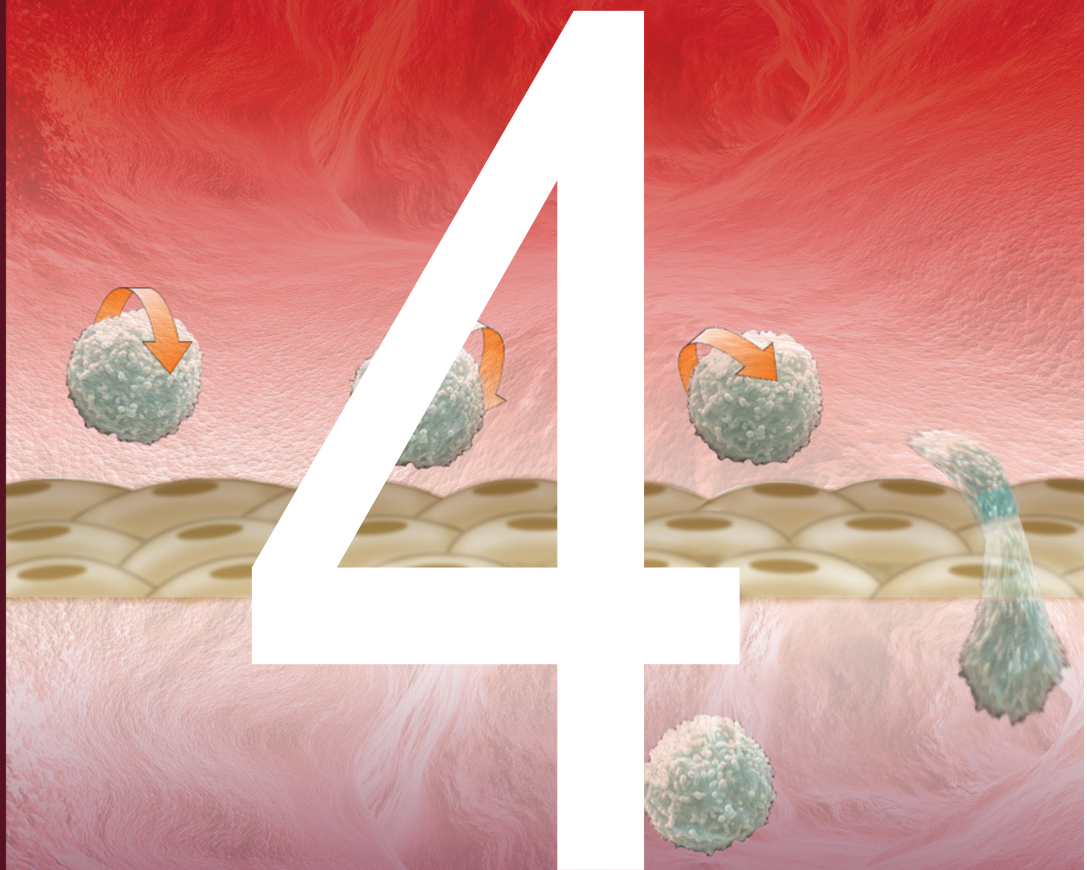
Mechanisms of Vascular Disease:

A Reference Book for Vascular Specialists



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Mechanisms of Vascular Disease

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A Reference Book for Vascular Specialists

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Abbreviation List

a1-PI	a1-protease inhibitor
5-HT	5-Hydroxytryptamine/Serotonin
AAA	Abdominal aortic aneurysm
AAS	Acute aortic syndrome
AAV	Adeno-associated viruses
ACE	Angiotensin converting enzyme
ACS	Acute coronary syndrome
ACS	Abdominal compartment syndrome
ACTH	Adrenocorticotropic hormone
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
ADP	Adenosine diphosphate
AIDS	Acquired immune deficiency syndrome
ALI	Acute lung injury
AMP	Adenosine monophosphate
AMPA	α-amino-3 hydroxy-5-methylisoxazole
ANA	Anti-nuclear antibody
ANCA	Anti-neutrophil cytoplasmic antibody
AOD	Aortic occlusive disease
AP1	Activated protein 1
APC	Activated protein C
APC	Antigen presenting cell
APLAS	Antiphospholipid antibody syndrome
ApoAI	Apolipoprotein AI
ApoE	Apolipoprotein E
APS	Antiphospholipid antibody syndrome
APTT	Activated partial thromboplastin time

ARDS	Acute respiratory distress syndrome
AT	Antithrombin
ATP	Adenosine triphosphate
AVP	Ambulatory venous thrombosis
β2-GPI	β2-glycoprotein Ib
bFGF	Basic fibroblast growth factor
BKCa	Large conductance calcium activated potassium channel
BMPs	Bone morphogenetic proteins
BMS	Bare metal stent
CAD	Coronary artery disease
CaM	Calmodulin
CAM	Cell adhesion molecule
cAMP	Cyclic adenosine monophosphate
CCK	Cholecystokinin
cGMP	Cyclic guanine monophosphate
CD	Cluster of differentiation
CD40L	Cluster of differentiation 40 ligand
CEA	Carotid endarterectomy
CETP	Cholesteryl ester transfer protein
CFD	Computational fluid dynamics
CG	Cationized gelatin
CGRP	Calcitonic gene regulated peptide
CHD	Coronary heart disease
CI	Confidence interval
CIMT	Carotid intimal-media thickness
c-JNK	c-Jun N-terminal kinase
CK-MB	Creatinine kinase (Myocardial specific)
CNCP	Chronic noncancer pain
cNOS	Constitutive nitric oxygen synthase enzyme
COX-1	Cyclooxygenase-1
COX-2	Cyclooxygenase-2
CROW	Charcot restraint orthotic walker
CRRT	Continuous renal replacement therapy

CRP	C-reactive protein
CRPS	Complex regional pain syndromes
CT	Computational tomography
CTA	Computed tomographic angiography
CTD	Connective tissue disorders
CTGF	Connective tissue growth factor
CYP	Cytochrome P450
CVD	Cardiovascular disease
CVI	Chronic venous insufficiency
DAG	Diacylglycerol
DES	Drug-eluting stent
DRG	Dorsal root ganglion
DNA	Deoxyribonucleic acid
DSA	Digital subtraction arteriography
DTS	Dense tubular system
DVT	Deep vein thrombosis
EC	Endothelial cell
ECM	Extracellular matrix
EDCF	Endothelium-derived contracting factor
EDH	Endothelium-dependent hyperpolarisation
EDS	Ehlers-Danlos syndrome
EET	Epoxyeicosatrienoic acids
ELAM-1	Endothelial-leukocyte adhesion molecule-1
ELG	Endoluminal grafts
ELISA	Enzyme linked immunosorbent assay
E_K	Equilibrium potential
E_M	Membrane potential
eNOS	Endothelial nitric oxide synthase enzyme
EPC	Endothelial progenitor cells
EPCR	Endothelial protein C receptor
ePTFE	Expanded polytetrafluoroethylene
ERK	Extracellular signal-regulated kinase
ESR	Erythrocyte sedimentation rate

ET	Essential thrombocytosis
ET-1	Endothelin 1
EVAR	Endovascular aortic aneurysm repair
EVLA	Endovenous LASER ablation
FDA	Food and drug administration
FDPs	Fibrin degradation products (soluble)
FGF	Fibroblast growth factor
FGF-2	Fibroblast growth factor 2
FMN	Flavin mononucleotide
FVL	Factor V Leiden
GABA	Gamma-aminobutyric acid
GABA B	Gamma-aminobutyric acid subtype B
G-CSF	Granulocyte colony stimulating factor
GMCSF	Granulocyte-macrophage colony stimulating factor
GP	Glycoprotein
GPCR	G-protein coupled receptor
GSV	Great saphenous vein
HDL	High density lipoprotein
HDL-C	High density lipoprotein cholesterol
HIF	Hypoxia inducible factor
HIT	Heparin induced thrombocytopenia
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HMG Co-A	Hydroxymethylglutaryl coenzyme-A
HMW	High molecular weight
HPETE	Hydroperoxyeicosatetraenoic acid
HETE	Hydroxyeicosatetraenoic acids
HR	Hazard ratio
hsCRP	High-sensitive C-reactive protein
HSP	Heat shock protein
HUV	Human umbilical vein
IAH	Intra-abdominal hypertension

IAP	Intra-abdominal pressure
IAPP	Intra-abdominal perfusion pressure
ICAM-1	Inter-cellular adhesion molecule-1
ICAM-2	Inter-cellular adhesion molecule-2
ICP	Intra-compartmental pressure
ICU	Intensive care unit
IFN	Interferon
IGF-1	Insulin-like growth factor-1
IHD	Ischemic heart disease
IL	Interleukin
IL-1	Interleukin-1
IL-1α	Interleukin-1 alpha
IL1-β	Interleukin-1 beta
IL-6	Interleukin-6
IL-8	Interleukin-8
ILT	Intraluminal thrombus
IKCa	Intermediate conductance calcium-activated potassium channels
IMH	Intramural haematoma
IMP	Inosine monophosphate
iNOS	Inducible nitric oxide synthase enzyme
IP(3)	1,4,5-inositol triphosphate
IRI	Ischemia reperfusion injury
IVIG	Intravenous pooled immunoglobulin
IVUS	Intravascular ultrasound
KGF	Keratinocyte growth factor
KGF-2	Keratinocyte growth factor-2
LAP	Latency associated peptide
LCS	Limb compartment syndrome
LDL	Low density lipoprotein
LDS	Loeys-Dietz syndrome
LLC	Large latent complex
LEC	Lymphatic endothelial cells

