Background and Purpose—The simplistic view of atherosclerosis as a disorder of pathological lipid deposition has been redefined by the more complex concept of an ongoing inflammatory response.

Summary of Review—Apolipoprotein E and low-density lipoprotein (LDL)-receptor–deficient mice develop accelerated atherosclerosis allowing in-depth pathophysiological investigations. Atherosclerotic plaques in these mice contain large numbers of T cells and macrophages. Crossbreeding apolipoprotein E–deficient mice with T-cell–deficient mice and mice with impaired macrophage function (osteopetrotic op/op mice) disclosed the important impact of immune cells on atherosclerotic lesion development. In contrast to the detrimental role of T cells and macrophages, B cells appear to be atheroprotective. These basic experimental findings have partly been confirmed in studies of the human carotid artery system. Inflammation is not only instrumental in the development of human atheromatous plaques, but, importantly, plays a crucial role in the destabilization of internal carotid artery plaques, thus converting chronic atherosclerosis into an acute thrombo-embolic disorder. Humoral factors involved in internal carotid artery destabilization include cytokines, cyclooxygenase-2, matrix metalloproteinases, and tissue factor. Antibodies to oxidized LDL can reflect disease activity on one hand, but can also confer atheroprotection. Novel MRI techniques may aid in the in vivo assessment of acute plaque inflammation in humans.

Conclusions—The impact of inflammation on the development of atherosclerotic plaques and their destabilization opens new avenues for treatment. The effects of statins, acetylsalicylic acid and angiotensin-converting enzyme inhibitors on stroke prevention may partly be attributable to their profound anti-inflammatory actions. Vaccination against modified LDL and heat shock proteins halt plaque progression in experimental atherosclerosis. Their potential for prevention of human atherosclerosis is currently under investigation. (Stroke. 2006;37:1923-1932.)

Key Words: atherosclerosis ▪ carotid artery ▪ macrophages ▪ T cells

Atherosclerosis has traditionally been viewed to simply reflect the deposition of lipids within the vessel wall of medium-sized and large arteries. This concept has changed. It is now assumed that a complex endothelial dysfunction induced by elevated and modified low-density lipoproteins (LDL), free radicals, infectious microorganisms, shear stress, hypertension, toxins after smoking or combinations of these and other factors leads to a compensatory inflammatory response.1 Endothelial dysfunction is characterized by decreased nitric oxide synthesis, local oxidation of circulating lipoproteins and their entry into the vessel wall.2 Intracellular reactive oxygen species similarly induced by the multiple atherosclerosis risk factors lead to enhanced oxidative stress in vascular cells and further activate intracellular signaling molecules involved in gene expression.3 Upregulation of cell adhesion molecules facilitates adherence of leukocytes to the dysfunctional endothelium and their subsequent transmigration into the vessel wall. As outlined in this review, the evolving inflammatory reaction is instrumental in the initiation of atherosclerotic plaques and their destabilization. In the first part we summarize the evidence derived from experimental studies supporting a pathophysiological role of T cells, B cells and macrophages in the development of atherosclerosis in general. The second part more specifically addresses inflammation in plaques of the human internal carotid artery (ICA) and discusses immune mechanisms of plaque destabilization leading to thrombo-embolism. Moreover, we provide a short outlook on novel MRI techniques to visualize plaque inflammation in vivo. Finally, in the third part, we summarize evolving inflammation-based treatment strategies for stroke prevention.

