Inflammation and Infections as Risk Factors for Ischemic Stroke

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Background—Inflammatory processes have fundamental roles in stroke in both the etiology of ischemic cerebrovascular disease and the pathophysiology of cerebral ischemia. We summarize clinical data on infection and inflammation as risk or trigger factors for human stroke and investigate current evidence for the hypothesis of a functional interrelation between traditional risk factors, genetic predisposition, and infection/inflammation in stroke pathogenesis.

Summary of Review—Several traditional vascular risk factors are associated with proinflammatory alterations, including leukocyte activation, and predispose cerebral vasculature to thrombogenesis on inflammatory stimulation. Furthermore, accumulation of inflammatory cells, mainly monocytes/macrophages, within the vascular wall starts early during atherogenesis. During later disease stages, their activation can lead to plaque rupture and thrombus formation, increasing stroke risk. Inflammatory markers (eg, leukocytes, fibrinogen, C-reactive protein) are independent predictors of ischemic stroke. Chronic infections (eg, infection with Chlamydia pneumoniae or Helicobacter pylori) were found to increase the risk of stroke; however, study results are at variance, residual confounding is not excluded, and causality is not established at present. In case-control studies, acute infection within the preceding week was a trigger factor for ischemic stroke. Acute and exacerbating chronic infection may act by activating coagulation and chronic infections and may contribute to atherogenesis. Genetic predisposition of the inflammatory host response may be an important codeterminant for atherogenesis and stroke risk.

Conclusions—Inflammation contributes to stroke risk via various interrelated mechanisms. Infectious diseases, traditional risk factors, and genetic susceptibility may cooperate in stimulating inflammatory pathways. Final proof of a causal role of infectious/inflammatory mechanisms in stroke pathogenesis is still lacking and will require interventional studies. (Stroke. 2003;34:2518-2532.)

Key Words: anticholesteremic agents ■ chlamydia ■ human experimentation ■ infection ■ inflammation ■ leukocytes ■ NF-kappa B ■ stroke

The conventional stroke risk factors, including hypertension, diabetes mellitus, smoking, and cardiac diseases, do not fully account for the risk of stroke, and stroke victims, especially young subjects, often do not have any of these factors. Geographic heterogeneity, seasonal preponderance in stroke incidence during fall or winter months found in most studies, and the decline of stroke during the 20th century are only incompletely explained by conventional risk factors and their temporal trends.1-5 Inflammatory parameters and chronic and acute infectious diseases have been considered to modify stroke risk independent of conventional risk factors. Although the roots of this topic go back as far as the 19th century, the discussion has strongly intensified during the last 5 to 10 years, with many new insights being gathered almost every month. However, results are often conflicting, and it appears increasingly difficult to keep abreast of this rapidly advancing field. Stroke is an etiologically heterogeneous disease, but atherosclerosis contributes to a large proportion of cases either directly via aortic, cervical, or intracranial large-artery atherosclerosis or indirectly by cardioembolism, eg, as a result of cardiac arrhythmias caused by coronary heart disease (CHD) or emboli after myocardial infarction. Atherosclerosis is today perceived as a chronic inflammatory vascular condition,6 and infectious diseases are believed to contribute to its pathophysiology.

In this report we summarize the current knowledge of the role of inflammation and infection in the pathogenesis of ischemic stroke, and, because the fields are interrelated, we also review mechanisms of inflammatory vessel disturbance as a pathogenetic pathway in atherogenesis and stroke. The main focus is on clinical evidence, and only key observations from animal experiments and basic science are included. It is one of our general hypotheses that infection/inflammation, specific genetic predispositions, and traditional risk factors interact with each other and may cooperatively enhance the risk of stroke. Therefore, one focus is to examine the current
knowledge of the relationship between these conditions to develop an integrated view on how they influence stroke risk. We attempt to apply generally accepted external criteria to determine whether the link between the 2 entities is of causal or merely associative nature. This review begins with local inflammatory alterations at the vessel wall and finally deals with systemic inflammatory changes, with acceptance of the concept that both features are closely connected to each other.

**Inflammatory Conversion of Cerebral Vasculature**

**Inflammatory Cells in Cerebral Vessels**

Autopsy evidence of young children and even fetuses has revealed early fatty streaks in blood vessels in association with infiltration of foam cells and T cells, although this does not necessarily produce atherosclerosis. Increasing evidence supports the concept that migration of inflammatory cells to the vascular wall is intimately associated with the cause of vascular conversion leading to atherosclerosis. Immunological mechanisms are stimulated early and launch the development of inflammatory cell infiltrates within vascular walls of human arteries, and the origin of early atherosclerotic lesions is preceded by inflammatory cell deposition (macrophages, T lymphocytes) in the subendothelial layer of major cerebral arteries such as the carotid artery bifurcation as well as in the perivascular spaces of small brain vessels.