LFA-1	Lymphocyte function-associated antigen-1
LO	Lipoxygenase
LOX	Lysyl oxidase
LOPS	Loss of protective sensation
LPA	Lysophosphatidic acid
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
LTGFBP	Latent TGF binding protein
MAC-1	Macrophage-1 antigen
MAPK	Mitogen activated protein kinase
MCP-1	Monocyte chemoattractant protein-1
M-CSF	Macrophage-colony stimulating factor
MFS	Marfan syndrome
MHC	Major histocompatibility
MI	Myocardial infarction
MIP-1	Macrophage inflammatory protein-1
MLC_{20}	Myosin light $chain_{20}$
MLCK	Myosin light chain kinase
MLCP	Myosin light chain phosphatase
MMP	Matrix metalloproteinase
MODS	Multiple organ dysfunction syndrome
MRA	Magnetic resonance angiography
MRI	Magnetic resonance imaging
mRNA	Messenger RNA
MRSA	Methicillin resistant *Staphylococcus aureus*
MRSE	Methicillin resistant *Staphylococcus epidermidis*
MRTA	Magnetic resonance tomographic angiography
MTHFR	Methylenetetrahydrofolate reductase
MT-MMP	Membrane-type MMP
MVPS	Mitral valve prolapse syndrome
NADPH	Nicotinamide adenine dinucleotide phosphate
NGF	Nerve growth factor

NFκB	Nuclear factor kappa B
NiTi	Nitinol
NJP	Non-junctional perforators
NMDA	N-methyl-D-aspartate
NNH	Number needed to harm
NNT	Number needed to treat
NO	Nitric oxide
NOS	Nitric oxide synthase enzyme
NSAID	Non-steroidal anti-inflammatory drug
NV	Neovascularisation
OCP	Oestrogen/progesterone contraceptive pill
OPN	Osteopontin
OPG	Osteoprotegerin
OR	Odds ratio
OxLDL	Oxidised low density lipoprotein
PAD	Peripheral arterial disease
PAF	Platelet activating factor
PAI	Plasminogen activator inhibitor
PAI-1	Plasminogen activator inhibitor-1
PAR	Protease activated receptor
PAR-1	Protease activated receptor-1
PAR-4	Protease activated receptor-4
PAU	Penetrating aortic ulcer
PC	Protein C
PCA	Poly (carbonate-urea) urethane
PCI	Percutaneous coronary intervention (angioplasty)
PCWP	Pulmonary capillary wedge pressure
PDGF	Platelet-derived growth factor
PDGFβ	Platelet-derived growth factor-β
PDS	Polydioxanone
PECAM-1	Platelet-endothelial cell adhesion molecule-1
PEDF	Pigment epithelium-derived factor
PES	Paclitaxel-eluting stent

PET	Positron emission tomography
PF4	Platelet factor 4
PGI_2	Prostacyclin
PGG_2	Prostaglandin G_2
PGH_2	Prostaglandin H_2
$PGEI_2/PGI_2$	Prostaglandin I_2
PGN	Peptidoglycan
PHN	Postherpetic neuropathy
PHZ	Para-anastomotic hyper-compliant zone
PI3K	Phosphatidylinositol 3-kinase
PIP2	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C
PLOD	Procollagen lysyl hydroxylase
PMCA	Plasma membrane Ca^{2+} APTases
PMN	Polymorphonuclear leukocyte
POSS	Polyhedral oligomeric silsesquioxanes
PPAR	Peroxisomal proliferation activating receptor
PPI	Proton pump inhibitor
PRV	Polycythaemia rubra vera
PS	Protein S
PSGL-1	P-selectin glycoprotein ligand-1
PT	Prothombin time
PTCA	Percutaneous coronary angioplasty
PTFE	Polytetrafluoroethylene
PTS	Post-thrombotic syndrome
PUFA	Polyunsaturated fatty acid
PVI	Primary valvular incompetence
rAAA	Ruptured AAA
Rac	Ras activated cell adhesion molecule
RANTES	Regulated upon activation, normal T cell expressed and secreted
RAS	Renin angiotensin system
RCT	Randomised controlled trial

RF	Rheumatoid factor
RFA	Radiofrequency ablation
rhAPC	Recombinant human activated protein C
RNA	Ribonucleic acid
ROS	Reactive oxygen species
RR	Relative risk
RSD	Reflex sympathetic dystrophy
S1P	Sphingosine-1-phosphate
SAPK	Stress-activated protein kinase
SCF	Stem cell factor
SCS	Spinal cord stimulation
ScvO2	Superior vena cava venous oxygen saturation
SDF-1	Stromal-cell-derived factor-1
SERCA	Sarco/endoplasmic reticulum CaATPases
SEP	Serum elastin peptides
SES	Sirolimus-eluting stent
SEPS	Subfascial endoscopic perforator surgery
SFA	Superficial femoral artery
SFJ	Sapheno-femoral junction
SIRS	Systemic inflammatory response syndrome
SKCa	Small conductance calcium-activated potassium channels
SLE	Systemic lupus erythematosus
SMA	Smooth muscle alpha actin
SMC	Smooth muscle cell
SMP	Sympathetically maintained pain
SNARE	Soluble N-ethylmaleimide-sensitive factor activating protein receptors
SNP	Single nucleotide polymorphisms
SNRI	Serotonin/Noradrenaline reuptake inhibitors
SPJ	Sapheno-popliteal junction
SPP	Skin perfusion pressure
SR	Sarcoplasmic reticulum
SSRIs	Selective serotonin re-uptake inhibitors
SSV	Small saphenous vein

SVT	Superficial thrombophlebitis
STIM1	Stromal interacting molecule 1
TαCE	TNFα converting enzyme
TAAD	Thoracic aortic aneurysm disease
TAD	Thoracic aortic dissection
TAFI	Thrombin-activatable fibrinolysis inhibitor
Tc-99 MDP	Technetium-99 methylene diphosphonate
TCA	Tricyclic antidepressant
TCC	Total contact cast
TCR	T-cell receptor
TENS	Transcutaneous electrical nerve stimulation
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
TGF	Transforming growth factor
TGF-α	Transforming growth factor-alpha
TGF-β	Transforming growth factor-beta
TGL	Triglycerides
Th	T helper
TIA	Transient ischemic attack
TIMP	Tissue inhibitors of metalloproteinase
TLR	Toll-like receptors
TNF	Tumour necrosis factor
TNF-α	Tumour necrosis factor-alpha
tPA	Tissue-type plasminogen activator
TRP	Transient receptor potential
TRPC	Transmembrane receptor potential canonical
TRPV1	Transmembrane receptor potential Vanilloid-type
TXA2	Thromboxane A2
uPA	Urokinase
UT	University of Texas
VCAM	Vascular cell adhesion molecule
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor

VEGF-R	Vascular endothelial growth factor receptor
VIP	Vasoactive intestinal peptide
VLA-1	Very late activating antigen-1
VOCC	Voltage operated calcium channels
VPT	Vibratory perception threshold
VSMC	Vascular smooth muscle cells
VTE	Venous thromboembolism
VV	Varicose veins
vWF	von Willebrand factor
XO	Xanthine oxidase

4 • Mechanisms of Plaque Rupture

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INTRODUCTION

Atherosclerosis continues to cause considerable morbidity and mortality, particularly in the western world. While risk factors have been clearly identified, their precise roles in early atherogenesis are complex. The early development of the plaque is dependent upon interactions between damaged endothelial cells, vessel wall smooth muscle cells and circulating inflammatory cells mediated by the release of cytokines, growth factors and cell adhesion molecules. Plaque formation may represent a cell-mediated immune phenomenon, with a variety of potential antigenic agents identified. Shear stress and flow considerations also play a part.

Atherosclerosis begins in childhood, but it takes decades for atherosclerosis to evolve into the mature plaques responsible for the onset of ischaemic symptoms. Whilst plaque growth due to smooth muscle cell proliferation, matrix synthesis and lipid accumulation may narrow the arterial lumen and ultimately limit blood flow, uncomplicated atherosclerosis is essentially a benign disease. The final clinical outcome depends on whether a plaque becomes unstable, leading to acute disruption of its surface and exposure of its thrombogenic core to the luminal blood flow. The concept of a 'vulnerable plaque' was initially described in 1990[1,2] and though this initially gained wide acceptance, many authors now favour the broader concept of a 'vulnerable patient', whereby certain systemic and haematological conditions (e.g. relative hypercaogulability) must also be met before plaque rupture will result in symptomatic thrombosis.[3]

Mature atherosclerotic plaques are composed of a lipid core that is separated from the vessel lumen by a cap composed of fibrillar collagen. Disruption of this cap exposes the plaque's underlying thrombogenic core to the bloodstream, resulting in thromboembolism. This process of 'plaque rupture' is responsible for the majority of acute coronary syndromes (unstable angina, MI)[4-6,7] and ischaemic cerebral events (stroke, TIA, amaurosis fugax).[8-10]

Unravelling the complex biochemical and haemodynamic factors leading to plaque rupture is one of the greatest challenges facing contemporary medical research. The vital question in plaque pathogenesis is why, after years of indolent growth, life-threatening disruption and subsequent thrombosis should suddenly occur. Plaque stabilisation may prove to be an important clinical strategy for preventing the development of complications.[6] Identification of 'vulnerable plaques' (i.e., those most at risk of rupture)

and 'vulnerable patients' (i.e. those with predisposition to atherothrombotic occlusion) would allow pharmacotherapy to be targeted more effectively. Furthermore, a greater understanding of the mechanisms involved in plaque rupture will lead to improvements in preventative therapy.