Inflammation and Atherosclerosis in Animal Models

The Detrimental Role of Immune Cells in Atherosclerosis

Most evidence for a decisive role of inflammation in atherosclerosis is derived from studies in animal models using apolipoprotein E (apoE−/−) or LDL-receptor (LDL-R−/−)–deficient...
mice. ApoE−/− mice display massively increased cholesterol levels and develop accelerated atherosclerosis with extensive lipid deposition on major arteries such as the aorta.4 These atherosclerotic plaques are heavily infiltrated by CD4+ T cells,5 an important finding that had previously also been observed in human atherosclerotic plaques.6 A proportion of these T cells are autoreactive to components of the atherosclerotic plaque. Two major autoantigens, oxidized LDL (oxLDL) and heat shock proteins (HSP), have been implicated in the pathogenesis of atherosclerosis.6,7,8 After challenge with HSP, plaque-derived T cells expressed T-helper type 1 functions, became cytotoxic and induced tissue factor production in macrophages.8 The key question, whether T-cell inflammation represents an epiphenomenon only or an important pathophysiological step in atherosclerosis, has been addressed by crossbreeding transgenic mice. Severe combined immunodeficiency (SCID) mice lack T and B cells and thus are immunocompromised. Crossbreeding of apoE−/− with SCID mice lead to a dramatic reduction of fatty streak lesions within the aorta and substantiated a crucial role of the immune system in atherosclerotic lesion development.9 Re-constitution of the T-cell population in immunodeficient crossbreed apoE−/− SCID/SCID mice by CD4+ T cells derived from apoE−/− mice reversed this protection and increased atherosclerotic lesion development by 164%. Adoptively transferred T cells infiltrated the atherosclerotic lesions and led to increased circulating levels of the proinflammatory cytokine interferon-γ (IFN-γ). In further support of an important role of T-cell–derived IFN-γ, systemic application of IFN-γ to apoE−/− mice further accelerated atherosclerosis.10 T cells further respond to macrophage-derived interleukin (IL)-12 and IL-18. IL-12 is expressed at early stages of plaque development and IL-12−deficient apoE−/− mice are partly protected from atherosclerosis.11 IL-18 has been identified as the principal IFN-γ inducing factor. IL-18 likewise enhances atherosclerosis in apoE−/− mice.12 It appears, however, that the proatherogenic effects of IL-18 are partly independent from the presence of T cells. IL-18 directly stimulated IFN-γ secretion from macrophages, natural killer and vascular cells.13 In addition to IFN-γ, another cytokine with proinflammatory effects, tumor necrosis factor-α (TNF-α), is upregulated in atherosclerotic plaques.14 Functionally, blocking of TNF-α activity or disruption of its gene expression diminished the development of atherosclerosis in apoE−/− mice.15,16 Taken together these findings point to a functional role of T cells and proinflammatory cytokines in lesion progression during atherosclerosis.

It appears that T cells are attracted to the vessel wall by cell adhesion molecules and chemokines.17,18,19 Among other not yet defined antigens, T cells recognize oxLDL and HSP within the arterial wall, and thereby become locally restimulated. Cytokines released from locally activated T cells further stimulate macrophages,20,21 the main effector cells in atherosclerosis. Macrophages similarly enter the vessel wall during atherogenesis facilitated by upregulation of cellular adhesion molecules and chemokines on endothelial cells.22 Accordingly, gene therapy directed against the chemokine monocyte chemotactic protein-1 (MCP-1) or lack of the corresponding macrophage chemokine receptor CCR2 limited the progression of atherosclerosis in apoE−/− mice.23,24 Macrophages then upregulate scavenger receptors and phagocytose extracellular lipids such as oxLDL thereby transforming into foam cells.25,26 On activation by T cells and uptake of lipids, numerous genes are induced in macrophages leading to the production of tissue factor (TF), cytokines, and matrix metalloproteinases (MMP).27 All of these factors are involved in the progression of atherosclerosis and plaque destabilization (see below). The pivotal functional role of macrophages in atherosclerosis has been elucidated by crossbreeding op/op mice with apoE−/− mice. Op/op mice lack functional macrophages. Defective macrophages led to less atherosclerosis in apoE−/− mice.28

Cardiac allograft vasculopathy (CAV) is a unique form of accelerated atherosclerosis resistant to immunosuppression.29 CAV represents the main cause of death in long-term heart transplant survivors and selectively affects donor vessels without involvement of recipient arteries. Experimental studies have shown that in contrast to conventional atherosclerosis, CAV can develop in the absence of CD4+ T cells. Instead CD8+ T cells are instrumental by direct cytolytic and nontoxic effector mechanisms.30 The underlying pathophysiology of human CAV is unknown.