The question of which mechanisms trigger the development of inflammatory cell infiltrates within vascular walls remains to be answered definitively. However, the view of the blood-brain barrier (BBB) as a major obstacle for the entry of leukocytes is no longer considered dogma. Activity of monocyte/macrophage lineage cells and T lymphocytes in cerebral perivascular and parenchymal locations continues through maturity. Endothelium actively regulates this activity in both health and disease to serve host functions, such as immune surveillance and removal of cell debris. The potential significance of this type of surveillance for stroke generation was recently demonstrated by experiments in stroke-prone hypertensive rats.

Besides being expressed on the luminal endothelial surface, chemotactic factors such as monocyte chemotactic protein-1 (MCP-1) and macrophage inflammatory protein-1α (MIP-1α) have binding sites along the parenchymal surface of human cerebral microvessels. These factors can be released by resident glial cells and transported to receptors on the vascular endothelium. Chemotactic factors and molecules mediating leukocyte adhesion exert chemotaxis and transmigration across the BBB and orchestrate cell entry to perivascular/subendothelial spaces.

Although the reason for the early entry of inflammatory cells into “subendothelial” or “perivascular” domains of cerebrovascular tissue is not clear, they can powerfully signal to endothelial cells (EC) and smooth muscle cells (SMC), eg, by releasing proliferative or proteolytic substances during systemic challenges. Presumably these cells (macrophage/monocyte lineage cells, T lymphocytes) receive receptor stimulation and activate humoral changes and then influence the inflammatory state of that vascular segment by releasing inflammatory mediators or growth factors, such as interleukins, tumor necrosis factor-α (TNF-α), interferons, and transforming growth factor-β (TGF-β). Some of these mediators fuel the inflammatory process further and can make the luminal EC surface adherent (eg, by upregulation of intercellular adhesion molecule-1 [ICAM-1] and E-selectin) and procoagulant (eg, by upregulation of tissue factor [TF] and plasminogen activator inhibitor-1 [PAI-1] and downregulation of thrombomodulin and tissue plasminogen activator). Experimental data suggest that induction of immunological tolerance in lymphocytes by repeated expression of E-selectin can render them able to inhibit this early local inflammation of vascular segments.

At this pivotal point, novel gene expression has occurred, and the EC have been activated to express immunologically provoking molecules. This early inflammatory step presumably can also occur in small vessels. Accordingly, plasma levels of soluble adhesion molecules (sICAM-1, sE-selectin) were observed to be increased both in large-intracranial-artery disease and small-artery disease.

**Link Between Traditional Stroke Risk Factors and Inflammation**

Inflammatory cells positioned in vascular locations are capable of responding to known long-term risk factors for human stroke such as hypertension, hyperlipidemia, diabetes mellitus, obesity, and smoking, which have been linked to markers of EC inflammatory changes (eg, increased sICAM-1) in subjects with and without a history of cerebral infarction. Prevalent cardiovascular risk factors increase systemic levels of TNF-α, which strongly augments adhesion molecule–dependent transendothelial migration of lymphocytes in human cerebral endothelium in vitro. Circulating monocytes regulate the level of plasma TNF-α and may cause a similar response in vivo. Once the circulating monocyte has become a stationary tissue macrophage, oxidized LDL inhibits its chemotaxis, presumably to prevent its departure from the vascular domain. Cholesterol is also a factor that activates monocytes and is gradually accumulated in them as they mature into macrophages and eventually into foam cells. Hypercholesterolemia has been shown to increase several markers of activation in both inflammatory cells and endothelium. This may stem from an increase of nuclear transcription factor-κB (NF-κB), which regulates many of the inflammatory vascular effects in hypercholesterolemia as well.

Arterial hypertension is perhaps the sturdiest stroke risk factor; accordingly, antihypertensives are most potent in stroke prevention. The association of chronically or acutely elevated blood pressure with markers of inflammation is well documented. Circulating levels of sICAM-1, soluble vascular cell adhesion molecule-1 (sVCAM-1), and sE-selectin have been reported to be increased in patients with essential hypertension. Acute hypertension induced by cold pressor test in normotensive and hypertensive patients increased serum levels of sICAM-1, sVCAM-1, and sE-selectin but did not influence the expression of adhesion molecules in circulating monocytes and lymphocytes.

Chronic hypertension involving structural organ remodeling is also associated with
signs of activation in monocytes obtained from peripheral blood.29 Interestingly, another study suggested that circulating monocytes from patients with hypertension are preactivated compared with those in nonhypertensive controls.30 On stimulation with lipopolysaccharide (LPS) or angiotensin II, these preactivated monocytes released more TNF-α than those from normotensives. Risk factors may perturb vascular function through additive, “ping-pong,” or even synergistic effects, which involve systemic inflammation. Indeed, cross-sectional observations are consistent with the hypothesis that abnormal vascular function in type 2 diabetes in hypertensive subjects is at least in part secondary to increased inflammation, with associated EC and platelet activation.31

Cigarette smoking is generally held to be immunosuppressive, but, in association with a prothrombotic risk factor, smoking may be a proinflammatory factor. Monocyte expression of TF was found to be increased in smoking women and even more so in those using oral contraceptives, which was based on induction of NF-κB in monocytes.32 Smoking increased circulating levels of sICAM-1 and decreased the number of activated circulating monocytes, which may indicate augmented cell-cell adhesion.33 In a population already harboring ischemic cerebrovascular disease, those who smoked had increased levels of sICAM-1 and sE-selectin.15