EVIDENCE FOR THE 'PLAQUE RUPTURE' THEORY'

Coronary circulation

Evidence that plaque rupture leads to acute coronary syndromes has been provided from a number of sources. Early pathological studies using post-mortem specimens from fatal cases of acute myocardial infarction have revealed that virtually all cases of coronary thrombosis are related to rupture or fissuring of atheromatous plaques, along with evidence of distal embolisation.[7,11-13] Angioscopic findings in patients with stable angina have identified smooth atheroma within their coronary arteries, but disrupted irregular atheroma in the arteries of those with unstable angina.[14,15]

Radiological and histological studies have demonstrated that patients with a plaque morphology consisting of large lipid cores and thin fibrous caps are at increased risk of cardiovascular events.[16-18] In addition, these 'unstable' plaques are not necessarily the ones causing severely stenotic lesions.[19-21]

Cerebral circulation

A similar association between carotid plaque rupture and cerebrovascular events has been shown. In patients undergoing multiple TIAs or stroke progression, microemboli can be detected in the middle cerebral artery by transcranial Doppler.[10,22] Surface ulceration of carotid plaques seen on ultrasound imaging correlates well with symptoms[23] and echolucent (lipid-rich) plaques are at increased risk of causing future cerebrovascular events.

Early work utilising carotid plaques retrieved at carotid endarterectomy, highlighted the relationship between the presence of thrombus and the clinical status of patients.[24,25] This supported the theory that ischaemic attacks resulted from embolism rather than reduction in cerebral blood flow, particularly as few strokes occur in watershed areas.[26]

A number of subsequent studies demonstrated a relationship between the presence of intraplaque haemorrhage and patient symptoms.[27] Persson et al found that intraplaque haemorrhage appeared more frequently in symptomatic patients than asymptomatic patients,[28] while Lusby suggested a relationship between the onset of neurological symptoms and development of plaque haemorrhage.[29] Intraplaque haemorrhage may potentially arise *after* cap rupture, though it now seems most likely that it occurs *prior* to plaque breakdown[30] and may play an important role in disruption of the fibrous cap.

The most compelling evidence for an association between carotid plaque rupture and ischaemic cerebral events, is that carotid endarterectomy specimens removed from symptomatic patients are more likely to show histological evidence of rupture, compared to those from asymptomatic patients.[8,9] Van Damme and colleagues showed that 53% of complicated carotid plaques (intraplaque haemorrhage, haematoma, thrombus or ulceration) were symptomatic with a corresponding neurological deficit, compared to 21% of simple uncomplicated plaques.[31]

THE ROLE OF INDIVIDUAL COMPONENTS OF THE ARTERIAL WALL

A number of intrinsic and extrinsic factors have been identified that determine plaque vulnerability: the size and consistency of

the plaque core, the thickness and collagen content of the fibrous cap, and inflammation within the plaque. Further factors such as haemodynamic stress upon the plaque may ultimately contribute to cap disruption.

The evolution of a stable to unstable plaque with cap rupture and thrombosis can be outlined in the following simplistic terms (Figure 4.1): Endothelial damage allows passage of inflammatory cells and LDL into the vessel intima; free radicals are responsible for oxidation of the deposited LDL, and oxidized-LDL promotes cytokine and protease release from macrophages; proteases (in addition to other factors) degrade the fibrous cap causing disruption, allowing exposure of thrombogenic material to the blood; local thrombotic and fibrinolytic activity determine the degree of thrombus progression or dissolution.

Each component contributing to plaque rupture will be discussed in further detail. The relevant processes occur in the endothelium, the lipid core, the fibrous cap and the vessel lumen.

The endothelium

The origin of plaque destabilization can be traced back to endothelial dysfunction, or 'activation'. The endothelium is a single layer of highly specialised cells lining the vessel wall/lumen interface. It plays a vital role in modulating vascular permeability, perfusion, contraction and haemostasis. Leukocytes do not bind to normal endothelium. However, endothelial activation leads to the early surface expression of cell adhesion molecules, including VCAM-1, ICAM-1, E-selectin and P-selectin, which permit leukocyte binding. Many of the known atherosclerosis risk factors (e.g., smoking, hyperlipidaemia, hyperglycaemia, hypertension, hyperhomocysteinaemia) exert their damaging effects by causing endothelial activation.[32-37]

Activated endothelial cells express chemoattractant cytokines such as MCP-1, M-CSF, IL-1, IL-6 and TNF-α, as well as cell adhesion molecules. This pro-inflammatory environment, in conjunction with the altered permeability of the dysfunctional endothelium, mediates the migration and entry of leucocytes (mainly monocytes and lymphocytes) into the intima.[38-40]

The degree of endothelial dysfunction depends upon the balance between endothelial activation and endothelial 'passivation' (see Figure 4.2). Nitric oxide is the predominant molecule responsible for passivation, and the endothelium acts as an autocrine organ in its production.[41] Nitric oxide is an antioxidant, but has other plaque-stabilizing properties including reducing cell adhesion molecule expression,[42] platelet aggregation and SMC proliferation. Endothelial nitric oxide synthase, the enzyme responsible for nitric oxide production, is increased in people undergoing regular physical exertion, which may partly explain the benefits of exercise in atherosclerosis prevention.[43]

Endothelial cells are exposed to 3 different types of mechanical force. Hydrostatic forces (generated by the blood) and circumferential stress (generated by the vessel wall) are responsible for endothelial injury and activation. The third force is haemodynamic shear stress (generated by the flow of blood), which is inversely related to atherosclerosis formation – areas of high shear stress being relatively protected.[44] Despite the systemic nature of atherosclerosis, it is an anatomically focal disease with certain sites having a propensity for plaque formation. Arterial bifurcations exhibit slow blood flow, sometimes even bi-directional flow, resulting in decreased shear stress. The activity of endothelial nitric oxide synthase is decreased in these areas of non-laminar blood flow.[45,46] In addition, there is increased oscillatory and turbulent shear stress at bifurcations,

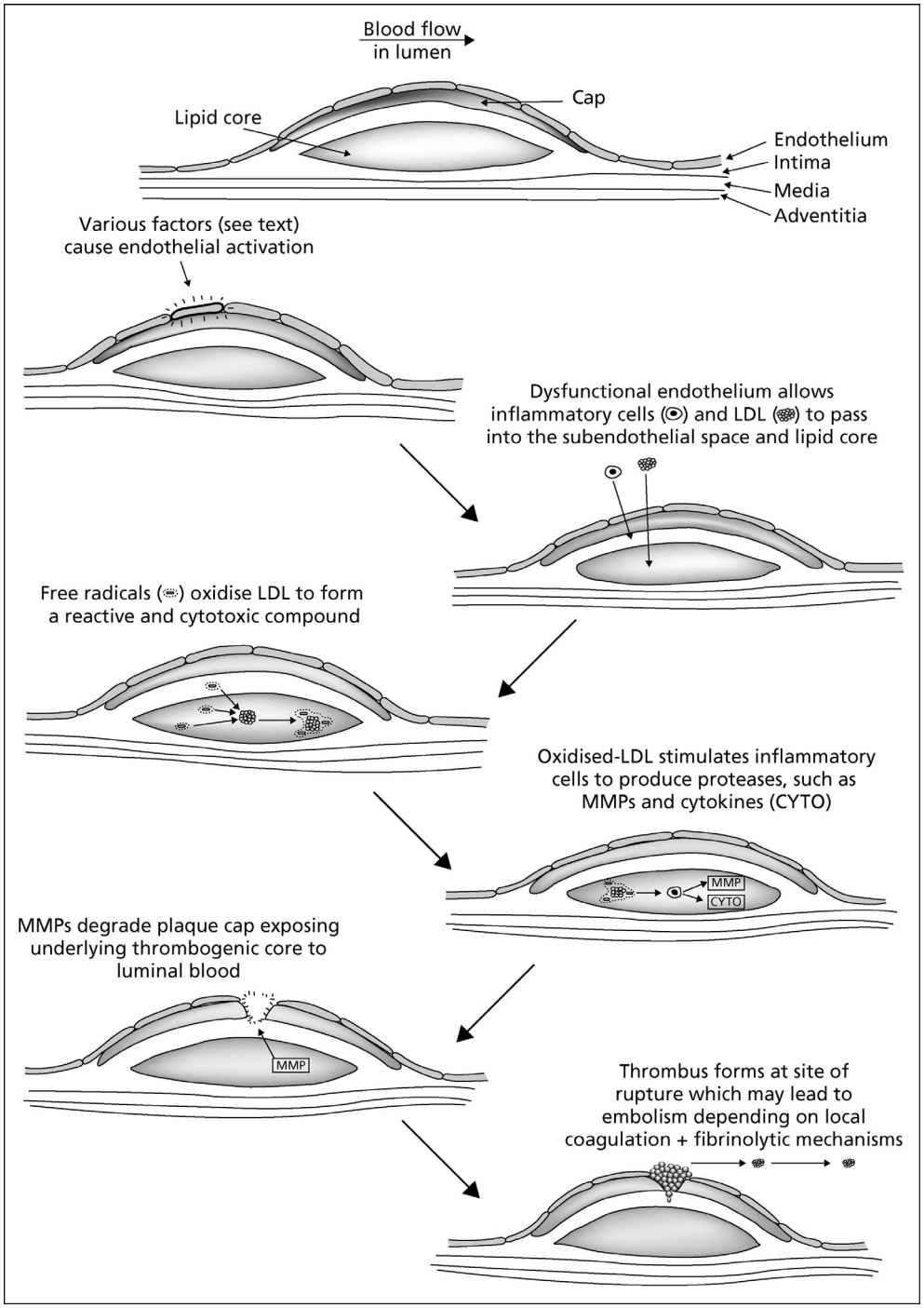

FIGURE 4.1: The stages of plaque rupture.

