Immunogenetic Susceptibility of Atherosclerotic Stroke
Implications on Current and Future Treatment of Vascular Inflammation

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Abstract—The understanding of the pathophysiology governing atherosclerosis supports a prominent role for inflammation pathways in plaque initiation and progression that result in stroke and myocardial infarction. Elevated levels of inflammatory markers in the blood, such as C-reactive protein and CD40 ligand/CD40, in concert with increased expression of adhesion molecules, chemokines, cytokines, matrix metalloproteinases (MMP), and inflammatory cells in the plaque, characterize the symptoms of atherothrombotic state. Advances in predictive capabilities of vascular events using a number of these biomarkers are beginning to remodel our clinical practice in the use of medications such as statins and angiotensin receptor blockers for stroke prevention. Although the general inflammatory features of atherosclerosis are becoming widely recognized, factors resulting in individual variability in plaque formation and instability remain poorly defined. Emerging literature points toward several acquired and innate susceptibility factors in the immune pathways that may provide insight into why many plaques rapidly evolve from a “stable” to an “unstable” or symptomatic state. First, exposure of plaque memory T-lymphocytes to infectious or endogenous antigens may result in rapid clonal expansion of T-cell variable β chain subtypes and stimulate macrophages to release MMPs, causing plaque destabilization. The effects of infectious agents can further be influenced by an individual’s major histocompatibility complex class II molecule profiles, which can affect susceptibility to specific organisms. Second, functional polymorphisms of genes that regulate the immune pathway can predispose patients to a more robust inflammatory expression after risk factor exposure. Identification of a susceptibility gene profile and immunologic mediators that promote T-cell activation provides a unique opportunity for early identification of stroke risk and targets for future therapy. (Stroke. 2004;35[suppl I]:2712-2719.)

Key Words: atherosclerosis ■ genetic susceptibility ■ inflammation ■ stroke ■ T-lymphocyte ■ therapeutics

Atherosclerosis is being redefined from its original perception as a disorder of lipid deposition to one of an ongoing inflammatory process. Exposure of the vessel wall to oxidative stress by hyperlipidemia is just one of a number of classic risk factors that can promote the disease state. Features of inflammation have been identified at all stages of atherosclerosis. Studies continue to identify markers that will help guide therapy to modify the inflammatory profile through the reduction of the effects of these classic risk factors.

Despite the basic understanding of putative pathological risk factors, manifestation of atherosclerotic disease and thromboembolic events varies greatly within the population. Some patients with minimal risk factor exposure display extensive atherosclerotic development, whereas others with significant risk factor burden can manifest limited vascular disease. In addition, evidence indicates that there is nearly a 10-fold increased risk of stroke in patients with previous events compared with asymptomatic patients with similar degrees of carotid stenosis, suggesting an additional component of the inflammatory pathway beyond degree of plaque stenosis and risk factor burden. A similar incongruity is seen in coronary disease in which inflammatory profile appears to be the more prominent factor for acute coronary syndromes than plaque size or luminal stenosis. The fundamental gap in knowledge is represented by the incomplete elucidation of the basic mechanisms that underlie the individual differences in phenotypic responses to vascular risk factors.

This article reviews the current concepts of atherosclerosis as an inflammatory disease, outlines the emerging literature regarding immunologic and genetic susceptibility factors in the development and progression of atherosclerosis, and addresses strategies that use existing medications and novel therapeutics directed at the inflammatory processes.

Inflammatory Profile of Atherosclerosis: Response to Injury

The initiation and formation of atherosclerotic plaque represents a complex interplay of environmental factors and genetic susceptibility that results in a chronic inflammatory...
process, eventually leading to stroke. Exposure of the endothelial surface to a variety of risk factors such as hypertension (shear stress forces), hyperglycemia, oxidized low-density lipoprotein (LDL), cigarette smoke toxins, infections, and other inflammatory compounds results in endothelial injury, the migration of lipids into the subintimal ground space of large arteries, and the expression of surface adhesion molecules such as intercellular adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1), and P-selectin. Expression of these adhesion molecules and release of chemokines results in the migration of circulating mononuclear cells into the vessel wall. Monocyte-derived macrophages and T lymphocytes contribute to the release of a number of inflammatory mediators that perpetuate the inflammatory and chemotactic state of the growing atherosclerotic plaque (Figure 1). They further release factors that promote smooth muscle and fibroblast proliferation, which leads to the deposition of an interstitial fibrous matrix, including collagen, elastin, proteoglycans, and other proteins, which form a fibrous cap purported to stabilize the underlying necrotic lipid core.