Cross-sectional studies also showed associations between vascular risk factors, including diabetes mellitus, smoking, and hyperlipidemia, and inflammatory indexes such as leukocyte count, C-reactive protein (CRP), and fibrinogen.34,35

In experimental studies, Hallenbeck and coworkers36 showed that aged, hypertensive, or diabetic rats but rarely young and healthy rats develop ischemic stroke on intrathecal LPS application, which indicated that conventional vascular risk factors may predispose to inflammation-induced proagulant mechanisms. Further work by the same group supported the hypothesis that perivascular immunoreactive cells are more abundant and capable of exaggerated cytokine signaling to the endothelium in animals with stroke risk factors.37,38 These findings are instrumental for understanding the longitudinal link between traditional risk factors, inflammatory mechanisms, thrombosis, and stroke.

To summarize, stroke risk factors may influence the interaction between inflammatory cells and the surrounding resident cerebrovascular cells, leading to increased susceptibility to inflammatory stimulation and to the formation of atheromatous plaques in large arteries and intimal thickening with local thrombosis in smaller arterioles (Figure). Inflammatory and prothrombotic alterations in arterial vessel walls due to stroke risk factors may also add to our understanding of why risk factors are linked with stroke, particularly in patients with atherosclerosis that is not yet discernible.

Continuous Inflammation in Matured Atherosclerotic Plaques

As noted by Virchow,39 development of atherosclerotic vascular lesions includes an immune-mediated inflammatory response. Migration of inflammatory cells, mononuclear cells, mast cells, and lymphocytes into the vascular wall is today held as a hallmark of a human atherosclerotic plaque in cerebrovasculature as in other vessels40–44 (reviewed in Reference 6). This ongoing process is fortified by the progressive deposition of modified lipids in the subendothelial layers. When LDL is caught in an artery, it becomes oxidized and phagocytosed by macrophages, which leads to the formation of lipid peroxides and the accumulation of cholesterol esters and the formation of foam cells.6,45 Oxidized LDL stimulates chemotactic effects and can increase the expression of macrophage colony-stimulating factor and MCP-1 synthesized by EC.46,47 Oxidized LDL can also upregulate the expression of adhesion molecules on human EC38 and promote the transmigration of monocytes.49 LDL may thus augment the inflammatory response by stimulating chemokines and recruiting new monocytes into the atheromatos lesion, as part of an ongoing process.

Some individuals with mature carotid artery disease have a systemic predisposition to irregularity and rupture of atherosclerotic plaques that is independent of traditional vascular risk factors, which was concluded after an analysis of 5393 carotid bifurcation angiograms from 3007 patients.50 Mechanisms involving inflammation may explain this. Continued dysfunction of vascular endothelium in the presence of macrophages and T cells leads to compensatory vascular changes, SMC proliferation, and recruitment of more macrophages and lymphocytes from the blood to eventually multiply within the atherosclerotic lesion. This further enhances the inflammatory changes of the endothelium and plaque maturation by locally released factors such as cytokines, chemokines, and growth factors.51 As the atherosclerotic lesion grows, this leads to formation of the so-called fibrous cap encapsulating the lipid-laden plaque core.51 Asymptomatic carotid plaques are more commonly morphologically so-called fibrous “hard” plaques, whereas symptomatic plaques are more commonly lipid-laden “soft” plaques,52,53 although this view has been disputed.54 However, it is believed that the formation of a fibrous “cap” in human carotid arteries may be associated with increased intimal expression of adhesion molecules,55 may protect the plaque from rupturing and in situ thrombosis, and perhaps may also inhibit inflammatory cell fluxes. In human carotid arteries, mast cells capable of releasing matrix-degrading proteases, such as metalloproteinases and elastase, are present in the “shoulder” region of the fibrous cap and may thus contribute to plaque rupture.43,44 Clearly, much more abundant macrophages may also play an important role in plaque destabilization. Furthermore, plasma cells capable of releasing large amounts of immunoglobulins have been found in atherosclerotic plaques.56

Microbial Agents in the Pathogenesis of Atherosclerotic Cerebrovascular Disease and Stroke