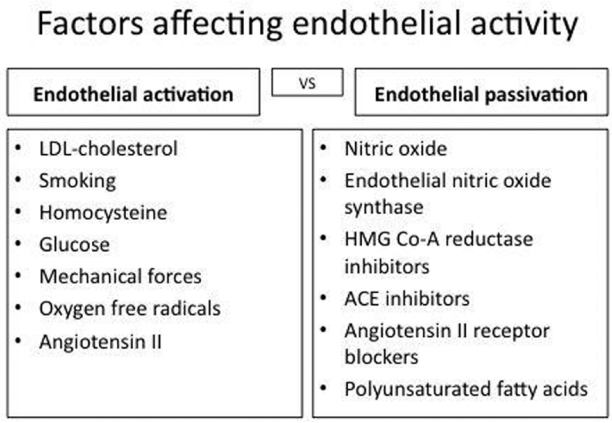

FIGURE 4.2: Factors affecting endothelial activity.

associated with an increase in oxygen free radical production[47] and monocyte adhesion.[48]

According to Laplace's law, the higher the blood pressure and the larger the luminal diameter, the more circumferential tension develops in the wall.[49] This phenomenon combined with a radial compression of the vessel wall may lead to excessive stress in vulnerable regions of the plaque, particularly the cap and shoulder.[50] For fibrous caps of the same tensile strength, those caps covering moderately stenotic plaques are probably more prone to rupture than those covering severely stenotic plaques, because the former have to bear a greater circumferential tension.[51]

The propagating pulse wave causes cyclic changes in lumen size and shape with deformation and bending of plaques, particularly those with a large soft plaque core. Eccentric plaques typically bend at the junction between the relatively stiff plaque and the compliant vessel wall.[52] The force applied to this region is accentuated by changes in vascular tone.

High blood velocity within stenotic lesions may shear the endothelium away, but whether high wall stress alone may disrupt a stenotic plaque is questionable.[4] The absolute stresses induced by wall shear are usually much smaller than the mechanical stresses imposed by blood and pulse pressure.[53]

It is clear that the endothelium is much more than an inert arterial wall lining. It is, in fact, a dynamic autocrine and paracrine organ responsible for the functional regulation of local haemodynamics. Factors that disturb this delicate balance are responsible for the initiation of a cascade of events eventually leading to plaque rupture.

The lipid core

The size and consistency of the atheromatous core is variable and critical to the stability of individual lesions, with a large volume lipid core being one of the constituents of the vulnerable plaque (Figure 4.3). It appears that the accumulation of lipids in the intima renders the plaque inherently unstable.

Although extremely variable, the 'average' coronary plaque is predominantly sclerotic with the atheromatous core making up <30% of the plaque volume.[54] The variability in plaque composition is poorly understood, with no relationship to any of the identified risk factors for atherosclerosis. Gertz and Roberts examined the histological composition of post-mortem plaques from 17 infarct-related coronary arteries.[55]

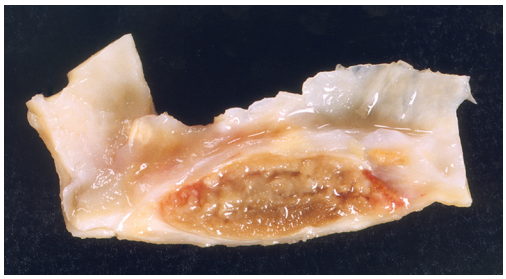

FIGURE 4.3: Longitudinal section of carotid plaque demonstrating a large volume lipid core

They found much larger proportions of the disrupted plaques to be occupied by atheromatous gruel in comparison to the intact plaques. Davies found a similar relationship in aortic lesions, with 91% of thrombosing plaques versus 11% of intact plaques exhibiting a lipid core that occupied >40% of the total plaque volume.[56]

Histological data regarding the necrotic core of carotid plaques is limited. There is, however, considerable evidence to link ultrasound-detected echolucent plaques (deemed to contain more soft or amorphous tissue) with symptomatology.[57,58] Feeley and colleagues demonstrated that symptomatic carotid plaques contained a significantly higher proportion of amorphous material than asymptomatic plaques,[59] with the lipid-rich core constituting 40% of overall plaque volume.[60]

LDL plays a more complex role in plaque instability than can be explained simply by the 'space-occupying' effect of accumulated lipid. A large core may produce a greater luminal narrowing, but plaque rupture sites are often characterized by 'outward remodelling' whereas those stenoses causing stable angina are more likely to be associated with 'inward remodelling'.[61] Indeed, it has been shown that in patients suffering acute coronary syndromes who had undergone angiography in the preceding months, the responsible lesion was recorded as causing a <70% stenosis in the majority of cases.[19,20,61] This is perhaps not surprising since, as mentioned earlier, a larger lumen places increased circumferential stress on the plaque, predisposing it to rupture.

As inflammatory cells cross the dysfunctional endothelium, cholesterol also enters in the form of LDL, and becomes trapped in the subendothelial space. This LDL is oxidized by free radicals creating a pro-inflammatory compound.[62] Oxidized-LDL is taken up by intimal macrophages – the process being mediated via receptors expressed on the macrophage surface,[63] although endocytosis of native LDL has also been demonstrated.[64] This process initially protects the surrounding smooth muscle and endothelial cells from the direct cytotoxic effects of oxidised-LDL, but leads to the formation of 'foam cells' (lipid-laden macrophages). Uptake of oxidized-LDL stimulates the expression of cytokines and proteolytic enzymes, propagating the cycle of inflammation.

The formation of a lipid core is a balance between LDL deposition of cholesterol in the damaged intima and removal by HDL (Figure 4.4). HDL and its carrier, apolipoprotein A-I, are responsible for so-called 'reverse cholesterol transport' – moving cholesterol from cells into the blood (from where it can be transferred to the liver for excretion in the bile).[65] However, it may also be capable of effecting lipid removal directly from the plaque, one of the possible explanations for plaque regression seen with increased HDL levels.[66] HDL may have other beneficial effects also, such as improving endothelial function,[67] decreasing cell adhesion molecule expression,[68] and inhibiting oxidation of LDL.[69]

In addition to the potential pro-inflammatory role of oxidised LDL, it has recently been proposed that cholesterol accumulation may lead to plaque rupture via a more direct physical pathway. Changes

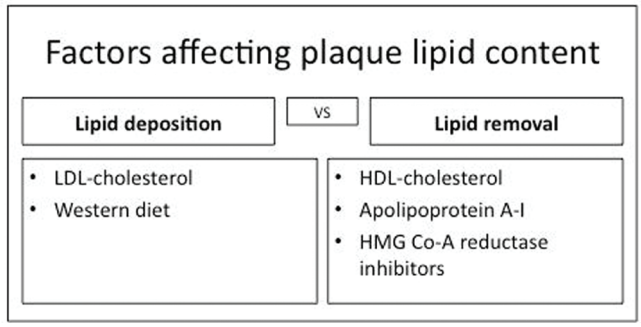

FIGURE 4.4: Factors affecting plaque lipid content

in local biological milieu such as decreased temperature or increased pH may cause in vitro precipitation of cholesterol into solid crystals. This alteration in state not only leads to a significant volume increase of up to 45%, but also leads to formation of sharp-tipped crystals that might be capable of damaging surrounding tissues and initiating plaque rupture. Using electron microscopy, Abela et al demonstrated that such crystal could be seen perforating the luminal surface of ruptured plaques from human coronary arteries.[70,71]

The cap of the plaque

The cap of the atherosclerotic plaque plays a vital role in isolating the plaque's thrombogenic core from the bloodstream. Since the thickness and collagen content of this cap are important determinants of overall plaque stability,[51] many authors now use the term 'thin-cap fibroatheroma' (TCFA) to identify those plaques most at risk of rupture. The accepted definition of TCFA is any plaque with a cap thickness of less than 65μm. Though the exact mechanisms that underlie progression from stable plaque to TCFA remain somewhat uncertain, it has been suggested that endothelial shear stress may play an important role, since TCFAs most often arise at sites of low endothelial shear stress (such as bifurcations and the concave side of arterial bends).[72]

Whatever the thickness of the fibrous cap, it is composed largely of fibrillar collagens (type I and type III[68]), though the relative proportion of collagen decreases as the cap thins. The fibrillar collagens have a lower thrombogenicity than the underlying core, but their exposure can be responsible for thrombus formation following erosion of the overlying endothelium.[73,74] This phenomenon accounts for one-third of acute coronary syndromes,[75] and the subsequent healing process of erosions can account for rapid and step-wise progression in plaque growth, leading to sudden increases in stenosis or occlusion.[76]

The most vulnerable area of the plaque is the shoulder region, where the cap is often at its thinnest.[7] Studies have shown a reduction in the collagen content of the cap around areas of plaque disruption, as well as steep transverse gradients of connective tissue constituents across ulcerated plaques.[77] This may result from a reduction in matrix production by smooth muscle cells, which exhibit diminished numbers in areas of plaque disruption,[56] or from increased degradation of matrix by proteolytic enzymes. It is most likely, of course, that a combination of excessive matrix degradation and reduced matrix production are responsible for cap thinning (Figure 4.5). A reduction in SMCs within the fibrous cap would certainly undermine its strength.[78] Recently there has been interest in the role of smooth muscle cell

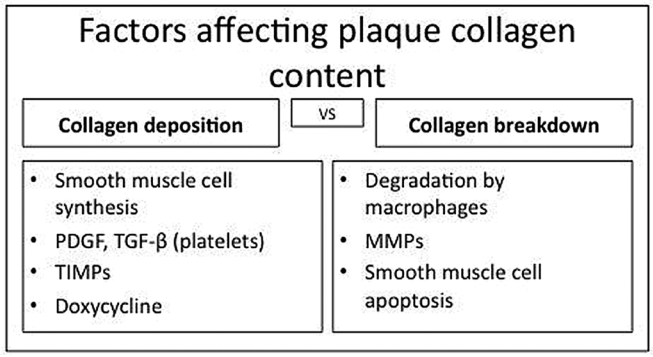

FIGURE 4.5: Factors affecting plaque collagen content

apoptosis in plaque cap weakening, caused by a combination of intrinsic and extrinsic factors, particularly macrophage and lipid derived products.[79,80]

More recently it has been suggested that plaque integrity may also be influenced by the development of minute, spherical microcalcifications within the fibrous cap. These microcalcifications are thought to represent accumulations of calcified macrophages or even post-apoptotic smooth muscle cells and result in highly focal increases in physical stress. The increased stress leads to areas of facial debonding, weakening the infrastructure of the cap and contributing to subsequent plaque disruption.[81]

Smooth muscle cells and collagen production

The SMC has a paradoxical role in plaque instability. On the one hand, SMCs are responsible for plaque matrix production and adverse arterial remodelling, while on the other, they produce collagens that give the plaque intrinsic strength. SMC inhibition therefore has potentially detrimental and beneficial effects.

