nature 417:750–754

Slowik MR, Min W, Ardito T, Karsan A, Kashgarian M, Pober JS (1997) Evidence that tumor necrosis factor triggers apoptosis in human endothelial cells by interleukin-1-converting enzyme-like protease-dependent and -independent pathways. Lab Invest 77:257–267

Smith SC, Smith EC, Taylor RL, Jr. (2001) Susceptibility to spontaneous atherosclerosis in pigeons: an autosomal recessive trait. J Hered 92:439–442

Song L, Leung C, Schindler C (2001) Lymphocytes are important in early atherosclerosis. J Clin Invest 108:251–259

Spronk HM, Soute BA, Schurgers LJ, Cleutjens JP, Thijssen HH, De Mey JG et al. (2001) Matrix Gla protein accumulates at the border of regions of calcification and normal tissue in the media of the arterial vessel wall. Biochem Biophys Res Commun 289:485–490

Stary HC, Malinow MR (1982) Ultrastructure of experimental coronary artery atherosclerosis in cynomolgus macaques. A comparison with the lesions of other primates. Atherosclerosis 43:151–175

Stary HC (1990) The sequence of cell and matrix changes in atherosclerotic lesions of coronary arteries in the first forty years of life. Eur Heart J 11:3–19

Stary HC, Chandler AB, Glagov S, Guyton JR, Insull W, Jr., Rosenfeld ME et al. (1994) A definition of initial, fatty streak, and intermediate lesions of atherosclerosis. A report from the Committee on Vascular Lesions of the Council on Arteriosclerosis, American Heart Association. Arterioscler Thromb 14:840–856

Stary HC (2000) Natural history of calcium deposits in atherosclerosis progression and regression. Z Kardiol 89:28–35

Steele PM, Chesebro JH, Stanson AW, Holmes DR, Jr., Dewanjee MK, Badimon L et al. (1985) Balloon angioplasty. Natural history of the pathophysiological response to injury in a pig model. Circ Res 57:105–112

Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL (1989) Beyond cholesterol. Modifications of low density lipoprotein that increase its atherogenicity. New Engl J Med 320:915–924

Steinberg HO, Chaker H, Leaming R, Johnson A, Brechtel G, Baron AD (1996) Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. J Clin Invest 97:2601–2610

Stemme S, Faber B, Holm J, Wiklund O, Witztum JL, Hansson GK. (1995) T lymphocytes from human atherosclerotic plaques recognize oxidized low density lipoprotein. Proc Natl Acad Sci USA 92:3893–3897

Stemme S (2001) Plaque T-cell activity: not so specific? Arterioscler Thromb Vasc Biol 21:1099–1101

Stender S, Zilversmit DB (1981) Transfer of plasma lipoprotein components and of plasma proteins into aortas of cholesterol-fed rabbits. Molecular size as a determinant of plasma lipoprotein influx. Arteriosclerosis 1:38–49

Subbanagounder G, Wong JW, Lee H, Faull KF, Miller E, Witztum JL et al. (2002) Epoxyisoprostane and epoxycyclopentenone phospholipids regulate monocyte chemotactic protein-1 and interleukin-8 synthesis. Formation of these oxidized phospholipids in response to interleukin-1beta. J Biol Chem 277:7271–7281

Suzuki H, Kurihara Y, Takeya M, Kamada N, Kataoka M, Jishage K et al. (1997) A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection. Nature 386:292–296

Syrjanen J, Peltola J, Valtonen V, Iivanainen M, Kaste M, Huttunen JK (1989) Dental infections in association with cerebral infarction in young and middle-aged men. J Intern Med 225:179–184

Tabas I (1997) Free cholesterol-induced cytotoxicity. A possible contributing factor to macrophage foam cell necrosis in advanced atherosclerotic lesions. Trends Cardiovasc Med 7:256–263

Tabas I (2002) Consequences of cellular cholesterol accumulation: basic concepts and physiological implications. J Clin Invest 110:905–911

Tangirala RK, Rubin EM, Palinski W (1995) Quantitation of atherosclerosis in murine models: correlation between lesions in the aortic origin and in the entire aorta, and differences in the extent of lesions between sexes in LDL receptor-deficient and apolipoprotein E-deficient mice. J Lipid Res 36:2320–2328

Taylor AJ, Farb AA, Angello DA, Burwell LR, Virmani R (1995) Proliferative activity in coronary atherectomy tissue. Clinical, histopathologic, and immunohistochemical correlates. Chest 108:815–820

Tomasian D, Keaney JFJr, Vita JA (2000) Antioxidants and the bioactivity of endothelium-derived nitric oxide. Cardiovasc Res 47:426–435

Tontonoz P, Nagy L, Alvarez JA, Thomazy VA, Evans RM (1998) PPAR gamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. Cell 93:241–252