Chlamydia pneumoniae

Infectious agents and mainly viruses have been implicated in atherogenesis for several decades.57 The present discussion on microbial agents and atherosclerosis is mainly centered on Chlamydia pneumoniae, a gram-negative intracellular bacterium that is distributed worldwide. Saikku et al58 first associated serological evidence of C pneumoniae infection with myocardial infarction. Thereafter, >40 studies ad-
dressed the same question. Most of them reported positive associations, but publication bias may be involved. With the use of such diverse techniques as polymerase chain reaction, immunohistochemistry, and electron microscopy, >40 studies detected *C pneumoniae* in coronary and carotid plaques but only rarely or not at all in normal vessel walls. However, the prevalence of positive findings varied widely between studies, and not all studies reported positive results, eg, the agent was not detectable in Australian patients. *C pneumoniae* was also detected in atherosclerotic plaques of intracerebral arteries. In some but not in other studies, *C pneumoniae* was particularly associated with more advanced atherosclerotic lesions and with plaque thrombosis. Viability of *C pneumoniae* was shown by detection of specific mRNA in a substantial proportion of carotid plaques and by culturing the pathogen from a few specimens, whereas other studies did not detect viable bacteria. *C pneumoniae* can infect and was detected in EC, macrophages, and SMC. Interestingly, human macrophage/monocyte lineage cells infected with *C pneumoniae* degenerated into foam cells, an early hallmark of atherosclerotic plaque formation. *C pneumoniae* can induce proatherogenic and prothrombotic changes involving activation of the transcription factor NF-κB. Aspirin inhibited *C pneumoniae*-induced NF-κB activation and chlamydial growth. *C pneumoniae*-reactive T lymphocytes were detected in carotid plaques, and cross-reactivity between human and chlamydial heat shock protein-60 may also play a role in the atherosclerotic process. Chlamydial heat shock protein-60 induced the production of TNF-α and matrix-degrading metalloproteinases by plaque macrophages, mechanisms that may contribute to plaque rupture and thrombosis. Clinical data support that the presence of *C pneumoniae* in carotid stenoses increases local thrombogenicity and the risk of infarction, but not all results favor this hypothesis.

Some but not all seroepidemiological studies reported an association between past *C pneumoniae* infection and asymptomatic carotid atherosclerosis or increased intima-media thickness. A prospective study found an independent association between *C pneumoniae* seropositivity and (1) progression of the intima-media thickness and (2) ischemic events, effects that were particularly expressed in patients with increased CRP. In several case-control studies, elevated anti-chlamydial antibodies (mostly IgA) were associ-
ated with stroke, whereas a population-based case-control study that assessed only IgG antibodies found no correlation. In 2 prospective studies, C pneumoniae seropositivity predicted the risk of future stroke or other ischemic events, whereas 2 other studies were negative, with 1 possibly influenced by a recent C pneumoniae epidemic and 1 measuring only IgG antibodies. Of note, endovascular presence and serological results were not correlated. In an increased proportion of stroke patients, C pneumoniae antigen was detected in circulating immune complexes. In contrast, C pneumoniae DNA was found in circulating leukocytes in patients with carotid stenosis but not in control subjects, and C pneumoniae DNA was more common than seropositivity.

It remains unclear whether C pneumoniae DNA correlates better with findings in plaques than serology. Seroepidemiological studies are limited by the high prevalence of antibodies in adults, by the failure to distinguish between subjects with prior infection and those with chronic infection, and by the discordance with findings in vessel walls. Furthermore, residual confounding, mainly by childhood and adult socioeconomic factors, may partly explain the associations found. In light of the more important tissue study results, the association between C pneumoniae and atherosclerosis is firmly established; however, causality is not yet proven, mainly because of a lack of interventional studies.

Animal experiments and therapy studies may help to determine the nature of the relationship. To this end, intranasal inoculation of C pneumoniae initiated lesions similar to early atherosclerosis. In animals prone to develop atherosclerosis because of diet or genetic makeup, C pneumoniae could accelerate atherogenesis, an effect reversed by antibiotics. The infectious component of C pneumoniae, the metabolically inactive elementary body, is not affected by antibiotics. Therefore, eradication of C pneumoniae is demanding and may require extended therapy periods. Presently, antibiotics should not be advised for reduction of stroke risk outside of a scientific study.

**Helicobacter pylori**

Helicobacter pylori, a gram-negative spiral bacterium, is acquired mostly during childhood, generally persists during a lifetime, and can cause chronic gastritis, peptic ulcer disease, and gastric cancer. Seroepidemiological studies on H pylori and CHD yielded widely varying results, but the larger studies and those that adjusted for potential confounders were mostly negative or reported moderate effects in multivariate analyses. Regarding stroke, a small, nested, case-control study found an increased risk in univariate but not in multivariate analysis. In 4 other case-control studies, seropositivity was associated with the risk of atherothrombotic and/or microangiopathic stroke. The studies used spouses as controls and adjusted for social class or school education; however, confounding mainly by childhood socioeconomic factors that are important regarding H pylori infection was not sufficiently excluded by these studies. Furthermore, the studies were small and did not possess sufficient statistical power to exclude the play of chance in subgroup analyses. In a prospective study, seropositivity for H pylori—as for C pneumoniae, cytomegalovirus (CMV), and herpes simplex virus (HSV)—did not predict cardiovascular events in women. H pylori strains bearing the cytoxin-associated gene-A (CagA) are particularly virulent and were associated with increased inflammation. Seroprevalence against CagA strains but not H pylori in general was increased in large-vessel stroke but not in cardioembolic stroke after adjustment for parental social class among other factors. An association between seropositivity against the CagA strain and increased intima-media thickness was rendered nonsignificant after controlling for cardiovascular risk factors. Antibodies against CagA cross-react with vascular wall antigens, and this may possibly represent a pathogenetic link between H pylori infection and atherosclerosis.