In the normal arterial wall, SMCs are present in the media and express a differentiated phenotype. They are contractile and do not divide or migrate.[82] In atherosclerosis, when stimulated by the milieu of growth factors and cyokines, they 'dedifferentiate' and express a synthetic phenotype.[83] In the media, SMCs are surrounded by a basal lamina consisting of type IV collagen. Proteolytic enzymes secreted by macrophages are responsible for digestion of this supporting framework. The released SMCs are then able to migrate to the intima, where they secrete new extracellular matrix.[84] SMCs play a crucial role in stabilising atherosclerotic plaques, as they are responsible for the production of the cap fibrillar collagens.[82] In this respect, SMCs are important not only in initial formation of the fibrotic cap, but also in repair of subclinical plaque rupture. SMCs accumulate at the rupture site and secrete fibrous proteins. This restores plaque integrity, but may also lead to rapid growth of the plaque causing vessel stenosis. Certain platelet factors, including PDGF and TGF-β, are felt to be particularly important in stimulating collagen synthesis by SMCs, whereas γ-interferon (from activated T-cells) has the opposite effect.[85]

Since SMCs are the only cells producing fibrous tissue for inclusion in the atherosclerotic plaque, the balance between recruitment and degradation of these cells is clearly of great significance in plaque stability. It had previously been accepted that all SMCs involved in atherosclerosis were

derived from the local vessel media or intima. However, many groups are now examining the possibility that they may also be recruited from a circulating pool of SMC progenitor cells.[86] The prospect of manipulating the activity of these progenitor cells to increase plaque stability is an attractive therapeutic target, though further work is still required in this area.

Whatever the true origin of plaque SMCs, they play a vital role in maintaining the structure of the plaque and SMC apoptosis leads to decreased collagen production, thinning of the fibrous cap and increased volume of the necrotic core.[87-89] A recent, though small, study demonstrated that the proportion of SMCs undergoing apoptosis and the frequency of cytoplasmic remnants of apoptotic cells were significantly increased in unstable versus stable angina atherectomy specimens.[90] Apoptosis of SMCs and macrophages has been identified within plaques, but only in advanced disease with dense macrophage infiltration. Apoptotic cells are deemed to have become susceptible to a form of cell death which is distinct from necrosis and is characterised by a series of morphological changes, starting with shrinkage of the cell membrane and leading on to condensation of nuclear chromatin, cellular fragmentation and eventually engulfment of apoptotic bodies by surrounding cells.[79]

Pro-apoptotic proteins are present in advanced plaques, and it has been observed that cells derived from the plaque, but not the adjacent media, die when brought into culture.[80,91] Intimal cell apoptosis may account for the low density of smooth muscle cells in unstable plaques, and may contribute to the events leading up to plaque disruption. Though further study is still required, prevention of smooth muscle cell apoptosis may prove to be an important therapeutic target in the treatment of atherosclerotic disease.

Macrophages and collagen degradation

It is now known that inflammation plays a major role in plaque progression and especially in the period just prior to its rupture.[92] Macrophages control many of the inflammatory processes within the plaque,[93] and are responsible for the production of proteolytic enzymes capable of degrading the extracellular matrix.[94,95] The predominant proteolytic enzymes involved in plaque disruption are the matrix metalloproteinases or MMPs.[96]

The MMPs are a family of proteolytic enzymes characterised by the presence of zinc ions at their active sites. All degrade components of the extracellular matrix, and are divided into 4 main classes on the basis of their substrate specificity (Table 4.1).

MMPs are essential in normal healthy individuals, playing a key role in processes such as wound healing.[97,98] However there is growing interest in their role in disease states where ECM breakdown plays a predominant role.[99] Early interest focused on a pathological role for MMPs in the resorption of periodontal structures in periodontal disease,[100] the destruction of joints in rheumatoid arthritis,[101] and the local invasive behaviour of malignancies.[102] In vascular disease, they have been implicated in many of the stages of atherosclerosis but most particularly in acute plaque disruption.[103] The site of rupture is characterised by an intense inflammatory infiltrate consisting predominantly of macrophages,[94] that undergoes activation resulting in increased MMP expression. This shifts the delicate equilibrium towards proteolysis and away from matrix accumulation, making plaque disruption more likely (Figure 4.5).

MMP activity is tightly controlled at several levels and expression of MMPs is determined at the transcriptional level by various cytokines and growth factors.[104]

TABLE 4.1: THE MATRIX METALLOPROTEINASE FAMILY

MMP	Alternative names	Principal substrates
Collagenases		
MMP-1	Collagenase-1, Interstitial collagenase	Collagens I,II,III, gelatin, MMP-2 & 9
MMP-8	Collagenase-2, Neutrophil	Collagens I,II,III, gelatin
MMP-13	collagenase	Collagens I,II,III, gelatin, PAI-2
MMP-18	Collagenase-3	Collagen I
	Collagenase-4, Xenopus collagenase	
Gelatinases		
MMP-2	Gelatinase-A, 72 kDa gelatinase	Gelatin, collagens IV,V,VII,X,XI,XIV, elastin, fibronectin, aggrecan
MMP-9	Gelatinase-B, 92 kDa gelatinase	Gelatin, collagen types IV,V,VII,X, elastin
Stromelysins		
MMP-3	Stromelysin-1	Collagens III,IV,IX,X, gelatin, aggrecan, MMP-1,7,8,9 & 13
MMP-10	Stromelysin-2	Collagens III,IV,V, gelatin, MMP-1 & 8
MMP-11	Stromelysin-3	
Matrilysins		
MMP-7	Matrilysin-1, Pump-1	
MMP-26	Matrilysin-2, Endometase	
Membrane types		
MMP-14	MT1-MMP	Collagens I,II,III, gelatin, MMP-2 & 13
MMP-15	MT2-MMP	MMP-2, gelatin
MMP-16	MT3-MMP	MMP-2
MMP-17	MT4-MMP	
MMP-24	MT5-MMP	
MMP-25	MT6-MMP	
Others		
MMP-12	Macrophage elastase	
MMP-19	No trivial name	
MMP-20	Enamelysin	
MMP-21	XMMP (Xenopus)	
MMP-23		
MMP-27		
MMP-28	Epilysin	

In a variety of tissue types, IL-1, PDGF and TNF-α stimulate expression,[105,106] while heparin, TGF–β and corticosteroids inhibit expression.[107,108] In recent years, there has also been considerable interest in the regulatory role of extracellular matrix metalloproteinase inducer (EMMPRIN). EMMPRIN was initially identified as a tumour-derived protein that facilitated cancer cell invasion by stimulating MMP production in epithelial cells and fibroblasts.[109] However, subsequent studies have demonstrated that EMMPRIN also stimulates production of MMPs by smooth muscle cells and monocytes, making it highly relevant in atherosclerosis and plaque instability. In addition, EMMPRIN may also

lead to increased production of inflammatory cytokines which further augment MMP activity as described above.[110]

MMPs are initially secreted as latent inactive proenzymes and converted to the active state by cleavage of a propeptide domain.[111] The major physiological activator is plasmin, which in turn is regulated by PAI.[112] Thrombin has been shown to activate MMP-2 in vitro[113] and could provide a mechanism for MMP activation at sites of vascular injury. Reactive oxygen species also modulate enzyme activation.[114,115]

Metalloproteinase activity is further governed by naturally occurring MMP inhibitors. These 'tissue inhibitors of metalloproteinases' (TIMPs) provides a further level of control and overall proteolytic activity depends on the ratio of activated MMPs to TIMPs.[116]

Early studies showed that MMPs were present at increased levels in atherosclerotic arteries. Raised levels of gelatinase activity were demonstrated in the aortas of patients with occlusive disease compared to healthy controls, and zymography revealed that this was predominantly MMP-9.[117] Subsequently, quantitative studies using ELISA revealed a six-fold increase in MMP-9 levels in atherosclerotic aortas.[118] The level and expression of MMP-2 is also increased in atherosclerotic aortic tissue compared with normal aorta.[119] While expression of MMP-2 has been detected in normal arteries, it appears that most MMPs are expressed only in atherosclerotic tissue.[120] The colocalisation of MMP-1, -2, -3 and -9 to the vulnerable shoulder of the plaque provided further evidence of their potential role in acute disruption.[120]

More recent studies have demonstrated an association between MMP levels and markers of plaque instability. Increased immunostaining for MMP-9 was seen in 12 atherectomy specimens retrieved from patients with unstable angina compared to the stable form.[121] A larger study, involving 75 carotid endarterectomy specimens, demonstrated a close association between raised plaque levels of MMP-9 and a number of indicators of plaque instability, including symptomatology, cerebral embolisation and histological features of plaque rupture.[9]

Convincing evidence therefore exists of increased levels of MMP-2 and -9 in unstable plaques. However, intact type I and type III collagen molecules, which account for the load-bearing strength of the plaque cap, are not substrates for MMP-2 and -9. While it has been reported that high concentrations of MMP-2 can degrade type I collagen in an in vitro environment devoid of TIMPs,[122] it is likely that in vivo only the collagenases, MMP-1, -8 and -13, are capable of degrading fibrillar collagens.