**Protective Immunity Against Atherosclerosis**

Immune responses are not universally detrimental during development of atherosclerosis, but can rather be protective. The first line of evidence is derived from immunization experiments. Hypercholesterolemic rabbits and LDL-R−/− mice immunized with modified or native LDL developed less atherosclerosis than sham-immunized littermates.31,32 This protective effect was independent from antibody titers against LDL, suggesting an antiatherogenic effect of the cellular rather than the humoral immune response.32,33 Similarly, pneumococcal vaccination decreased atherosclerotic lesion formation in LDL-R−/− mice because of an unexpected molecular mimicry between epitopes of oxLDL and *Streptococcus pneumoniae*.34 In apoE−/− mice only early LDL immunization modulated humoral and cellular immune responses and reduced plaque size and macrophage infiltration.35 Further investigations elucidated the atheroprotective capacities of B cells. In apoE−/− mice, after splenectomy leading to reduction of B cells, atherosclerosis was dramatically aggravated.36 Contrastingly, adoptive transfer of spleen cells significantly reduced lesion development. Immunomagnetic separation of spleen-derived T and B cells revealed that the atheroprotective effect was conferred by B cells, but not T cells. Similar results were obtained in LDL-R−/− chimeric mice after transplantation of bone marrow from mice with a disrupted B-cell receptor gene. These mice possess <1% of their normal B-cell population and exhibited a 30% to 40% increase in atherosclerotic lesions in the aorta.37 As an alternative approach to B-cell transfer, nasal vaccination with HSP 65 can also induce atheroprotection.38 HSP-tolerized LDL-R−/− deficient mice showed a dramatic decrease in the size of atheromatous plaques, increased expression of the anti-inflammatory cytokine IL-10 and reduced numbers of plaque-infiltrating T cells and macrophages. Taken together these studies show that autoimmunity during atherogenesis is
Inflammation and Atherosclerotic Plaques of the Human ICA

The Potential Role of Inflammation in Plaque Destabilization

Chronic atherosclerotic disease is often complicated by conversion into an acute stage. This is characterized by thrombomembran embolism evolving from atherosclerotic plaques or by thrombotic vessel occlusion. In coronary arteries this leads to the acute coronary artery syndrome. Similarly, stable atherosclerotic plaques of extracranial vessels, mainly the ICA, can convert into a source of thromboembolism with subsequent transient ischemic attacks or stroke. Large comparative trials of patients with clinically symptomatic and asymptomatic high-grade (>70%) ICA stenoses have revealed that on symptom onset symptomatic ICA stenoses carry a high risk of recurrent thromboembolic events of around 12% per year.42,43 In contrast, previously asymptomatic stenoses appear to be in a quiescent state with a low risk of 1% to 2% per year.44,45 These investigations led to the broadly accepted concept to treat high-grade symptomatic ICA stenosis either by carotid endarterectomy or angioplasty. In lower grade symptomatic ICA stenosis (<70%) the necessity and benefit of interventions are still a matter of debate. These studies, besides providing important guidelines for clinical practice, disclosed important pathophysiological aspects of ICA destabilization. In the group of symptomatic ICA stenoses receiving best medical treatment, but no intervention, the risk for further ischemic events spontaneously declined over time reaching the lower-risk level of the previously operated ICA stenoses at 2 to 3 years despite the persistence or even progression of ICA stenosis.46 These observations indicate that there is an acute phase of plaque destabilization that is reversible despite the persistence of the atherosclerotic ICA stenosis.