**Other Bacterial Infections**

Symptoms of chronic bronchitis were an independent predictor of CHD in a large cohort study from Finland. In a case-control study, chronic bronchitis was also independently associated with stroke or transient ischemic attack; however, this was not confirmed in a larger study (A.J. Grau, MD, PhD, et al, unpublished data). Periodontitis is among the most common human infections and results from a complex interplay between chronic bacterial infection and the inflammatory host response. Bacteria from periodontal pockets can enter the bloodstream, eg, during chewing or tooth brushing, leading to recurrent bacteremia. Periodontal pathogens were identified in carotid plaques. Several but not all studies reported periodontitis to be a risk factor for CHD. The association between periodontitis and stroke has been under less intensive investigation. Small case-control studies and a cross-sectional study indicated an association between stroke and periodontitis or poor dental status. In post hoc analyses of 2 cohort studies, periodontitis was an independent stroke risk factor, whereas self-reported periodontal disease was not. Adjustment for socioeconomic classes and other confounders was incomplete in these studies, and it cannot be excluded that such factors have biased the results.

**Viruses**

Viral pathogens and mainly herpes viruses have been discussed for decades to contribute to atherogenesis. They were
shown to infect cells of vascular lineage, to induce proliferation of SMC, to render EC prothrombotic, and to induce adhesion receptors on EC. Antibodies against CMV, a herpes virus, were associated with future risk of carotid intima-media thickening in a population-based cohort study and with carotid atherosclerosis in a cross-sectional study. CMV and HSV-1 could be detected in carotid atheromas by some but not all authors. Antibody titers were not correlated with tissue findings. CMV seropositivity did not predict stroke or cardiovascular diseases, nor did HSV titers. In animal experiments, CMV infection led to an increased injury in the intima despite the absence of the virus in the vascular wall, suggesting a role for inflammatory and immune responses. Hepatitis A virus seropositivity was independently associated with CHD, but data on stroke are not yet available. Viruses, including varicella zoster virus and CMV, are also known to cause vasculitic stroke.

Atherosclerotic plaques often harbor multiple pathogens, including CMV, HSV-1, and odontopathogenic agents, besides C pneumoniae. In agreement, seroepidemiological studies showed an association between the number of pathogens to which a subject had been exposed and (1) the extent of atherosclerosis, (2) future cardiovascular mortality, and (3) EC dysfunction. Similarly, chronic respiratory, urinary tract, dental, and other infections amplified the risk of developing carotid atherosclerosis. Therefore, multiple pathogens appear relevant, and the stroke risk may relate to the aggregate burden of microbial antigens. However, again, childhood and adult socioeconomic conditions were not sufficiently adjusted for in these studies, and important residual confounding cannot be excluded.

Mechanisms That Link Infection With Atherothrombosis

Intracellular pathogens, which can lead to a persistent lifelong infection and/or elicit a long-term immune response, appear to be particularly important. Atherosclerosis is a discontinuously developing disease, and recurrent acute infections or intermittent reactivation of latent chronic infection may contribute to the intermittent exacerbations of atherosclerotic vessel disease. A multitude of mechanisms may link chronic infection and atherothrombotic events. The risk rendered by prior infection may be influenced by the individual inflammatory response mounted by the host, with the highest risk pertaining to those with a strong response. Pathogens can exert direct effects on atherogenesis by residing in the vascular wall, most likely after being delivered to the vessel wall by circulating monocytes (Figure). These include increased SMC proliferation and migration; inhibition of apoptosis with excessive accumulation of cells; increased cholesterol loading in macrophages and SMC; EC dysfunction with procoagulant effects and inhibited vasodilator function; increased expression of proinflammatory cytokines, chemokines, adhesion receptors, and reactive oxygen species; and contribution to plaque rupture by increased metalloproteinase activity. However, effects independent of microbial invasion to the vessel coexist. Chronic infection may indirectly influence the risk of atherosclerosis and thrombosis (Figure) by the following: (1) increased systemic inflammation, which in turn may damage vascular walls (eg, by cytokines and proteases) and lead to a procoagulant state; (2) immune-mediated mechanisms, eg, molecular mimicry, possibly including a cross-reaction of antibodies between human and bacterial structures such as heat shock proteins; (3) recurrent bacteremia (eg, periodontitis), which may induce platelet activation and a procoagulant state; and (4) influence on risk factors, eg, alteration of serum lipids toward a more proatherogenic profile.