MMP-1 and -13 levels are higher in 'atheromatous' compared to 'fibrous' plaques,[123] and MMP-8 has been demonstrated in atheroma but not normal arteries.[124] The expression of MMP-1 is increased in areas of high circumferential stress.[125] It is likely that both mechanics and proteolysis play a role in the degradation and weakening of the collagen-rich extracellular matrix, and understanding their interaction may be crucial.[126]

Evidence from our laboratories suggests that active MMP-8 is significantly raised in unstable plaques retrieved at carotid endarterectomy (Figure 4.6). The ratio of active MMP-8 to TIMP-1 and -2 (its naturally occurring inhibitors) were also significantly higher in the more unstable plaques of the 159 specimens collected in this study. This implies net proteolysis of the types of collagen found in the cap of the plaque by MMP-8. Immunohistochemistry confirmed the presence of MMP-8 protein within the plaque, which colocalised with macrophages (Figure 4.7).

Genetic variation in the genes controlling MMPs could theoretically be responsible for the susceptibility of some individuals to atherosclerotic plaque rupture. Early work has identified a number of polymorphisms that may be influential in this regard. Price et al have identified a novel genetic variation in the MMP-2 gene.[127] Ye and colleagues detected a polymorphism in the promoter region of the MMP-3 gene that may lead to increased systemic levels.[128] This polymorphism was subsequently found to be more common in patients suffering MI, compared to a control group.[129] A single nucleotide polymorphism

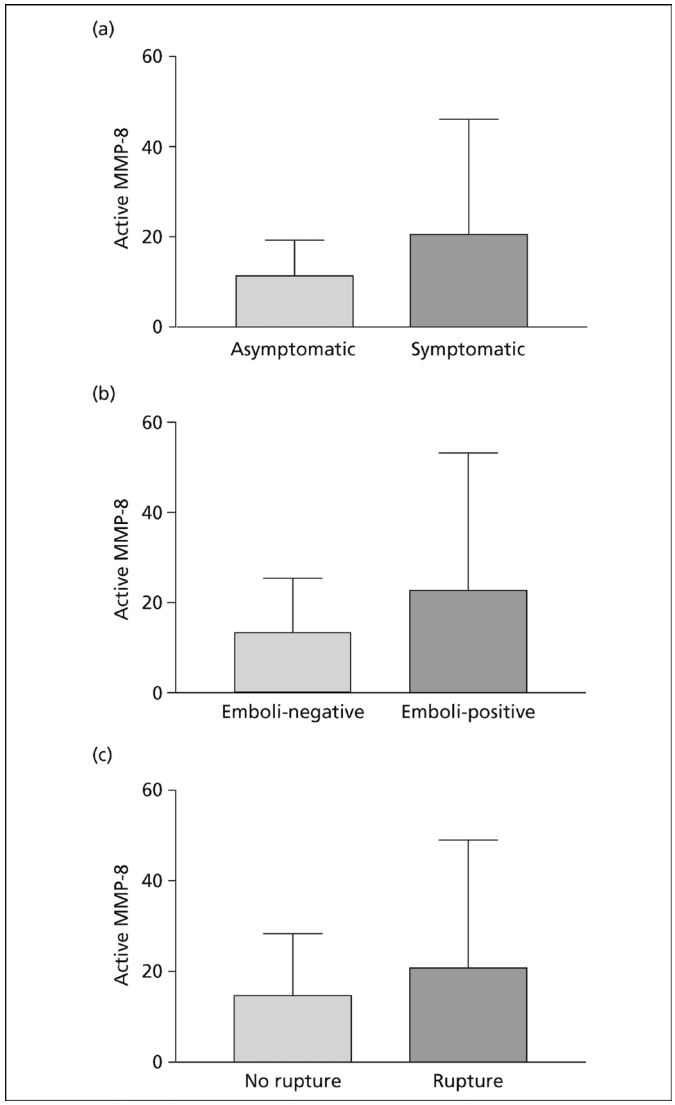

FIGURE 4.6: Plaque concentrations of active MMP-8 are significantly higher in symptomatic compared to asymptomatic carotid plaques: **(a)** from patients suffering carotid territory symptoms in the 6 months prior to surgery (p-value 0.0002), **(b)** from patients with pre-operative cerebral embolisation detected by transcranial Doppler (p-value 0.003) and **(c)** showing histological evidence of plaque rupture. Median values and interquartile ranges shown (p-value 0.003).

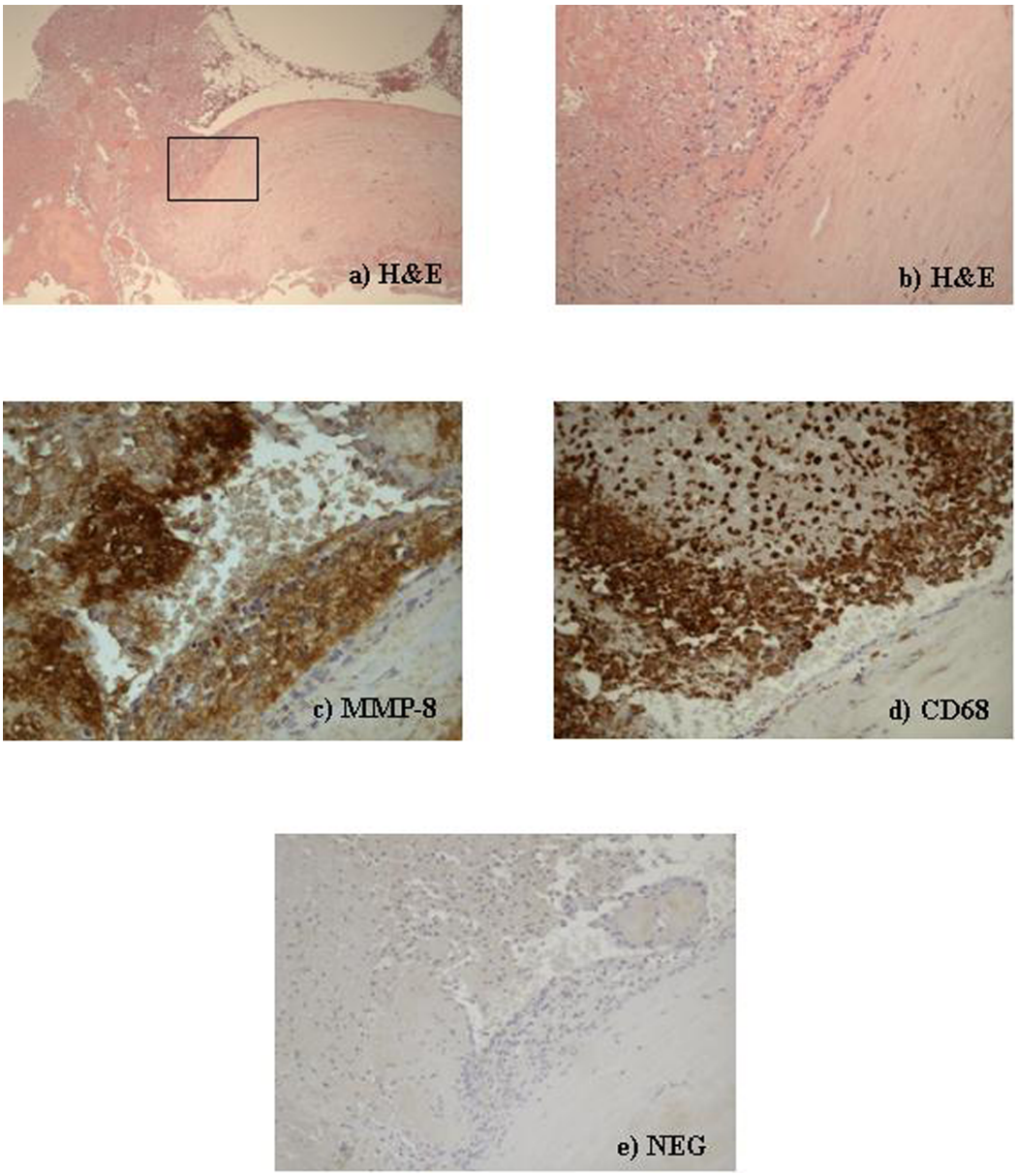

FIGURE 4.7: Histological sections taken from the shoulder region of a symptomatic carotid plaque. Some sections show disruption of the friable plaque.

(a) Low power H&E section with boxed area delineating high power view shown in **(b-e)**.
(b) High power H&E section demonstrating a cellular infiltrate.
(c) Strong reactivity for MMP-8 in cells.
(d) Positive staining for CD68 (macrophages).
(e) Negative immunohistochemistry control.

(C to T transition at position –1562) has been shown to influence MMP-9 transcription.[130] In this study by Zhang and co-workers, triple-vessel coronary artery disease was detected by angiography in 26% of patients with this polymorphism compared to 15% of those without.[130] Presenting a coherent picture of the interactions between various polymorphisms and the corresponding gene expression is difficult, and further complicated by environmental effects. However, it is clear that the potential exists to identify 'at risk' individuals in such a manner.

The vessel lumen

Disruption alone would not precipitate ischaemic syndromes without thrombus formation on the plaque surface, so plaque instability and thrombogenicity in tandem predispose to acute clinical events. Platelet adherence to the sub-endothelium after surface disruption leads to activation, with ADP and serotonin release stimulating further platelet recruitment and activation.