Histopathological studies have shown no obvious differences in the core size of atheromatous plaques between symptomatic and asymptomatic ICA stenoses except that the core was more closely located to the fibrous cap in symptomatic plaques.44,47–55 Further analysis of endarterectomy specimens disclosed plaque ulceration and rupture as the morphological characteristics of previously symptomatic stenoses compared with asymptomatic plaques. Specimens from symptomatic patients with plaque rupture and ulceration frequently showed luminal thrombosis,48,54,55 especially in the subgroup with preceding ischemic strokes.54 Human carotid artery plaques show T-cell and macrophage infiltration similar to atherosclerosis-prone laboratory animals.54,56–60 The comparison between previously symptomatic and asymptomatic endarterectomy specimens revealed that the plaque areas covered by inflammatory cells were significantly larger in symptomatic ICA plaques versus asymptomatic ones,54,58–60 indicating that acute inflammation could also be involved in plaque destabilization. Recent studies, moreover, suggest an impact of systemic immune activation on plaque destabilization in general, independently from the site of plaque formation at coronary or extracranial vessels. Higher serum levels of high-sensitivity C-reactive protein (CRP) and the acute-phase reactant serum amyloid A were significantly associated with a progression of atherosclerosis and destabilization in patients with coronary artery and ICA stenoses.51,62 Moreover, patients with unstable angina pectoris had a substantially increased risk of 42% versus 8% (stable group) to develop transient ischemic attack or stroke attributable to concomitant destabilization of a concurrent ICA stenosis.63

Acute T-cell and macrophage infiltration is most likely facilitated by an increased expression of cell adhesion molecules on the luminal surface of ICA stenoses such as the intercellular adhesion molecule-1.64 Intercellular adhesion molecule-1 was predominantly located in regions with severe narrowing of the lumen in symptomatic ICA stenosis. Interestingly, also the number of dendritic cells that present antigens to T cells and thereby activate them to proliferate were more numerous in symptomatic than asymptomatic plaques.65 Proinflammatory cytokines such as IL-18 that can activate T cells and macrophages are expressed at higher levels in symptomatic ICA plaques,66 providing evidence that the local cytokine milieu influences plaque stability. Conversely, stable atherosclerotic ICA plaques exhibited increased expression of the anti-inflammatory cytokine transforming growth factor-β-1 compared with unstable ICA plaques.67 Similarly, a proportion of advanced atherosclerotic ICA plaques showed increased IL-10 mRNA and protein expression, another anti-inflammatory cytokine, which was associated with reductions of proinflammatory inducible nitric oxide synthase activity.68 However, no differentiation was made between symptomatic and asymptomatic ICA stenoses in this study. Functionally, adenoviral gene transfer of IL-10 attenuated lesion progression in experimental atherosclerosis.69

At present the antigen specificity of the T cells infiltrating ICA plaques is unclear. As discussed above, LDL- and HSP-responsive T cells could be retrieved from atherosclerotic plaques. In search for other autoantigens as potential targets for T cells, the relation between numerous pathogens and atherosclerosis,70,71 and more specifically plaque destabilization of the carotid artery, has been extensively studied.60,72–76 In experimental settings cytomegalovirus infection aggravated atherogenesis in apoE−/− mice by local and systemic immune activation.77 In patients, elevated antibody titers against Chlamydia pneumoniae, Epstein-Barr virus and herpes simplex type-2 were associated with progression of atherosclerosis as indicated by an increase of intima-media thickness or progression of carotid artery stenosis.78 It has further been speculated that infections of the vessel wall with C. pneumoniae, cytomegalovirus or herpes viruses cause progression of atherosclerotic plaques to a symptomatic state. In C. pneumoniae–positive symptomatic ICA plaques, all T-cell subsets, but most prominently CD8+ T cells, were
more abundant than in negative samples. Overall, C pneumoniae were detectable in 15% of ICA plaques, but no difference in the percentage of C pneumoniae-positive plaques was found between symptomatic and asymptomatic patients. Similarly, a prospective study comparing serum antibody titers against C pneumoniae, herpes simplex and cytomegalovirus found no differences between patients with symptomatic and asymptomatic ICA stenosis. Moreover, RT-PCR examinations of these infectious agents performed directly on carotid endarterectomy specimens found no association to plaque destabilization. A possible link between infection and inflammation in atherogenesis is molecular mimicry between microbial antigens and human HSP or oxLDL sharing common immunogenic epitopes.