Chronic Infection and Stroke Risk: Summary and Critical Evaluation

A meta-analysis of prospective studies on CHD and C pneumoniae, H pylori, and CMV seropositivity as well as dental disease concluded that there is no good evidence to support the existence of any strong epidemiological association. The evidence regarding stroke is even smaller when only large prospective studies are taken into account. When the criteria of Bradford Hill for causality (Table) are applied, the overall concept of chronic infection as a contributor to stroke appears biologically plausible in view of numerous experimental studies and does not appear to “conflict with generally known facts on the natural history and biology of the disease” (coherence). A dose-response relationship is more difficult to establish and was not shown for any single pathogen, but preliminary evidence suggests a graded response between the number of chronic infections and stroke risk. However, seroepidemiological studies showed primarily associations of only moderate strength and yielded inconsistent results, and only a few prospective studies established the temporal sequence of infection occurring before stroke. There is certainly no specific link between any chronic infectious disease and stroke, but one-to-one relationships are generally infrequent in medicine. It appears much more likely that chronic infections are risk factors that act in cooperation with conventional risk factors and genetic predisposition (see below) and are neither necessary nor sufficient for disease generation. In such a concept, Koch’s postulates for causality between microbial agent and disease that include a specific link cannot be satisfied. Furthermore, most seroepidemiological studies were case-control studies that are prone to selection bias and to a publication bias toward underreporting of negative results. Even prospective studies may be biased by residual confounding and were based on only 1 measurement; therefore, random measurement errors may be substantial. A source of confounding that has not been sufficiently observed is the role of childhood and adult socioeconomic factors. Cardiovascular morbidity is more common in lower social classes, in which several infectious diseases are also more prevalent because of poorer housing and nutrition. Social factors could confound the link between chronic infection and stroke; future studies thus need to control for these factors. Furthermore, chronic infections may partly explain the association between social class and vascular diseases.

However, the most important evidence for a role of chronic infection in atherogenesis, if not in stroke, stems from tissue analyses and not from seroepidemiological studies. In regard...
to tissue analyses and *C. pneumoniae*, the criteria for causality are fulfilled to a higher degree, although causality is still not proven in the clinical setting (Table). The value of serological studies is also limited by a lack of good correlation between results from plaque analysis and serum titers. Other surrogate markers that may reflect vascular risk in association with chronic infection, such as antigen detection in leukocytes, are needed. In summary, although there is good evidence for an association between chronic infections and stroke, causality is needed. In summary, although there is good evidence for an association between chronic infections and stroke, causality is needed.

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Systemic markers of inflammation have been shown to be risk markers of stroke. In epidemiological studies, the leukocyte count was associated with the risk of first-time myocardial infarction and ischemic stroke, an effect that was independent of smoking and other vascular risk factors in a recent meta-analysis. The leukocyte count also predicts the magnitude of the disease risk. There is no evidence for a dose-response relationship for single pathogens. However, recent studies indicated that risk increases along with the number of chronic infectious diseases.

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indicating that the efficacy of antiplatelet therapy may be related to the level of inflammatory and thrombotic markers. A positive correlation between CRP levels and activation of the coagulation/fibrinolysis system and platelet function after stroke hints at a link between coagulation and inflammation. Statins lower CRP levels independent of lipid effects and reduce coronary events in subjects with below-median lipid levels but with above-median CRP levels. Statins may be a means for anti-inflammatory treatment strategies, but this option has been insufficiently evaluated, particularly in regard to stroke prevention.

In summary, prospective studies consistently showed an increasing risk of stroke along with increasing levels of systemic inflammatory parameters at baseline, although the overall strength of the association was mostly moderate (Table). However, studies usually relied on only 1 baseline value and may therefore underestimate “actual” associations due to so-called regression dilution effects. The link between inflammatory parameters and stroke is clearly non-specific, and the source of such persistent low-grade inflammation is unknown. It may mirror ongoing atherosclerotic processes or reflect chronic infectious diseases or a particularly strong host response to ubiquitous stimuli (see below).

At present, there is increasing evidence that inflammatory parameters, especially sensitive measurement of CRP, are useful risk markers in routine assessment of systemic cardiovascular risk in clinical practice. However, it is not yet established whether lowering of inflammatory indexes lowers stroke risk, and any causal role of these parameters in stroke pathophysiology is unproven.

Acute Infection and Stroke Risk
Acute infectious diseases had been linked to stroke in children and younger adults in the 19th century. In a case-control study, Syrjänen and coworkers showed that recent infection increased stroke risk in younger subjects. Similar and consistently strong associations have thereafter been shown by other groups (odds ratios, 3.4 to 14.5), indicating that infection during the preceding week is a risk factor for stroke (Table). Both viral and bacterial infections were independent risk factors, and the risk by infection was particularly, but not exclusively, increased in younger age groups.

Several different microbial agents were detected in patients with infection-associated stroke. Therefore, it is likely that the systemic inflammatory response in the host rather than the microbial invasion per se is responsible for this elevated stroke risk. Recent infection was found to be associated particularly with stroke due to cardioembolism and cervical artery dissection. However, all studies on stroke were retrospective case-control studies in which infection was ascertained after stroke. Studies that evaluated the risk of stroke after infections have not been performed thus far. Furthermore, study results may be biased by a low participation rate in control subjects with recent infection, although studies with different control groups (hospital controls, population controls, patients with previous stroke) and different risk of such bias showed similar results.