Tremoli E, Camera M, Toschi V, Colli S (1999) Tissue factor in atherosclerosis. Atherosclerosis 144:273–283

Vainio S, Ikonen E (2003) Macrophage cholesterol transport: a critical player in foam cell formation. Ann Med 35:146–155

van der Wal AC, Becker AE, Vanderloos CM, Das PK (1994) Site of intimal rupture or erosion of thrombosed coronary atherosclerotic plaques Is characterized by an inflammatory process irrespective of the dominant plaque morphology. Circulation 89:36–44

Vartio T, Seppa H, Vaheri A (1981) Susceptibility of soluble and matrix fibronectins to degradation by tissue proteinases, mast cell chymase and cathepsin G. J Biol Chem 256:471–477

Vasquez-Vivar J, Kalyanaraman B, Martasek P, Hogg N, Masters BS, Karoui H et al. (1998) Superoxide generation by endothelial nitric oxide synthase: the influence of cofactors. Proc Natl Acad Sci USA 95:9220–9225

Velican D, Velican C (1980) Atherosclerotic involvement of the coronary arteries of adolescents and young adults. Atherosclerosis 36:449–460

Virchow R (1856) Phlogose und Thrombose im Gefäßsystem. Gesammelte Abhandlungen zur wissenschaftlichen Medizin. Meidinger, Frankfurt am Main, p ff

Virchow R (1858) Die Cellularpathologie in ihrer Begründung auf physiologische und pathologische Gewebelehre. Verlag von August Hirschwald, Berlin

Virmani R, Burke AP, Farb A (1999) Plaque rupture and plaque erosion. Thromb Haemost 82:1–3

Virmani R, Kolodgie FD, Burke AP, Farb A, Schwartz SM (2000) Lessons from sudden coronary death: a comprehensive morphological classification scheme for atherosclerotic lesions. Arterioscler Thromb Vasc Biol 20:1262–1275

Vlassara H (1996) Advanced glycation end-products and atherosclerosis. Ann Med 28:419–426

von Eckardstein A, Langer C, Engel T, Schaukal I, Cignarella A, Reinhardt J et al. (2001) ATP binding cassette transporter ABCA1 modulates the secretion of apolipoprotein E from human monocyte-derived macrophages. FASEB J 15:1555–1561

von Rokitansky C (1852) Über einige der wichtigsten Krankheiten der Arterien. K. K. Hof- und Staatsdruckereien Wien, Vienna

Wang Y, Lindstedt KA, Kovanen PT (1995) Mast cell granule remnants carry LDL into smooth muscle cells of the synthetic phenotype and induce their conversion into foam cells. Arterioscler Thromb Vasc Biol 1995; 15:801–810

Wang Y, Kovanen PT (1999) Heparin proteoglycans released from rat serosal mast cells inhibit proliferation of rat aortic smooth muscle cells in culture. Circ Res 84:74–83

Wang N, Lan D, Chen W, Matsuura F, Tall AR (2004) ATP-binding cassette transporters G1 and G4 mediate cellular cholesterol efflux to high-density lipoproteins. Proc Natl Acad Sci USA 101:9774–9779

Wasserman SI (1990) Mast cell biology. J Allergy Clin Immunol 86:590–593

Watt S, Aesch B, Lanotte P, Tranquart F, Quentin R (2003) Viral and bacterial DNA in carotid atherosclerotic lesions. Eur J Clin Microbiol Infect Dis 22:99–105

Wedemeyer J, Tsai M, Galli SJ (2000) Roles of mast cells and basophils in innate and acquired immunity. Curr Opin Immunol 12:624–631

Weitkamp B, Cullen P, Plenz G, Robenek H, Rauterberg J (1999) Human macrophages synthesize type VIII collagen in vitro and in the atherosclerotic plaque. FASEB J 13:1445–1457

Whitman SC, Ravisankar P, Daugherty A (2000) Exogenous interferon-gamma enhances atherosclerosis in apolipoprotein E−/−mice. Am J Pathol 157:1819–1824

Whitman SC, Ravisakar P, Daugherty A (2002) Interleukin-18 enhances atherosclerosis in apolipoprotein E(−/−) mice through release of interferon-gamma. Circ Res 90:E34–E38

Widlansky ME, Gokce N, Keaney JF, Vita JA (2003) The clinical implications of endothelial dysfunction. J Am Coll Cardiol 42:1149–1160

Williams CM, Galli SJ (2000) The diverse potential effector and immunoregulatory roles of mast cells in allergic disease. J Allergy Clin Immunol 105:847–859

Williams H, Johnson JL, Carson KG, Jackson CL (2002) Characteristics of intact and ruptured atherosclerotic plaques in brachiocephalic arteries of apolipoprotein E knockout mice. Arterioscler Thromb Vasc Biol 22:788–792

Wu NZ, Baldwin AL (1992) Transient venular permeability increase and endothelial gap formation induced by histamine. Am J Physiol 262:H1238–H1247