**Mechanism of Intraluminal Thrombosis**

The inflammatory profile of the growing atherosclerotic plaque provides 2 pathways by which intraluminal thrombosis and cerebralvascular ischemic events can occur.

First, macrophage release of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) results in the conversion of the endothelium over the plaque from an anticoagulant to a prothrombotic state. This transformation is characterized by a reduction in tissue plasminogen activator and protein-S production with an increase in the expression of tissue factor, matrix metalloproteinases (MMPs), endothelin-1, platelet activating factor, plasminogen activator inhibitor-1, ICAM-1, VCAM-1, E-selection, P-selectin, IL-8, and monocyte chemotactic protein-1 (MCP-1). These changes are believed to be in part secondary to intraplaque signaling mediated by CD40–CD40L interaction associated with a variety of cell types including CD4 T-lymphocytes. Evidence that the inflammatory profile is associated with the symptomatic atherosclerotic state is supported by numerous studies that reveal intraplaque and endothelial surface protein expression of inflammatory mediators (ie, ICAM-1, CD40/CD40L, and macrophage and T-cell concentration) in symptomatic versus asymptomatic carotid plaques.

Second, breakdown of the elastin/collagen fibrous cap caused by T-cell–mediated and macrophage-mediated release of MMPs, including collagenases such as gelatinase-B (MMP-9), stromelysin (MMP-3), and gelatinase-A (MMP-2), is believed to result in plaque instability and rupture. Extrusion of the necrotic lipid core along with collagen material leads to intraluminal thrombosis and thromboembolic stroke.

**Biomarkers**

Numerous systemic markers have been associated with active atherosclerotic disease, including elevated levels of C-reactive protein, IL-6, ICAM-1, TNF-α, VCAM-1, E-selectin, homocysteine, and fibrinogen. Beyond representing markers of vascular disease, factors such as C-reactive protein and homocysteine have known atherogenic properties. Most notably, C-reactive protein has been reported to induce expression of leukocyte adhesion molecules, tissue factors, monocyte recruitment to arterial wall with the induction of MCP-1, and increased complement activation.

Soluble CD40 ligand is also emerging as an important marker for acute coronary syndromes and acute cerebral ischemia. Increased CD154 (CD40 ligand) on platelets and monocyte-laden CD40 result in the upregulation of numerous inflammatory factors and procoagulation factors as previously described. These markers not only are potential indicators of efficacy of antiinflammatory treatment but also are targets themselves for reduction of the inflammatory state in vascular disease.

**Immunologic and Genetic Susceptibility**

Vascular responses to risk factor exposure and subsequent compilation of inflammatory cells and mediators provide a vital substrate for plaque progression and thromboembolic events. However, burden of oxidative stress and general inflammatory composition alone does not completely explain the conversion of many plaques from an asymptomatic to an unstable or symptomatic state. Although it is clear that the profile of symptomatic plaque is one of increased inflammatory mediators and inflammatory cells, the question remains as to the mechanism that causes one high-grade stenotic plaque to become symptomatic whereas other plaques of equal stenosis remain clinically quiescent.