Several mechanisms have been indicated to link acute inflammation with a prothrombotic state and stroke. Macko et al found the level of circulating antithrombotic activated protein C (APC) to be decreased in stroke subjects, and those with an antecedent infection/inflammation had the lowest concentrations of APC. Stroke patients with recent infection/inflammation had elevated levels of C4b-binding protein, which binds the anticoagulant protein S, and a distinctively lower ratio of active tissue plasminogen activator to plasminogen activator inhibitor. Grau et al detected no differences among several factors regulating hemostasis and fibrinolysis between stroke patients with and without infection, whereas Ameriso et al found increased D-dimer levels in stroke patients with infections. Since systemic changes in hemostatic parameters have not been a consistent finding in these studies, it is possible that local procoagulant effects may dominate over robust systemic changes.

Septic states permit the entry of bacteria and LPS into the bloodstream, which has profound effects favoring thrombosis in vivo. As cited above, animal experiments indicated that vascular risk factors increased the risk of thrombosis and stroke on LPS stimulation, indicating a pathogenetic link between inflammatory stimuli, traditional risk factors, and stroke. TNF-α is released during septic states, resulting in procoagulant changes in vascular endothelium with increased expression of TF, which activates the extrinsic pathway of blood coagulation. TNF-α also reduces thrombomodulin, which is required for the anticoagulant effect of protein C, and increases PAI-1, which inhibits the fibrinolytic system. In septic patients, increased levels of systemic TNF-α have been correlated with antithrombin III and PAI-1. The aforementioned changes in hemostasis and fibrinolysis, eg, explain why the risk of ischemic stroke in patients with endocarditis is tremendously increased with high risk of recurrences.

Multiple links between inflammation and coagulation may also explain the particular association between recent infection and stroke due to cardioembolism that was mainly caused by a frequent presence of atrial fibrillation in infection-associated stroke, whereas endocarditis was rare. In atrial fibrillation, coagulation is persistently activated, and, as shown recently, CRP is elevated, suggesting that inflammation may promote the persistence of atrial fibrillation. Not only septicemia but also milder infections are accompanied by a measurable activation of coagulation. Infection as an additional trigger factor could increase the prothrombotic state in atrial fibrillation and other sources of cardioembolism and finally lead to thrombosis and embolism. This underscores the concept that inflammatory mechanisms are important not only in stroke due to large-artery atherosclerosis.

Novel therapeutic strategies may arise. Vaccinations may offer a means to prevent infection-associated stroke. Influenza vaccination was found to be associated with reduced stroke risk, however, it is unclear whether this reflected a specific effect or was due to residual confounding, mainly because of differences in health awareness. Induction of natural immune tolerance to endogenous inflammatory stimuli is also worth further studies in stroke prevention.

In a critical appraisal, several case-control studies consistently showed strong associations between recent infection
and ischemic stroke, and a framework of biochemical studies provides a background for the plausibility of the association. As with chronic infections, any association between acute infection and stroke appears nonspecific. A biological gradient in the association is difficult to establish because there is no clear measure for the severity of infection, but febrile infections and septicemia are associated with a higher risk than infections in general.172,183 Although infections very likely preceded stroke in these studies, the temporal sequence is insufficiently established, and there is a need for prospective studies of the risk of stroke after infections of any kind and more vigorously designed studies of potential preventive strategies (eg, vaccination) before a causal relationship is firmly established.

**Genetic Factors, Inflammation, and Stroke Risk**

As noted above, the risk of atherosclerosis and ischemic events may depend not only on the infectious burden itself but also on the severity and the type of the immune response of the host.144 For example, susceptibility to CMV-related CHD was restricted to women with a humoral immune response and was not present in women with a cellular response.144 A hypothesis currently under investigation considers that a genetically determined strong response to inflammatory stimuli (eg, acute or chronic infection) may be associated with increased risk of stroke. The gene encoding for mannose-binding lectin codetermines individual susceptibility to certain infectious agents. Infection-susceptibility alleles were significantly associated with increased carotid plaque area and may influence interindividual differences in atherosclerotic risk.189 Polymorphisms of the P-selectin glycoprotein ligand-1 associated with lower capacity of neutrophils to bind activated platelets were linked to a reduced risk of cerebral ischemia.190 Polymorphisms in the genes of cathepsin G, a neutrophil-derived protease, and plasma platelet-activating factor (PAF) acetylhydrolase, leading to reduced inactivation of PAF, were associated with increased stroke risk.191,192 A common polymorphism in the promoter of the monocytic LPS receptor CD14 gene with potential association with higher receptor density was described as a risk factor for myocardial infarction.193,194 This polymorphism was not a risk factor for stroke in general,195–197 but it was associated with atherothrombotic and lacunar stroke in a German197 but not in a Japanese population.195 Patients with a stroke at a young age (<50 years) showed a persistently increased release of interleukin-8 by leukocytes independent of coexisting risk factors.198 Interleukin-6 possesses both neuroprotective and neurotoxic properties; a polymorphism in the gene promoter associated with lower interleukin-6 plasma levels was recently linked to the risk of lacunar stroke.199 Most studies of inflammatory genes and stroke risk were small and require confirmation in larger studies that include analyses of etiologic stroke subgroups. However, genetic variability regulating the individual host response to immune challenges and stroke risk is an exciting field of research.