Any model on a role of inflammation in plaque destabilization should provide a link between intramural cell infiltration and thrombosis/thrombo-embolism developing on the luminal surface of the vessel wall. As mentioned above, histopathological investigations on carotid endarterectomy specimens have revealed 2 consistent features, namely plaque rupture and ulceration, as indicators of acute plaque destabilization. On plaque rupture atheromatous material gets into contact with the blood stream and activates the extrinsic pathway of coagulation (see below). Macrophages are an important source of MMP that can degrade extracellular matrix components and thereby can destroy the fibrous cap of the atheromatous plaque. Among a number of MMP tested, MMP-9 protein levels and activity were closely related to the recent (<4 weeks) occurrence of cerebrovascular events in high-grade ICA stenosis, suggesting a role in the destruction of the fibrous cap of atherosclerotic ICA plaques. Expression of an autoactivating form of MMP-9 in macrophages in vitro greatly enhanced elastin degradation and induced significant plaque disruption when overexpressed by macrophages in advanced atherosclerotic lesions of apoE−/− mice in vivo. These data for the first time show that enhanced macrophage proteolytic activity can induce acute plaque disruption and highlight MMP-9 as a potential therapeutic target for stabilizing rupture-prone plaques. Increased plaque infiltration and MMP expression in human atherosclerotic plaques were related to local overexpression of cyclooxygenase-2 (COX-2) and prostaglandin E synthase. Prostaglandin E can induce MMP expression pointing to a potential role of the arachidonic acid metabolism in plaque destabilization.

Disruption of advanced atherosclerotic plaques triggers the formation of thrombi several times larger than thrombi generated by exposure of other components of the arterial wall. Macrophages surrounding and forming atheromateous plaques contain large quantities of TF. In vitro, T cells and the proinflammatory cytokine IFN-γ induce TF production, providing a possible link between plaque inflammation and increased expression of TF in atherosclerotic plaques. TF binds to activated coagulation factor VII and activates an enzymatic cascade that induces thrombus formation. In fact, in ICA endarterectomy specimens the extent of TF immunoreactivity in plaque-associated macrophages was significantly higher in symptomatic compared with asymptomatic patients. In vitro, antibody-mediated blocking of TF activity decreased spontaneous platelet and fibrinogen deposition, and thrombus formation on atherosclerotic plaque specimens. It should also be noted that endothelial cells activated by proinflammatory cytokines or bacterial lipopolysaccharide can directly express TF. The contribution of endothelial TF to plaque symptomatology has, however, not yet been determined.