**Acquired Susceptibility/T-Lymphocyte Variable β Chain Repertoire in Atherosclerotic Plaques**

One potential mechanism promoting the rapid progression of inflammatory mediators in atherosclerotic plaques is the exposure of plaque T lymphocytes to antigens previously recognized by T-cell receptors. Although T cells can be
“Burden” of Infectious Disease

Table 1: Analysis of Serum Antibodies to Infectious Pathogens in Patients with CAD

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Number of Patients Positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>10</td>
</tr>
<tr>
<td>HSV-1</td>
<td>15</td>
</tr>
<tr>
<td>EBV</td>
<td>20</td>
</tr>
<tr>
<td>R. Influenza</td>
<td>5</td>
</tr>
<tr>
<td>C. pneumonia</td>
<td>20</td>
</tr>
<tr>
<td>M. pneumoniae</td>
<td>10</td>
</tr>
</tbody>
</table>

Mean F/U = 3 years

Outcome: CV death

Figure 2. Death from cardiovascular causes was increased during a mean follow-up period of 3 years with increasing presence of seropositivity of infectious organisms. CMV indicates cytomegalovirus; HSV, herpes simplex virus; EBV, Epstein-Barr virus; CAD, coronary artery disease. Reprinted with permission from Circulation.34 Copyright Lippincott Williams and Wilkins, 2001.

many of the genes that regulate inflammatory cytokine and adhesion molecules are polymorphic in nature. Increased disease. Although known to stimulate an innate immune response by activation of macrophages, oxidized LDL has also been identified as a specific antigen that can stimulate T-cell proliferation.35 Studies in our laboratory using flow cytometry on T cells harvested from human carotid atherosclerotic plaque at the time of endarterectomy revealed clonal expansion of Vβ T-cell families compared with T-cell profiles in the peripheral blood.36 These emerging data strongly support the development of an acquired susceptibility as the natural course of the initiation and progression of atherosclerotic plaque. Although no specific organism or group of organisms has yet been directly identified as common to all symptomatic atherosclerotic events, the repertoire of previous infectious exposure and increased inflammatory cell concentration increases the likelihood of vascular events in patients with atherosclerosis.

Innate Susceptibility to Inflammatory/Infectious Cause

In addition to antigenic T-cell activation resulting from exposure of infectious organisms, inflammatory responses can be significantly influenced by MHC class II polymorphisms.37,38 Evidence indicates that activation of T-cell populations and Vβ-specific T-cell repertoire changes are influenced by the preferential binding of specific antigens in bacterial infections such as Staphylococcus aureus and Streptococcus pyogenes to specific T-cell receptor-independent binding sites on the MHC molecule. This preferential susceptibility to an inflammatory response represents an innate susceptibility that is MHC class II-mediated. MHC class II differences have been demonstrated to impart variants in susceptibility to rheumatic heart disease and hepatitis C.39 Given the variety of infectious organisms associated with the initiation and progression of atherosclerosis, and given the polymorphic nature of MHC class II determinants, further investigation is warranted to study innate susceptibility profiles of the MHC class II system in patients with atherosclerosis.

Genetic Susceptibility From Inflammatory Gene Polymorphisms

Development of atherosclerotic plaques represents a complex interplay of environmental risk factor exposure and a multi-genic profile with intermediate phenotypes.40 It is becoming more widely accepted that individual patient phenotypic variants in plaque instability is unexplained solely by risk factor exposure and that differences in distribution of polymorphisms in genes coding for inflammatory factors may account for the patterns of behavior in carotid atherosclerotic plaque. Although large epidemiological studies confirm the hereditary contribution to cerebrovascular risk,41,42 the quantification of genetic factors for large-vessel disease remains to be defined.43 Therefore, a need exists to develop a comprehensive profile of genetic markers and single nucleotide polymorphisms that define with greater specificity the susceptibility of an individual for atherosclerosis development based on gene presence as well as gene–gene interaction. Many of the genes that regulate inflammatory cytokine and adhesion molecules are polymorphic in nature. Increased
frequency of specific alleles for cytokines such as TNF-α and IL-1β have been associated with other inflammatory diseases. Animal models demonstrate the role of inflammatory genes on risk factor exposure in atherosclerosis development. Mice bred for susceptibility or resistance to atherosclerosis demonstrate that an inbred resistance strain (C3H/HEJ) with low-inflammatory gene expression did not form atherosclerosis when fed a high-cholesterol diet. This is contrasted to a high-susceptibility strain (C57BL/6J), which has high levels of inflammatory gene expression and significant fatty streak formation. When these 2 strains of mice were inbred, fatty streak formation segregates with the high levels of inflammatory gene expression in a nonmendelian pattern of inheritance, Further supporting that multiple genes modify inflammatory mediator expression that influences responses to the western atherogenic diet.