**Tempering the Inflammation: The Lesson in Statins**

Statins are a group of antihyperlipidemic compounds that inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase (eg, simvastatin, pravastatin, fluvastatin, cerivastatin) and that effectively decrease the blood level of LDL and triglycerides and raise HDL. Statins were found in several large-scale studies to substantially reduce cardiovascular morbidity (by approximately 30%) and mortality in mildly hyperlipidemic or even normolipemic cohorts.200 Importantly, the risk of stroke was also significantly decreased by statins in several studies201 (reviewed in Hess et al202). It is now commonly believed that the beneficial effects of statins are not mediated solely by lipid lowering, but effects on systemic inflammatory parameters have also been observed in clinical studies.203 Through recent investigations of the effects of statins on both systemic parameters and local carotid plaque composition,204 we have begun to understand the clinical significance of the multifaceted inflammatory processes contributing to atherosclerosis and associated thrombotic events.

Pravastatin was reported to decrease the plasma concentration of CRP,205 a sensitive systemic marker of inflammation found to be independently associated with myocardial infarction and stroke.158,206 Statin therapy also reduced the concentrations of circulating soluble adhesion molecules P-selectin and ICAM-1 in hyperlipemic patients207 and reduced cytokine production.208 There is recent evidence that statins (lovastatin, simvastatin) inhibit the expression of MCP-1 by human EC and monocytes on stimulation by LPS or whole bacteria,209 which suggests that statins may inhibit the early stationing of inflammatory cells in vascular sites. In accordance, atorvastatin inhibited the expression of proinflammatory regulator NF-κB and the chemokines interferon-inducible protein 10 and MCP-1 in isolated SMC and mononuclear leukocytes.210 Lovastatin was reported to bind to the I domain of human leukocyte function–associated antigen-1, thereby inhibiting leukocyte adhesion through interaction of leukocyte function–associated antigen-1 and ICAM-1.211 Furthermore, statins inhibit the production and gene expression of cyclooxygenase-2, interleukin-1β, and interleukin-6 in human EC.212 Targets of statin effects also include platelet-thrombus interaction, hemostasis, and nitric oxide–dependent EC function.213,214 Recently, cerivastatin was shown to inhibit the production of MCP-1 and interleukin-8 by monocytes coinoculated with C pneumoniae.215

Taken together, these data suggest that the substantial ameliorating effect of statins on the risk of cardiovascular insults and stroke is mediated largely through multifaceted anti-inflammatory systemic and local vascular effects in addition to lipid-lowering effects. Angiotensin-converting enzyme inhibitors also reduce vascular inflammation216 and may be another class of drugs that protect against vascular injury through anti-inflammatory mechanisms. Future studies may reveal additional aspects of the effects of different statins or angiotensin-converting enzyme inhibitors on the inflammatory pathogenesis of atherosclerosis that may help to target and tailor their use in a selected patient group chronically predisposed to unstable plaques.20

**Conclusions**

Starting from the continuous or occasional presence of inflammatory cells in the cerebral vasculature, stimuli that are currently not clearly defined lead to activation of the endo-
thelium in either small cerebral microvessels or intracranial or extracranial arteries. Long-standing risk factors and chronic infectious diseases, possibly in conjunction with genetic predispositions, may lead to gradual activation of circulating mononuclear cells and their entry to subendothelial/perivascular locales and may aggravate proinflammatory and procoagulant EC effects. Responsiveness of the cerebrovascular tree to a systemic or local inflammatory challenge, eg, acute infection, is thus increased and may lead to local thrombosis. Infections with microbes such as C pneumoniae may actively prime this process in larger arteries and contribute to maturation of atherosclerotic plaques. Inflammatory cells are always present in these plaques and respond to further systemic stimuli by releasing proteases and procoagulant factors, which can trigger plaque rupture and thromboembolism. Measurement of sensitive indexes of plasma CRP may respond to an aggregate burden of microbial and nonmicrobial proinflammatory transformation of the vasculature and may be considered in routine clinical practice to identify individuals with elevated inflammatory risk for cardiovascular disease and stroke. It could also prove useful in monitoring treatment effects.

There is preliminary evidence for an association between chronic infections and atherosclerosis or stroke, but causality is not sufficiently established. The association between chronic infection and atherosclerotic diseases may be explained partly by residual confounding, eg, by childhood and adult socioeconomic conditions, which were insufficiently controlled for in several studies. On the basis of current knowledge, chronic infections appear to be risk factors that may act in cooperation with conventional risk factors and genetic predispositions and are neither necessary nor sufficient for disease development. In such a concept, Koch’s postulates for causality, which require a specific link between microbial agent and disease, among others, cannot be satisfied. However, more general criteria for causality are still insufficiently fulfilled, and the role of chronic infection and inflammation in stroke pathogenesis is still incompletely defined. Future studies need to address whether inhibition of inflammation or long-standing antimicrobial therapies will reduce the risk of ischemic stroke to offer effective adjuncts to platelet inhibitors, anticoagulants, and statin therapies already in clinical use.

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