**Identification of Plaque Inflammation by Iron-Enhanced MRI**

MRI and MR angiography of the carotid arteries are commonly applied to assess the extent of vessel-narrowing caused by the plaque. High-resolution MR techniques allow further in vivo visualization of plaque composition, such as inteplaque or juxtaluminal hemorrhages, the fibrous cap, and the lipid-rich plaque core. Even though these techniques provide information about plaque morphology, they do not visualize plaque inflammation, which could have an impact on the classification as stable or unstable plaque. It appears, however, that inflammatory markers such as CRP are related to the extent of plaque formation as revealed by MRI. The contributions and limitations of the different imaging modalities such as MRI, ultrasound and nuclear imaging in identifying vulnerable ICA plaques have most recently been reviewed. We here focus on a selected aspect, the in vivo visualization of plaque inflammation by MRI using superparamagnetic iron oxide particles. Briefly, these iron particles on systemic application are phagocyted by hematogenous mononuclear cells in the circulation and thereby label a proportion of macrophages. When macrophages are attracted by chemokines to sites of acute tissue inflammation, they thereby carry intracellular iron particles to these sites. Accumulation of iron-labeled macrophages in inflamed tissues results in a signal loss on T2*-weighted MR images, i.e., they appear markedly hypointense. The feasibility of in vivo MRI of acute inflammation by either small (SPIO) or ultrasmall superparamagnetic iron particles (USPIO) has been demonstrated both in neurological as well as in non-neurological disorders, such as nerve injury, stroke, experimental neuritis and encephalitis, as well as in arthritis or transplant rejection. In experimental atherosclerosis, hyperlipidemic rabbits revealed a marked signal loss in aortic plaques on MRI after application of USPIO particles. On histology, these areas corresponded to dense accumulations of iron laden macrophages within the plaques predominantly located in the intima of the vessel. These findings have been confirmed by clinical investigations: in several clinical studies the application of iron oxide particles resulted in a focal signal loss of the vessel wall at the site of the carotid artery plaque. Importantly, only a proportion of macrophages identified by immunocytochemical staining of tissue sections revealed iron uptake on Perl’s stain. Similar to the experimental studies described above, this suggests that on systemic USPIO application macrophages in the circulation are “pulse-labeled” with iron particles, and signal loss on MRI represents recent migration of these iron-laden cells to the
plaques. In contrast, macrophages that were already present within the plaques before application of USPIO particles are not labeled. Accumulation of USPIO particles in an atherosclerotic lesion thus appears to be indicative of an acute ongoing inflammatory response within the plaque. The fact that iron accumulation in ICA plaques was significantly more often seen in rupture-prone and already ruptured lesions, but rarely in stable plaques, suggests that USPIO-contrast MRI could be an important means to assess plaque instability in vivo in the future.

**Therapeutic Implications and Perspectives**

Although our knowledge on the mechanisms of plaque destabilization is still incomplete and largely hypothetical, currently available data provide a conceptual framework that can be used for the design of further pathophysiologically oriented investigations and novel treatment strategies. At present, they already help to explain unexpected stroke protection of statins seen in patients with normal blood cholesterol. Other promising immune-based therapeutic strategies effective in experimental animals await further clinical development before application in human atherosclerosis.

**Statins**

Statins are mainly used in patients as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA)–reductase inhibitors. In patients with coronary artery disease, statins reduced the annual stroke incidence by ≈30%, an effect that was attributed to the decrease in cholesterol levels. However, patients with normal serum levels of cholesterol likewise benefited from statin treatment suggesting alternate, probably anti-inflammatory therapeutic mechanisms. Statins downregulate important coregulatory signals involved in interactions between T cells and macrophages/dendritic cells such as major histocompatibility complex class II molecules, lymphocyte-function associated antigen-1, B7-1, B7-2 and CD40 molecules, thereby shifting T cells toward a T-helper–2 response characterized by the induction of mainly anti-inflammatory cytokines. Thus, statins can prevent and even reverse ongoing inflammation and tissue damage. Statins appear to be similarly effective in primary stroke prevention, possibly through inhibition of inflammatory activity of macrophages or an immune deviation that resembles an anti-inflammatory tolerization that creates regulatory T cells. Another mechanism could be reduction of TF and MMP expression. In heritable hyperlipidemic rabbits, cerivastatin decreased TF and MMP expression in atheroma-associated macrophages. Accordingly, in the Atorvastatin and Thrombogenicity of the Carotid Atherosclerotic Plaque (ATROCAP) study intermittent atorvastatin treatment between subsequent bilateral carotid endarterectomy reduced TF plaque activity, when specimens obtained at the first and second operation were compared. In a retrospective study, patients with symptomatic stenoses and concomitant statin treatment before carotid endarterectomy showed a reduced in-hospital mortality and ischemic stroke rate compared with patients without statins. Importantly, this benefit was not seen in asymptomatic patients, indicating that statins contributed to plaque stabilization in patients with vulnerable plaques. Accordingly, endarterectomy specimens from statin-pretreated symptomatic ICA plaques showed less macrophage and T-cell infiltration, reduced MMP-2 immunoreactivity, increased expression of MMP tissue inhibitor (TIMP), and a higher collagen content. Statins, additionally, exert profound down-regulatory effects on systemic markers of inflammation in patients with atherosclerosis such as CRP and serum amyloid A levels, as reviewed recently.