To explain the presence of these seemingly injurious proinflammatory gene polymorphisms, genes that increase proinflammatory expression from an evolutionary perspective may have provided an advantage to our ancestors, including allowance for wound healing and eradication of infection during periods of nutritional deprivation. These same genes may now confer selective disadvantages in modern times, particularly given the average life span increases, environmental stresses, and western diet/lifestyle that could lead to insulin resistance and atherosclerosis.

Numerous gene polymorphisms associated with the development of coronary and carotid atherosclerotic disease have now been identified. For example, a large population-based study revealed a strong association in the gene encoding phosphodiesterase 4D (PDE4D) for carotid and cardiacogenic stroke. Haplotype identification was essential for determining phenotypic expression. This gene revealed 3 distinct groups characterized by wild-type, at-risk, and protective haplotype classification.

Other genes showing significant association with the development of atherosclerotic disease include IL-1 receptor antagonist, in which the homozygous carrier state for allele 2 imparts an adjusted odds ratio (OR) of 13.78 for a greater likelihood of atherosclerosis. Also, Toll-like receptor-4 polymorphism Asp299Gly is associated with an alteration in inflammatory expression and acute coronary events.

Although individual single nucleotide polymorphism identification provides a framework for genetic susceptibility, the characterization of single gene polymorphisms is insufficient to describe the overall effects of genetics on the phenotypic state of atherosclerosis and other complex diseases. Studies have shown that optimal understanding of phenotypic expression will require knowledge of haplotype expression and gene–gene interactions that can have a profound effect on expression of individual polymorphisms. This is demonstrated in a study by Palo et al who found a synergistic effect of the −174 G/C IL-6 gene promoter polymorphism with 469 E/K ICAM-1 gene polymorphism. Although the GG genotype of the ICAM-1 polymorphism imparted increased risk for ischemic stroke, the IL-6 GG/ICAM-1 EE double-homozygous subjects had a significantly greater increased odds ratio (OR, 10.1; P=0.004). This demonstrates the need for future studies to take into account essential gene–gene interactions when using gene profiles for predictive value.

**Conventional Medications With Antiinflammatory Effects: Statins**

A fundamental change has occurred in the treatment of atherosclerosis. In addition to the classic risk factor modification, we now must apply strategies that selectively reduce the inflammatory characteristics of atherosclerotic plaque. In the past decade, statins have emerged as a class of medications that reduce cardiovascular and cerebrovascular events. This effect is mediated not only by the classic lowering of cholesterol burden but also through pleiotropic effects that include a profound antiinflammatory and immune modulatory profile, believed to stabilize active atherosclerotic plaque (Figure 3). Antinflammatory effects have been demonstrated in multiple drugs within this class of medications, including the reduction of chemokines, proinflammatory cytokines such as IL-1β and TNF-α, and leukocyte adhesion molecules necessary for inflammatory cell and endothelial interaction. Many of these statins’ effects are believed to be secondary to an alteration of intercellular signaling through Rho A, rac 1, and other GTPases responsive proteins.

Furthermore, statins have an antithrombotic effect through their reduction in tissue factor and upregulation of production of tissue plasminogen activators. Plaque stabilization is believed to be, in part, mediated by the reduction of MMPs, which cause disruption of the elastic/collagen fibrous cap in atherosclerotic plaques. Believed to be equally important is the optimization of blood flow from vasodilatory effects through endothelial nitric oxide synthase upregulation and induction of angiogenesis mediated through phosphatidylinositol 3 kinase/AKT-dependent mobilization of bone marrow endothelial progenitor cells.

