The Harms and Benefits of Inflammatory and Immune Responses in Vascular Disease

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Accumulating experimental evidence strongly supports a role for inflammatory, innate immune and adaptive immune mechanisms in many facets of vascular disease. This brief review will highlight recently published insights into inflammation and immune system involvement in stroke biomarker identification, atherosclerosis, abdominal aortic aneurysm (AAA) formation, thrombosis, ischemic tolerance, progression of ischemic brain injury, and peristroke infections.

Inflammatory Biomarkers and Stroke

Inflammation plays a role in the genesis of brain ischemia and inflammatory processes and may facilitate serious and life-threatening complications in stroke patients. However, efforts to disentangle good from bad effects of inflammation in cerebrovascular disease reveal frequent discrepancies between preclinical and clinical data. A clinical goal for several decades has been the identification of reliable inflammatory biomarkers of impending stroke in asymptomatic subjects and clinical prognosis in stroke patients. So far, most markers have shown moderate utility at the bedside as the result of low sensitivity and specificity. The list of biomarkers includes high-sensitivity C-reactive protein (hsCRP), fibrinogen, serum amyloid A, matrix metalloproteinase (MMP)-9, P-selectin, sCD40L, myeloperoxidase, vascular cell adhesion molecule (VCAM)-1, intercellular adhesion molecule (ICAM)-1, tumor necrosis factor (TNF)-α, interleukin (IL)-1, IL-6, and IL-8. Novel markers evaluated in 2005 are PARK7, 3 nucleoside diphosphate kinase A (NDKA), 3 and adiponectin. 4 PARK7 is a redox-sensitive molecular chaperone activated by oxidative stress; it increases within 30 minutes to 3 hours of stroke onset. Sensitivities are of 54% to 91%, for PARK7, and 70% to 90%, for NDKA, and specificities are 80% to 97%, for PARK7, and 90% to 97%, for NDKA. Adiponectin is an adipocytokine with anti-inflammatory and antiatherogenic properties that correlates inversely with infarction volume and neurological impairment in acute stroke patients. 4

Recent preclinical data showed that the administration of anti-TNF-α neutralizing mAb treatment in rats decreased upregulation of TNF-α in parallel with reduced infarct volume and cerebral edema after transient focal ischemia. However, TNF-α plasma levels did not predict the risk of edema-related malignant cerebral infarction in patients. Similarly, clinical stroke studies suggested that higher levels of VCAM-1 were predictors of bad outcome, but anti-VCAM-1 therapy showed no significant protection in stroked rats. The population-based Rotterdam Scan Study illustrates that the study of biomarkers provides a deeper understanding of cerebrovascular disease; higher levels of hsCRP identified the presence and progression of white matter disease on MRI. Also, higher plasma levels of hsCRP, IL-6, or ICAM-1 were observed in Japanese and Austrian subjects with asymptomatic white matter infarctions. These studies reinforce the role of inflammation in small-vessel disease, a view also stressed in a recent case-control study in which a Gly174Cys polymorphism of the IL-6 gene correlated with lacunar stroke only. Future studies will assess additional biomarkers singly and in combination as predictors of stroke occurrence and prognosis.

Immune System and Atherosclerosis

From modern approaches to its molecular pathobiology, atherosclerosis emerges as perhaps the most common chronic inflammatory disease. Chronic exposure to low-density lipoprotein (LDL) modified by oxidation or enzymatic attack can activate endothelial cells and cells in the underlying intima to express adhesion molecules and inflammatory genes that promote monocyte accumulation and macrophage differentiation in developing atherosclerotic plaques. Pattern recognition receptors play a key role in this innate immune response that leads to local inflammation and both innate and adaptive immune responses. Major scavenger receptors, CD36 originally identified as a platelet integral membrane glycoprotein receptor for thrombospordin-I and scavenger receptor A family members, bind and internalize modified LDL and activate macrophages. CD36 null and scavenger receptor A null gene modifications show robust suppression of atherosclerosis in apoE−/− and LDL receptor−/− mice. Toll-like receptors (TLR) discovered in 1997 as sharing homology with the toll receptor that is essential for dorsoventral patterning and antifungal immunity in Drosophila initiate and orchestrate inflammatory and immune re-

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sponses in the plaque. TLR signal transduction in response to endogenous ligands released or formed under conditions of cellular stress (alarm or danger signals) or pathogen-associated molecular patterns can turn on immune and inflammatory responses. Of 11 known TLRs, genetic deficiency of TLR4 or TLR2 has been shown to reduce development of atherosclerosis in murine models. Also, the Asp299Gly TLR4 polymorphism, which attenuates TLR4 receptor signaling, is associated with a decreased risk of atherosclerosis in clinical studies. Novel approaches to atheroprotection involve several forms of immunomodulation. Based on observations indicating that native IgM anti-oxidized LDL antibodies reduce circulating levels of oxidized LDL, a series of oxidized LDL immunization studies in animal models of atherosclerosis were performed. Production of IgG antibodies to oxidized LDL and clear-cut reductions in plaque burden were noted in these studies. More recently, detailed molecular characterization of the complex oxidized LDL antigen has identified 2 epitopes that are atherogenic, malondialdehyde-ApoB-100 peptide sequences and oxidized phospholipids containing a phosphorylcholine head group. Immunization with these defined antigens reduces atherosclerosis in murine models, and future clinical studies are expected. Also, mucosal tolerization to mycobacterial heat shock protein 65, a molecular mimic of native heat shock protein 60, by nasal or oral administration generates regulatory T cells (Treg) that suppress local inflammatory and immune reactions and decrease development of atherosclerosis in preclinical models.