Wu T, Trevisan M, Genco RJ, Dorn JP, Falkner KL, Sempos CT (2000) Periodontal disease and riskof cerebrovascular disease: the first national health and nutrition examination survey and its follow-up study. Arch Intern Med 160:2749–2755

Xu Q, Kleindienst R, Waitz W, Dietrich H, Wick G (1993) Increased expression of heat shock protein 65 coincides with a population of infiltrating T lymphocytes in atherosclerotic lesions of rabbits specifically responding to heat shock protein 65. J Clin Invest 91:2693–2702

Xu QB, Kleindienst R, Schett G, Waitz W, Jindal S, Gupta RS et al. (1996) Regression of arteriosclerotic lesions induced by immunization with heat-shock protein 65-containing material in normocholesterolemic, but not hypercholesterolemic, rabbits. Atherosclerosis 123:145–155

Xu XX, Tabas I (1991) Lipoproteins activate acyl-coenzyme A:cholesterol acyltransferase in macrophages only after cellular cholesterol pools are expanded to a critical threshold level. J Biol Chem 266:17040–17048

Yancey PG, St.Clair RW (1992) Cholesterol efflux is defective in macrophages from atherosclerosis-susceptible White Carneau pigeons relative to resistant show racer pigeons. Arterioscler Thromb 12:1291–1304

Yancey PG, St.Clair RW (1994) Mechanism of the defect in cholesteryl ester clearance from macrophages of atherosclerosis-susceptible white carneau pigeons. J Lipid Res 35:2114–2129

Yang ZH, Richard V, von Segesser L, Bauer E, Stulz P, Turina M et al. (1990) Threshold concentrations of endothelin-1 potentiate contractions to norepinephrine and serotonin in human arteries: a new mechanism of vasospasm? Circulation 82:188–195

Yao PM, Tabas I (2000) Free cholesterol loading of macrophages induces apoptosis involving the fas pathway. J Biol Chem 275:23807–23813

Yao PM, Tabas I (2001) Free cholesterol loading of macrophages is associated with widespread mitochondrial dysfunction and activation of the mitochondrial apoptosis pathway. J Biol Chem 276:42468–42476

Yeagle PL (1991) Modulation of membrane function by cholesterol. Biochimie 73:1303–1310

Yee KO, Schwartz SM (1999) Why atherosclerotic vessels narrow: the fibrin hypothesis. Thromb Haemost 82:762–771

Young JD, Liu CC, Butler G, Cohn ZA, Galli SJ (1987) Identification, purification, and characterization of a mast cell-associated cytolytic factor related to tumor necrosis factor. Proc Natl Acad Sci USA 84:9175–9179

Yura T, Fukunaga M, Khan R, Nassar GN, Badr KF, Montero A (1999) Free readical-generated F2-isoprostane stimulates cell proliferation and endothelin-1 expression on endothelial cells. Kidney Int 56:471–478

Zhang SH, Reddick RL, Piedrahita JA, Maeda N (1992) Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. Science 258:468–471

Zhou X, Caligiuri G, Hamsten A, Lefvert AK, Hansson GK (2001) LDL immunization induces T-cell-dependent antibody formation and protection against atherosclerosis. Arterioscler Thromb Vasc Biol 21:108–114

Zhou X, Hansson GK (2004) Vaccination and atherosclerosis. Curr Atheroscler Rep 6:158–164

Zhu J, Quyyumi AA, Norman JE, Csako G, Waclawiw MA, Shearer GM et al. (2000) Effects of total pathogen burden on coronary artery disease risk and C-reactive protein levels. Am J Cardiol 85:140–146

Zhu J, Nieto J, Home BD, Anderson JL, Muhlestein JB, Epstein SE (2001) Prospective study of pathogen burden and risk of myocardial infarction or death. Circulation 103:45–51

Zimmerman MR (1993) The paleopathology of the cardiovascular system. Tex Heart I J 20:252–257

Zuckerman SH, Ackerman SK, Douglass J (1979) Long-term human peripheral blood monocyte cultures: establishment, metabolism and morphology of primary human monocyte-macrophage cell lines. Immunology 38:401–411

HEP (2005) 170:71–105

Risk Factors for Atherosclerotic Vascular Disease

A. von Eckardstein

Institute of Clinical Chemistry, University Hospital Zurich, Raemistrasse 100, 8093 Zurich, Switzerland
arnold.voneckardstein@usz.ch

Abstract Several controlled interventional trials have shown the benefit of anti-hypertensive and hypolipidaemic drugs for the prevention of coronary heart disease (CHD). International guidelines for the prevention of CHD agree in their recommendations for tertiary prevention and recommend lowering the blood pressure to below 140 mm/90 mm Hg and low density lipoprotein (LDL)-cholesterol to below 2.6 mmol/l in patients with manifest CHD.