Once formed, thrombus can behave in three ways, dependent on the physical nature of the rupture and the balance between local fibrinolytic and coagulation processes. Firstly, the initial thrombus may progress to cause occlusion of the vessel. Secondly, the thrombus may disintegrate resulting in distal embolisation. Thirdly, the clot can undergo rapid dissolution, with the healed rupture resulting in a variable decrease in vessel lumen diameter.[76]

Tissue factor is a major regulator of haemostasis.[131] It is the most thrombogenic component of atherosclerotic plaques[132] and is expressed by numerous cell types, including endothelial cells. The level of tissue factor in coronary plaques from patients with unstable angina is more than twice the value observed in those plaques from stable angina patients.[133] Positive immunostaining for tissue factor correlates with areas of intense macrophage infiltration and SMCs, suggesting a cell-mediated increased thrombogenicity in unstable plaques. The increase in tissue factor levels seems to be linked to expression of the CD-40 receptor on the macrophage cell surface. The CD-40 ligand is expressed on activated T-lymphocytes, and other atheroma-associated cells,[134] which can therefore induce tissue factor production by macrophages via this signalling system. Expression is also regulated by cytokines and oxidised LDL.[135,136] It has been reported that a blood-borne pool of tissue factor exists,[137] though in the context of plaque disruption, macrophage production of tissue factor is predominantly responsible for plaque thrombogenicity.[133,138,139] It is interesting to note that many of the recognised cardiovascular risk factors increase the expression of tissue factor.[140,141]

THE ROLE OF ANGIOGENESIS IN PLAQUE RUPTURE

Angiogenesis is essential for normal growth and development. Neovascularisation has been observed in plaques[142] and it is postulated that it may play a role in atherosclerosis by providing growth factors and cytokines to regions of plaque development.

A study of coronary atherectomy specimens revealed the presence of neovascularisation in 50% of specimens from patients with unstable angina compared to 10% of specimens from patients with stable angina,[29] suggesting a possible role in plaque instability. Angiogenesis may contribute to plaque instability by causing intraplaque haemorrhage or extravasation of erythrocytes and inflammatory mediators into the centre of the plaque. Once red blood cells have leaked into the plaque, cholesterol from the cell membrane may become incorporated into the lipid core increasing its volume.[143]

This is supported by the finding that lipid-rich plaques have a significantly higher microvessel density than fibrous plaques.[144] The associated delivery of inflammatory cells may also lead to plaque degradation by stimulating MMP activity as described earlier in this chapter.

Perhaps more importantly, most neovascularisation occurs at the vulnerable shoulder area of the plaque. Immunostaining for inflammatory cells showed a close association between angiogenesis and inflammatory infiltration. In addition, a parallel increase in the expression of leukocyte adhesion molecules in the same vulnerable areas was demonstrated.[144]

Angiogenesis involves interactions between endothelial cells and components of the basement membrane matrix. MMP activity is required for such interactions, especially MMP-2 and MT1-MMP.[145] TIMPs have been shown to reduce angiogenesis, while up-regulation of MMP activity stimulates its increase.[146] However, whilst neovascularisation may promote and sustain inflammatory infiltration, the converse may also be true, whereby changes in the plaque associated with inflammation may themselves promote angiogenesis. Further work in this area is required.

THE ROLE OF INFECTIOUS AGENTS IN PLAQUE RUPTURE

The role of infectious agents in atherosclerosis and plaque rupture is controversial. Definitive proof of a causal relationship is lacking, although studies have reported associations between plaque development and *Chlamydia pneumoniae*,[147-149] *Helicobacter pylori*,[150] cytomegalovirus,[151,152] Herpes simplex virus types 1 and 2,[153] and hepatitis A virus.[154]

Certain infectious agents can evoke cellular and molecular changes supportive of a role in atherogenesis.[155] Work has shown that Chlamydial interaction with monocytes results in upregulation of TNF-α and IL-1β,[156,157] both of which are associated with plaque development. Chlamydial production of the HSP-60 antigen activates human vascular endothelium, and increases TNF-α and MMP expression in macrophages.[158,159] Once again, these are factors that influence plaque stability.

It has also been proposed that infective pathogens may exert their effects via direct infection of cells in the vessel wall. This establishes localised inflammation, leading to increased smooth muscle cell migration and greater uptake of oxidised low-density lipoprotein.[160]

There is some doubt about the methods employed for Chlamydia detection,[161] and also the role of potential confounding factors in epidemiological studies.[162] A large-scale prospective study of 15,000 healthy men in the United States which was controlled for age, smoking, socio-economic status and other cardiovascular risk factors, failed to show any association between Chlamydia seropositivity and the risk of MI.[163]

More recently, the STAMINA trial[164] demonstrated that eradication therapy (amoxicillin/ azithromycin, metronidazole and omeprazole) administered for 1-week after an acute coronary syndrome, significantly reduced cardiac death and acute coronary syndrome readmission rates over the following 12 months. These effects were unrelated to *Chlamydia pneumoniae* or *Helicobacter pylori* seropositivity, however, suggesting that the trial therapy prevented lesion progression by a mechanism unrelated to its antibiotic action.

Though the role of infection in atherosclerosis is still unclear, it seems that any causal relationship is likely to be highly complex and involve both direct and indirect pathways. Important factors may also include the patient's susceptibility to infection and

their innate inflammatory and immune responses.[165]

RISK PREDICTION OF PLAQUE INSTABILITY

Imaging

Angiography can demonstrate ulceration[166] but does not appear to be able to adequately distinguish between stable and unstable plaques.[167] In addition, the degree of stenosis detected by angiography does not correlate well with the future risk of events[19-21] because, as already discussed, it is often not the most stenotic plaques that are at highest risk of rupture.

Conventional ultrasound studies have shown an association between carotid plaque morphology and neurological symptoms[168] but have been unable to predict the risk of future events.[23] Intravenous ultrasound (IVUS), however, has been shown to have much greater resolution (100 μm) and provides detailed cross-sectional images of the arterial wall. It is also able to identify the increased echolucence of lipid-rich plaques and for a time it was thought that it might prove useful in detection of rupture-prone plaques.[169] Unfortunately, sensitivity and specificity were found to be low with this technique and it has largely been superseded by intravenous ultrasound virtual histology (IVUS-VH). The improved spectral analysis offered by this technology allows more detailed plaque characterization and can provide detail on lipid content, calcification and volume of the necrotic core.[170] Recent studies have shown this may be a clinically useful tool and demonstrated that IVUS-VH identified more TCFAs in patients with acute coronary syndrome than in those with stable angina pectoris.[171]

In parallel with the development of IVUS-VH, many groups have now begun to use optical coherence tomography (OCT). Also an intravenous modality, OCT is analogous to ultrasound imaging (using light rather than sound waves) and provides excellent spatial resolution (10-15μm). This allows detailed assessment of the arterial wall and can identify those plaques with a fibrous cap less than 65μm thick (i.e. TCFA) as well as areas of increased echolucency.[172] Whilst this technique has yielded very encouraging results in the identification of culprit atherosclerotic lesions, it is not without its limitations. Since blood attenuates the optical signal, the vessel under investigation must be proximally occluded for considerable periods to allow accurate imaging. An updated version of the technology has therefore been developed in recent years. This second generation of OCT is known as optical frequency domain imaging (OFDI) and involves much higher frame rates (>100 frames/sec). The higher frame rate allows rapid three-dimensional imaging of long arterial segments using high-speed pull-back of the probe. This means there is no need for proximal occlusion of the vessel and the artery can simply be purged with saline just prior to imaging[173] Further investigation will be needed to assess the true clinical utility of this technique.

Since increased inflammatory activity occurs prior to plaque rupture, attempts have been made to detect this increase, using local temperature measurements. Thermography studies have shown that temperature correlates well with macrophage cell density in human carotid plaques.[174] The temperature of coronary vessels in patients with ischaemic heart disease, in particular acute coronary syndromes, is higher than in normal controls.[175] In addition, increased local plaque temperature has been shown to be an independent predictor of adverse clinical outcome.[176]

High-resolution MRI appears to characterize the atherosclerotic plaque better than other imaging techniques.[177] It is more

accurate then angiography in measuring the degree of stenosis and, unlike angiography and IVUS, is non-invasive. However, a multicentre trial of imaging in coronary artery disease found whilst that MRI could reliably identify significant intraluminal lesions and rule out proximal or three-vessel disease, specificity was low.[178] This led to the suggestion that MRI may be more sensitive and specific if combined with intravascular enhancing agents such as gadolinium. Using such a marker improved MRI specificity and facilitated identification of carotid TCFA.[179]

MRI is still technically limited in many cases by small vessel size and movement artefact, and studies have not yet demonstrated the ability to predict risk of future cardiovascular events. Nonetheless, advances in the technique suggest a potential future role for MRI in detection of the high-risk plaque.

Just as enhancing agents may increase the accuracy of MRI, they may also prove useful in identifying atherosclerotic lesions using positron emission tomography (PET) and there has been increasing interest in the use of ^{18} fluorodeoxyglucose (^{18}FDG). Uptake of this glucose analogue is increased in metabolically active cells and early animal studies suggest it enriches in plaque macrophages and indicates areas of neovascularisation.[180] However, the clinical application of this technique has yet to be demonstrated.

Blood markers

It has long been established that adverse lipid profiles correlate with increased risk of MI and stroke though this is not a direct predictor of plaque rupture. Raised CRP levels have also been associated with increased cardiovascular risk in apparently healthy patients,[181,182] though its use as a prognostic marker of clinically significant thrombosis remains controversial.

MMP-2 and MMP-9 are raised in the peripheral blood of patients suffering from acute coronary syndromes,[183] while plasma MMP-9 is raised in patients with unstable carotid plaques.[184] A recent study of 1127 patients with coronary artery disease identified baseline plasma MMP-9 levels to be a novel predictor of cardiovascular mortality.[185]

Similarly, raised serum levels of soluble intercellular adhesion molecule-1 (slCAM-1) have been shown to be an independent predictor of future coronary event in patients with coronary heart disease.[186]

Many other molecules have also been investigated as potential prognostic markers in progression of atherosclerosis, including cytokines, lipoproteins, myeloperoxidases and placental growth factor. Though some have yielded promising results, none has yet been widely accepted as a reliable predictor of plaque rupture or clinical events.[187]

THERAPY AIMED AT PLAQUE STABILISATION

Pharmacotherapy to induce plaque stabilisation could be targeted at different aspects of the complex pathway leading up to plaque rupture, in particular:

1. the endothelium – by increasing endothelial passivation
2. the lipid core – by reducing LDL deposition/ augmenting LDL removal
3. the fibrous cap – by increasing collagen deposition/ preventing collagen degradation
4. the vessel lumen – by altering the thrombogenicity of the local environment.