The clinical relevance of the statins’ on the pathophysiology of unstable atherosclerotic plaque was demonstrated in a study by Crisby et al. This study revealed that in patients undergoing carotid endarterectomy for symptomatic plaque, preoperative Pravastatin (40 mg every day for 3 months) significantly reduced the concentrations of oxidized LDL,
MMP-2, T cells, and macrophages while increasing collagen and inhibitors of MMPs. This study demonstrated the dramatic effect on the pathogenesis of active atherosclerotic plaque in a brief period of time, highlighting the potential stabilizing short-term effects of this class of drugs.

Finally, HMG-CoA reductase inhibitors appear to have a direct effect on the T-helper cell profile in vitro and in vivo. Studies indicate that the statins increase Th2/TH3 (regulatory T cell) cytokines while inhibiting Th1 (helper cell) inflammatory effects. This, in combination with the interference of the CD40/CD40 ligand system, demonstrates a more specific effect on T-cell–mediated inflammation that is believed to be a pivotal step in the activation of atherosclerotic plaque from the quiescent to the unstable prothrombotic state.

Attempts to identify markers of inflammation, which may be a surrogate for the antiinflammatory efficacy of the statins, are being sought. C-reactive protein, which has been strongly associated with active vascular disease and recurrence of myocardial ischemic events, has been shown to be reduced in a number of studies involving the use of statins. However, not all studies show a direct correlation between the beneficial effects of vascular event reduction and C-reactive protein lowering. The Prove It Trial, which showed a significant reduction in vascular events associated with intense statin therapy, showed both treatment groups with similarly robust reduction in C-reactive protein. However, despite the inconsistencies, C-reactive protein has proven itself to be not only a marker but also a direct mediator of inflammatory activity by increasing adhesion molecules, thrombotic agents, and activation of complement. This protein is likely, in the future, to be part of a panel of inflammatory markers that may better-predict primary prevention effects.

Angiotensin Receptor Blocker
Plaque stabilizing effect was identified by Cipollone et al. who demonstrated in patients undergoing carotid endarterectomy for high-grade symptomatic carotid stenosis angiotensin II type 1 receptor blocker, irbesartan significantly reduced the concentration of macrophages, T lymphocytes, human leucocyte antigen-DR, COX-2, MMP-2, and MMP-9 and increased the collagen content compared with chlorthalidone. This study supports that the antiinflammatory profile of angiotensin receptor blockers reported in vitro translates to an in vivo benefit of human atherosclerotic disease. It may further explain the results of the HOPE trial in which patients treated with the antihypertensive angiotensin-converting enzyme inhibitor, ramipril, experienced a significant reduction in vascular events compared with placebo. This effect was seen even in patients without significant decrease in blood pressure, suggesting an alternative mechanism was involved in the benefit.

Antiplatelet Agents
The antiplatelet agents by virtue of their ability to reduce platelet aggregation and interaction with circulating activated monocytes can reduce signaling at the endothelial surface associated with the proinflammatory profile of atherosclerosis. Studies have further demonstrated that the drug dipyridamole may reduce inflammation and plaque rupture by suppressing the synthesis of IL-8, MCP-1, and MMP-9.

Recent studies have demonstrated that clopidogrel may have an effect on expression of adhesion molecules involved in the inflammatory pathways. The influence of these antiplatelet agents need to be studied further with respect to their relative effects on the proinflammatory characteristics of atherosclerotic plaque in relation to risk reduction.

Future: Targeting T-Cell Activation and Genetic Susceptibility
Given the prominent role of specific antigen-driven T-cell activation in the destabilization of atherosclerotic plaque, strategies that directly inhibit Th1 proinflammatory cytokine production could prove instrumental in selective antiinflammatory treatments of stroke-prone patients. The induction of oral and nasal tolerance, a process that specifically suppresses the cellular or humoral immune response to an antigen given repetitively at low doses, could be used to locally suppress inflamed vascular tissue. Data recently presented by Takeda et al revealed that repetitive nasal installation of E-selectin (a protein specifically expressed on activated endothelium) reduced the development of both ischemic and hemorrhagic strokes in spontaneously hypertensive stroke-prone rats. Tolerance is believed to be based on the production of regulatory T cells in response to chronic low-dose exposure to a specific antigen (Figure 4). By using an antigen specifically expressed only on activated endothelium, such as E-selectin, the migration of regulatory T cells, activated by the presence of that antigen, could locally suppress the Th1 response in atherosclerotic plaque. Studies by others have used the same strategy to reduce atherosclerotic plaque development in an LDL receptor-deficient mouse model of aortic arch inflammatory atherosclerosis. The use of nasal vaccination of heat shock protein 65 in the LDL receptor-deficient mice significantly reduced the inflammatory processes associated with atherosclerosis. These data provide a new immunologic approach for the treatment of atherosclerosis that capitalizes on the identification of T-cell–specific plaque activation.
T-Lymphocyte Vβ Antagonists