**COX Inhibition**

COX exists in 2 isoforms, COX-1 and COX-2. Acetylsalicylic acid (ASS) inhibits both COX isoenzymes. The direct antithrombotic effect of ASS is mediated by inhibition of COX-1 in platelets resulting in a decreased production of thromboxane A2. Although most of the benefits of ASS in the prevention of cardio- and cerebrovascular events have been attributed to this mechanism, ASS probably in addition exerts platelet-independent effects by inhibition of COX-2. COX-2 is induced at sites of inflammation and is expressed in human atherosclerotic lesions including carotid artery plaques in conjunction with COX-2 synthase. ASS decreases the adherence of monocytes and T cells to human coronary artery endothelial cells, inhibits TNF-induced nuclear factor-κB mobilization, an important transcription factor regulating immune responses, and blocks the expression of immunological cell adhesion molecules.

**Inhibition of the Renin-Angiotensin System**

In animal models of atherosclerosis, angiotensin-converting enzyme inhibitors exerted consistent beneficial effects on plaque progression. One identified mechanism was the reduction in MCP-1 expression and concomitant macrophage plaque infiltration. Reduced MCP-1 levels have also been measured in patients with myocardial infarction treated by angiotensin-converting enzyme inhibitors. However, in the Prevention of Atherosclerosis with Ramipril-2 (PART-2) study, there was no difference throughout a 4-year follow-up in carotid artery–wall thickness or plaque formation between ramipril or placebo-treated patients.

Cipollone and colleagues inhibited the angiotensin II pathway with irbesartan in patients with symptomatic carotid artery disease for 4 months before endarterectomy. Analysis of plaque specimens revealed that irbesartan decreased T-cell and macrophage inflammation and inhibited MMP activity in comparison to controls. Similarly, concurrent treatment with renin-angiotensin blockers and ASS reduced nuclear factor-κB activation and CRP expression in human symptomatic and progressive asymptomatic ICA plaques as revealed by Western Blot Analysis of endarterectomy specimens in comparison to ASS alone.

**Suppression of Cytokines**

The fact that proinflammatory cytokines are instrumental in the progression of atherosclerosis, as revealed by numerous animal studies and suggested by their expression in atherosclerotic human plaques, opens the therapeutic prospect of targeting cytokine expression and cytokine-signaling proteins. In experimental settings blockade of IFN-γ and TNF ameliorated the development of atherosclerosis. Pentoxifyl-
line, a TNF-antagonist, significantly decreased plaque formation in apoE−/− mice by shifting T cells toward T-helper–2 differentiation characterized by increased production of the immunosuppressive cytokine IL-10.125 Although based on its hemorheologic properties pentoxifylline has been approved in humans for treating claudication126, its potential as an anti-inflammatory agent to prevent atherosclerosis in humans has not yet been tested or reported. As another approach, suppressors of cytokine signaling proteins could ameliorate cytokine-induced chronic inflammation in the vessel wall.127 Although corticosteroids (GC) have the capacity to suppress T-cell, macrophage, and cytokine responses, to the best of our knowledge, GC have not yet been formally tested as therapeutics to slow the development of atherosclerosis and to stabilize plaques in humans despite some older promising evidence derived from animal studies.128 Effects of GC during atherosclerosis, however, appear to be more complex than expected. In a recent experimental study, pharmacological inhibition of the enzyme 11β-hydroxysteroid dehydrogenase (11β-HSD1), converting inactive into active cortisol in cells, paradoxically decreased atherosclerotic lesion progression in apoE−/− mice rather than increasing plaque size as one would have predicted.129 Although lowering of serum lipids and glucose may have contributed to decreased atherosclerosis by 11β-HSD1 inhibition, the extent of atheroprotection was disproportionate to metabolic improvements. It was, therefore, concluded that 11β-HSD1 inhibition, leading to a decrease in cellular GC expression, beneficially influenced local processes within the arterial wall during plaque formation.