Most recent interest has focussed on the role of HMG Co-A reductase inhibitors, which appear capable of influencing plaque stabilisation at all these levels.

HMG Co-A Reductase Inhibitors

HMG Co-A reductase inhibitors, or statins, are well known for their lipid-lowering action. They are the most effective group of therapeutic agents for lowering LDL and raising HDL levels. However, recent evidence suggests that they are also capable of decreasing cardiovascular events in those with normal cholesterol levels.[188,189] The Oxford Heart Protection Study[189] was a randomised controlled trial of simvastatin versus placebo in 20,536 individuals at high-risk of cardiovascular disease. Coronary death rate and other vascular events were significantly reduced in the simvastatin groups, even in patients with lipid levels below currently recommended targets (<5mmol/l total cholesterol and <3mmol/l LDL-cholesterol).

In the lipid lowering arm of the Anglo-Scandinavian Cardiac Outcomes Trial (ASCOT),[188] 10,305 individuals with total cholesterol levels <6.5mmol/l were randomised to either atorvastatin or placebo. The trial was stopped 1.7 years before the planned 5-year follow-up target was reached, as there were significantly fewer cardiovascular events in the atorvastatin group. The observed clinical benefit is probably a combination of lipid lowering below levels previously considered 'normal' and additional lipid-independent plaque stabilising actions. Several studies have reported effects other than lipid-lowering properties, including anti-proteolytic and anti-inflammatory mechanisms.[190,191]

Statins increase nitric oxide synthase activity[192] and encourage endothelial passivation (Figure 4.2). As discussed earlier, nitric oxide causes vasodilatation, inhibition of SMC proliferation and platelet aggregation and has widespread anti-inflammatory and anti-oxidant properties. Statins also reduce the expression of cell adhesion molecules,[193] interfering with the adherence of monocytes to the endothelium.

Statins may also have direct anti-inflammatory and anti-proteolytic actions, which contribute to increased plaque stability. In cell culture and animal models, statins have been shown to reduce macrophage secretion of MMP-1, -2, -3 and -9,[194] and increase the collagen content of the plaque.[195] Also, CRP levels are decreased by statins in a lipid-independent manner.[191,196]

Work from our laboratories suggests that statin therapy stabilises carotid plaques by lowering the levels of MMP-1, MMP-9 and IL-6. In an observational non-randomised study of 137 patients, we found that patients on statin therapy were significantly less likely to have suffered carotid territory symptoms within the month prior to carotid endarterectomy. The number of patients undergoing spontaneous pre-operative cerebral embolization was also significantly lower in the statin group.

HMG Co-A reductase inhibitors also have the potential to reduce thrombogenicity by decreasing tissue factor activity[197] and lowering levels of PAI-1.[198,199]

MMP Inhibition

The realisation that tissue remodelling due to increased MMP activity plays a key role in disease states has led to considerable interest in the potential for MMP inhibition. Most clinical and pre-clinical data regarding therapeutic manipulation of the extracellular matrix has been in the fields of arthritis, periodontal disease and cancer.[103] MMP inhibition aimed at plaque stabilisation aims to redress the imbalance between enzymes and inhibitors, which causes excessive tissue degradation. Potential methods of MMP inhibition include the administration of:

Tissue Inhibitors of Metalloproteinases (TIMPs)

The level of TIMPs can be increased either by the exogenous administration of recombinant TIMPs or by stimulating their local production through gene therapy. Increased TIMP-1 raised the collagen, elastin and smooth muscle content of atherosclerotic lesions in animal models,[200] while local gene transfer of TIMP-2 has been shown to decrease vascular remodelling in conjunction with lowered MMP activity (experimental models).[201]

It is difficult to extrapolate these data to potential applications in humans. The major drawback associated with TIMPs would be tissue delivery, since exogenous products would be metabolised and denatured with minimal tissue penetration at the intended site of action. Systemic stimulation of TIMPs would almost certainly have significant side effects precluding clinical use. Therefore, treatment would have to take the form of local tissue delivery or gene therapy. Clearly either system will be very expensive to develop, so more interest has concentrated on the development of synthetic MMP inhibitors.

Synthetic MMP inhibitors

Synthetic peptides work by binding to the zinc ion at the active site of the MMP, thus preventing cleavage of substrate collagen molecules.[202] Batimastat showed promise in decreasing tumour development and metastasis (animal models)[203] and limiting aneurysm expansion (experimental models),[204] but is not available in an oral form. Marimastat, which is available orally, was shown to limit intimal hyperplasia[205] and aneurysm expansion in vivo.[206] It also showed promise in early human cancer studies, but caused significant musculoskeletal side effects in 30% of patients.[207] Recent studies of MMI270, a more specific inhibitor (of MMP-2, MMP-8 and MMP-9), have shown a similar side effect profile.[208] Furthermore, recent animal studies of broad-spectrum synthetic MMP inhibitors have found them to be generally deleterious in terms of both plaque growth and plaque stability.[209]

Doxycycline

Doxycycline, a member of the tetracycline antibiotic family, is also a non-selective MMP inhibitor,[210] with a proven safety profile. Clinical trials have shown that doxycycline is capable of decreasing cartilage MMP levels when given to patients prior to hip surgery.[211] It has also been shown to limit intimal hyperplasia[212] and aneurysm expansion in vivo,[213] by reducing MMP-9 activity. Furthermore, when given to patients prior to AAA repair the expression of MMP-2 and MMP-9 was reduced in the aortic wall.[214]

A randomised clinical trial of doxycycline versus placebo in patients prior to carotid endarterectomy demonstrated decreased plaque MMP-1 levels and a potential for clinical benefit.[215] A phase II study of doxycycline administration to patients with small AAAs recently showed that it was reasonably well-tolerated (92% completed the 6-month course) and reduced plasma MMP-9 levels.[216] Further studies are on going to evaluate its effects on small aneurysm expansion.

ACE Inhibitors

ACE inhibitors (ACEI) and angiotensin II receptor antagonists decrease cardiovascular events, independently of their effects on blood pressure control. The ACEI, trandalopril, and the experimental angiotensin receptor antagonist, HR720, decrease the area

of atherosclerotic lesions in the thoracic aorta of cholesterol-fed monkeys.[217] This was achieved without alteration of mean blood pressure or cholesterol levels. The Heart Outcomes Prevention Evaluation (HOPE) study demonstrated a decrease in cardiovascular events in high-risk patients given ramipril as opposed to placebo.[218] This effect could only be partly explained by the modest decrease in mean blood pressure seen between the 2 groups (3/2mmHg).

Angiotensin II promotes endothelial activation,[219] and therefore the mechanism of action of ACEIs could be through endothelial passivation (leading to a reduction in cell adhesion molecule expression and macrophage infiltration). ACEIs may also exert their effects through bradykinin potentiation, resulting in decreased smooth muscle cell migration, decreased inflammation and decreased production of oxygen free radicals (COPPOLA 2008). Navalkar et al provided biochemical evidence to support these hypotheses by demonstrating that irbesartan (an angiotensin II receptor blocker) can decrease plasma levels of VCAM-1, TNF-α and superoxide.[220]

With ever more detailed understanding of the human genome, gene therapies have also come under investigation in the search for anti-atherosclerotic therapies. Hans et al have demonstrated that polyADP-ribose polymerase (PARP-1), a DNA-repair protein, stimulates apoptosis in the presence of local inflammation and plays an important role in plaque dynamics. They went on to show that inhibition of PARP-1 resulted in a reduction in plaque size, decreased collagen degradation and increased plaque smooth muscle content in ApoE(-/-) mice.[221] These findings suggest that PARP-1 inhibition may also represent a valuable therapeutic tool, though its applicability in humans has yet to be demonstrated.

Since the clinical significance of any plaque rupture is also governed by the intravascular environment, investigators continue to seek new therapies that may decrease the thrombogenicity of blood. Until now, this has been achieved with a combination of aspirin and another antiplatelet agent – most commonly clopidogrel – but drug resistance and side effect profiles can limit its applicability. The latest class of antiplatelet drugs is the $P2Y_{12}$ blockers, which inhibits platelet activation via blockade of the $P2Y_{12}$ ADP-receptor. Though these drugs (such as prasugrel and ticagrelor) may still have significant side effect profiles, they seem to be associated with far less unwanted bleeding and may be effective in patients who do not respond to clopidogrel.[222]

Though a number of therapeutic targets have shown promise in preventing plaque rupture, substantial work is still needed in this area since many of the potential therapeutic targets (such as smooth muscle cells and macrophages) have the ability to play both detrimental and beneficial roles in the complex process of atherosclerosis.

SUMMARY

Acute plaque disruption precedes the onset of clinical ischaemic syndromes. Exposure of the highly thrombogenic core to luminal blood results in platelet adherence and thrombosis. Inflammation is clearly involved in the process of plaque development and acute disruption, though the precise mechanism by which the inflammatory process is initiated remains unclear. The roles of angiogenesis, cellular apoptosis and infectious agents also require further clarification. Unstable plaques have a large lipid core and a thin fibrous cap with reduced collagen content. A major component of plaque destabilisation appears to be increased matrix degradation, the primary regulators of which are the MMPs and their inhibitors. There are a number

of potential therapeutic options aimed at preventing plaque disruption. In particular, MMP inhibition is an attractive target for such pharmacotherapy.

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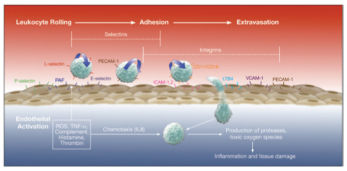

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Mechanisms of Vascular Disease

Edited by Robert Fitridge and Matthew Thompson

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