If individual T-cell Vβ families can be demonstrated to be specific with respect to atherosclerosis activation, then identification of clonal populations of T cells within the plaque may provide another opportunity for specific reduction of inflammatory cells. Data presented support the contention that specific MHC class II profiles, along with T-cell Vβ-specific families, increase susceptibility to particular infectious organisms. If such a profile is found to be associated with the majority of atherosclerotic plaque within a given individual, then direct antagonism of that T-cell receptor Vβ family may reduce the inflammatory nature of atherosclerosis without causing generalized T-cell immune suppression. Relevance of this strategy has been demonstrated in a study revealing the beneficial effect in patients with myasthenia gravis who spontaneously produced antibodies against T-cell receptor Vβ 5.1 (the inducer of MHC DR3β associated anti-AchR humoral response). Patients with the highest levels of antibodies against their T-cell receptor variable-region beta chain 5.1 had a significantly milder course of myasthenia gravis and responded better to medications.73 These data support the notion that “vaccination” of peptide fragments of specific T-cell receptor subtype could be used to block harmful T-cell activity if a preferential Vβ family is associated within atherosclerotic plaque.36,73

In addition, potential benefits of using mucosal tolerization against common organisms that express antigens that mimic oxidized LDL may be beneficial in reducing the response of oxidized LDL-specific T cells within atherosclerotic plaque. This strategy exploits the understanding that epitopes of organisms such as Streptococcus pneumoniae mimic oxidized LDL antigenicity and can be used via tolerization to reduce the innate immune response incited by hypercholesterolemia.74

In general, the understanding of T-cell specificity within the inflammatory process seen in atherosclerosis provides a unique opportunity to specifically reduce the inflammatory profile by identifying individual susceptibility.

Gene Profile-Directed Therapy

Identification of a gene polymorphism profile that predicts atherosclerotic plaque formation and activation susceptibility opens new vistas of exploration for gene-directed therapy. Studies demonstrate that gene profiling can significantly influence responses to drugs such as the statins. A polymorphism in the gene Toll-like receptor-4 that influences innate immunity is associated with an increased beneficial effect in risk reduction of cardiovascular events in patients treated with pravastatin.75 Mutant allele Asp299Gly polymorphism was found to have significantly fewer cardiovascular events in the treatment group than patients with the wild-type gene profile. These data and other studies support the concept that a comprehensive genetic profile will lead to a more efficient use of current and future medications in patients with atherosclerotic disease. Data further indicate that genetic profiling in combination with serotyping of specific organisms shows the interrelation between genetic and immune response in the increased risk for atherothrombotic stroke.76

In summary, the understanding of the immunogenetic profile of patients is emerging as the next pathophysioligic facet in atherosclerosis to be modified to reduce the risk of stroke and heart attack. Studies that better define T-cell clonal populations in individuals along with profiling of gene polymorphism haplotypes that alter a patient’s response to the classic risk factors will lead to the next generation of therapies that potentially will dramatically reduce the burden of stroke from atherosclerosis.

References

The document contains a series of references to scientific literature, discussing various topics in the field of stroke and cardiovascular disease. The references are cited in the context of research studies, clinical trials, and reviews. The text is formatted in a way that is typical of academic papers, with proper citation of previously published works. The references are written in a style that is consistent with the scientific community's conventions for citing sources.


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