**Immune System and AAA**

Interestingly, although stenotic atherosclerotic lesions are predominantly associated with Th1 cytokine profiles, recent evidence implicates Th2-predominant inflammation in AAA formation. In a murine allografted aorta aneurysm model, interferon-γ receptor–deficient (GRKO) hosts (with a consequent Th2 immune deviation) developed severe AAA formation associated with augmented elastolytic activity primarily attributable to expression of increased matrix metalloproteinase 12 (MMP-12). Allografts in GRKO recipients treated with anti–IL-4 antibody or allografts in GRKO hosts that were congenitally deficient in IL-4 did not develop AAA. This identifies IL-4, a Th2 cytokine, as an important stimulus for AAA formation.

**Inflammation and Thrombosis**

Inflammation and coagulation intermingle in many disease states; better understanding of this relationship might result in the development of safer and more efficacious drugs for acute treatment and secondary prevention of stroke. A major player in this network is tissue factor (TF), an extrinsic coagulation pathway activator in humans with cellular and soluble subtypes. In a recent study, soluble TF was expressed and released from human endothelial cells in response to TNF-α and IL-6, a finding confirmed in another study that showed thrombus formation was driven primarily by TF derived from blood vessel wall instead of leukocytes. Thrombomodulin (TM) has shown novel anti-inflammatory properties in addition to its ability to activate Protein C. Abeyama and colleagues have identified an N-terminal lectin-like domain (D1) of TM with potent anti-inflammatory properties that include the binding and inhibition of high mobility group box 1 protein. The latter has powerful cytokine-like activity mediated by the receptor for advanced glycosylation end products, thus suggesting possible therapeutic potential of D1 of TM.

CD40/CD40L is a membrane glycoprotein belonging to the TNF receptor superfamily. Its expression is increased in platelets and monocytes of patients with acute stroke, an effect that can facilitate the production of proinflammatory cytokines. In a model of focal ischemia/reperfusion, mice deficient in either CD40 or CD40L had less leukocyte and platelet recruitment, reduced brain injury, and less endothelial barrier dysfunction than wild-type animals. Further research on the therapeutic value of molecules targeting CD40/CD40L in patients with acute stroke would seem reasonable.

**Immune System and Ischemic Tolerance**

Based on the capacity of proinflammatory innate immune system mediators to induce cross-tolerance to ischemia, a novel unifying concept of ischemic tolerance that involves TLR function has been proposed. Preconditioning with lipopolysaccharide, a TLR4 ligand, and downstream cytokine effector molecules, IL-1 and TNF, has been shown to confer robust cytoprotection in subsequent severe brain ischemia. Activation of the TLR4 signal transduction cascade has been shown to upregulate multiple feedback inhibitors that include signaling inhibitors, decoy receptors, and anti-inflammatory cytokines. Generation of feedback inhibition of TLR signal transduction by a preconditioning stress exposure that activates TLR receptors may be important in ischemic tolerance.

**Immune System and Stroke Progression**

The literature on the cytoprotective mechanisms of immunomodulation by mucosal tolerization of locally expressed antigens has been recently extended. Nasal vaccination with myelin oligodendrocyte (MOG) glycoprotein to prime Treg with a brain-specific antigen targeted IL-10-secreting CD4+ Treg to ischemic brain in a transient middle cerebral artery occlusion model. The observed reduction in ischemic brain damage was associated with a local increase in IL-10, reduction in interferon-γ and reduced accumulation of CD11b+ cells (macrophages, neutrophils). Nasal MOG vaccination was ineffectual in IL-10−/− mice; adoptive transfer of CD4+ MOG-specific Treg from Wt mice reduced infarct volume in contrast to Treg from IL-10−/− donors. Thus, IL-10-secreting CD4+ Treg reduce injury after stroke.

**Infection and Stroke**

Infections may cause stroke and frequently complicate the clinical course of that disease; the mechanisms and best treatment response are being studied. Patients with stroke and preceding infection reveal a significantly increased proportion of platelet-leukocyte aggregates and higher P-selectin expression by flow cytometry assay compared with noninfected stroke patients. Blocking ICAM like P-selectin or the P-selectin glycoprotein ligand may provide clinical benefits. The Early Systemic Prophylaxis of Infection After Stroke
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(ESPIAS) Trial provides insights on the management of infection in acute stroke and highlights the relevance of appropriate antibiotic selection. Prophylactic levofloxacin (500 mg/100 mL/d for 3 days) was found not better than optimal care to prevent infections, and the drug lessened clinical recovery rates. Based on current knowledge, antibiotics that deserve clinical testing in acute stroke are minocycline, moxifloxacin, and ceftriaxone.

References


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