MMP Inhibition

As outlined above, MMP (eg, MMP-1 and MMP-9) contribute to atherosclerosis and destabilization of ICA plaques.59,79,80 Thus, MMP represent a potential target for therapeutic interventions by restoring the physiological balance between MMP and their tissue inhibitors, TIMP. Pseudopeptide inhibitors mimic the cleavage site of the MMP substrate and compete with tissue substrates of MMP.130 However, they are poorly selective and inhibit the activity of many MMP. First clinical results in cancer patients yielded promising results. Their therapeutic potential in atherosclerosis, however, awaits further investigation. Doxycycline, a member of the tetracycline family, exhibits a nonselective inhibitory effect on MMP. Pretreatment of patients scheduled for ICA endarterectomy with 200 mg doxycycline led to a reduction of MMP-1, but not MMP-2 and MMP-9 expression in carotid specimens.131 There was no clinical difference in patient outcome, but a larger cohort would be required for a definite answer. Similarly, doxycycline had no effect on the development of atherosclerosis in LDL-R−/− mice, but markedly reduced the incidence of aortic aneurysms.132 As mentioned above, statin treatment also profoundly decreased MMP expression in carotid plaques.117 Similarly, percutaneous delivery of TF pathway inhibitor blocking the extrinsic coagulation cascade concomitantly reduced the neointimal expression of MMP-2 and MMP-9 activity in a rabbit femoral artery model of vascular injury.133 More specific inhibitors of MMP are mainly being developed as anticancer drugs, but may also provide a new antiatherosclerotic therapy in the future.130

Cannabinoids

Derivatives of cannabinoids such as δ-9-tetrahydrocannabinol (THC) modulate immune functions. Recently, it was shown that the main cannabinoid receptor (CB2) is expressed in atherosclerotic plaques of humans and mice.134 Oral treatment of apoE−/− mice with low doses of THC inhibited atherosclerosis progression through pleiotropic immunomodulatory effects on lymphoid and myeloid cells suggesting that the CB2 receptor could be a novel target for treating atherosclerosis.

Vaccination Strategies

The fact that immune mechanisms are involved in atherosclerosis opens the fascinating possibility to develop atheroprotective vaccines.135 In animal models of accelerated atherosclerosis, immunization with an antigenic epitope of apoB-100, the protein part of LDL, reduced progression of atherosclerotic lesions by 40% and plaque inflammation by 80%.136 Importantly, immunization retarded progression of aortic atherosclerosis also when started after manifest atherosclerosis. Atheroprotective immunity could be adoptively transferred through splenocytes to nonimmunized mice. The clinical potential of vaccination approaches is currently under investigation.135

Summary

Inflammation plays an important role in the progression of atherosclerosis and ICA plaque destabilization converting a chronic process into an acute disorder with ensuing thromboembolism. During atherosclerosis, T cells and macrophages infiltrate the vessel wall triggered by endothelial dysfunction, and locally interact in a synergetic manner. Autoreactive T cells recognize oxLDL, HSP and shared microbial antigens by molecular mimicry and locally release proinflammatory cytokines. Macrophages on stimulation by T-cell–derived cytokines and transformation into foam cells after uptake of oxLDL secrete MMP predisposing the plaques to subsequent rupture. Plaque-associated macrophages, moreover, are an important cellular source of TF. On plaque rupture TF-rich plaque material gets in contact with the circulation and activates the extrinsic coagulation pathway. Novel MRI techniques may help to visualize active phases of plaque infiltration in vivo, and to better define ICA plaques at risk for impending destabilization. Immunity can also be atheroprotective. Lack of B cells accelerated atherosclerosis in experimental animals. Vaccination against modified LDL and HSP can slow development of atherosclerotic plaques. Current therapeutics effective in preventing atherosclerosis and stroke such as statins, ASS and renin-angiotensin system inhibitors may exert part of their effects by modulating inflammatory responses in the vessel wall. As alternative approaches, inhibitors of MMP activity and vaccination strategies are currently under clinical development.

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