# Immunomodulation Strategies for Preventing Vascular Disease of the Brain and Heart. Workshop Summary

John Hallenbeck, Gregory del Zoppo, Tom Jacobs, Antoine Hakim, Stephen Goldman, Ursula Utz, Ahmed Hasan and for the Immunomodulation Workshop Participants

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# Immunomodulation Strategies for Preventing Vascular Disease of the Brain and Heart

## Workshop Summary

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***Abstract*—This workshop examined the opportunities for translational research directed at immune and inflammatory mechanisms. This summary presents the background data in 3 general areas: (1) inflammation and hemostasis in cerebrovascular and cardiovascular disease, (2) immune interactions in the central nervous system and heart, and (3) translation of immune modulation in the brain and heart, all of which supported a consensus derivation of the opportunities for future research in these areas. The summary concludes with 11 recommendations. (*Stroke*. 2006;37: 000-000.)**

**Key Words:** acute stroke ■ cerebrovascular disease ■ immunology ■ inflammation ■ ischemia ■ stroke

There have been a number of advances in understanding the roles of both cellular and humoral types of inflammation in ischemia of the central nervous system (CNS) and heart. Most recently, this has been extended to insights about the role of innate and adaptive immune system activation in many facets of cerebrovascular and cardiovascular disease (eg, atherosclerosis, initiation of vascular compromise, progression of ischemic or hemorrhagic damage, and induction of ischemic tolerance; see reviews[1–3]). There is increasing evidence that inflammation and immunity play major roles in the outcomes of our most devastating diseases, including coronary heart disease, atherosclerotic disease, and stroke. We are at a point where further understanding is needed to formulate effective research and therapeutic approaches to modulate and reduce this disease burden. Already, preclinical work has suggested novel avenues that may have treatment benefit. Measures that induce development of regulatory T cells ($T_{reg}$) that cause a type 1 T-helper cell (Th1)-to-Th2 immune deviation of T cells (such that their cytokine profiles shift from proinflammatory to immunomodulatory), interrupt leukocyte adhesion, inhibit proteases, and modulate hemostasis have shown promise.

To formulate possible future directions for translational research involving immune and inflammatory mechanisms, a workshop entitled *Immunomodulation Strategies for Preventing Vascular Disease of the Brain and Heart* was sponsored jointly by the National Institute of Neurological Disorders and Stroke (NINDS), the Canadian Stroke Network (CSN), and the National Heart, Lung, and Blood Institute (NHLBI). The meeting was held on March 10–11, 2005, in Silver Spring, Md.

The goal of the workshop was to bring together talented immunologists, vascular biologists, cardiologists, hematologists, neurologists, and neuroscientists to discuss the state of the art in immune and inflammatory strategies so as to identify those processes and approaches that are most promising for clinical translation to CNS and myocardial ischemia.

## Inflammation and Hemostasis in Cerebrovascular and Cardiovascular Diseases

The traditional view that cerebral blood vessels are passive conduits providing nutritional flow to neurons and glia has undergone major revision during the past several years. In the CNS, neurovascular coupling, which assumes interacting vascular, glial, and neuronal elements, operates within the conceptual framework of the neurovascular unit[4] (Figure 1). Components of the neurovascular unit include the endothelial cells with tight junction proteins, claudins and occludin, a basal lamina, astrocyte endfeet, and pericytes embedded in the basal lamina. In addition to these layers, neurons send processes to the neurovascular units that influence their behavior. Cellular members of the neurovascular units appear to be responsible for functional specificities along the microvascular axis that lead to heterogeneity of function, the

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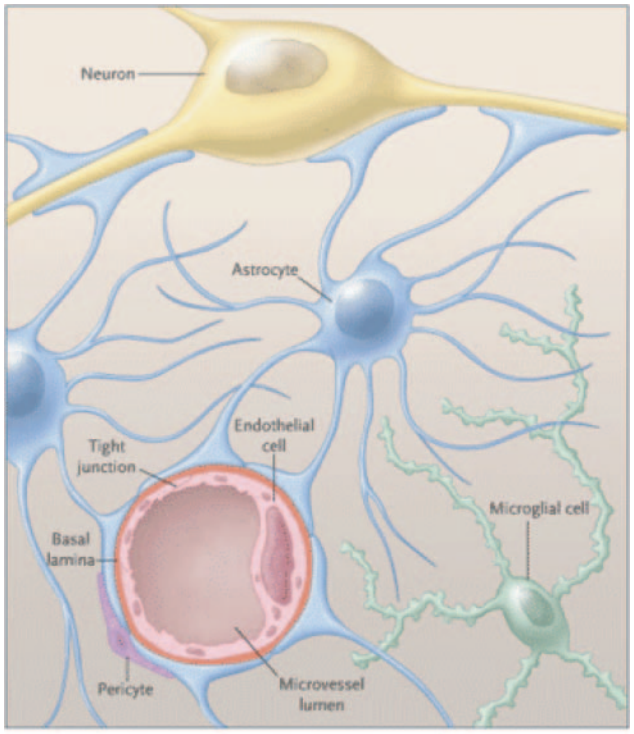


**Figure 1.** The neurovascular unit. A conceptual framework, the neurovascular unit comprises neurons, the microvessels that supply them, and their supporting cells. Cerebral microvessels consist of the endothelium (which forms the blood-brain barrier), the basal lamina matrix, and the endfeet of astrocytes. Microglial cells and pericytes may also participate in the unit. Communication has been shown to occur between neurons and microvessels through astrocytes. With permission from del Zoppo G. Stroke and neurovascular protection. *N Engl J Med.* 2006; 354: 553–555. Copyright © 200× 2006 Massachusetts Medical Society. All rights reserved.

presence of the 2 unique permeability barriers (interendothelial tight junctions and basal lamina), and procoagulant substances (tissue factor) along the glia limitans. In the setting of adult neurogenesis, formation of new vessels (vasculogenesis) produces a vascular niche that generates molecular cues needed for the differentiation of neuronal and glial precursors.[5,6] Structurally, neurons, astrocytes, and vascular cells are in close contact,[7] and the integrity of the brain depends on such close association.[8] Functionally, neural, glial, and vascular functions are intimately related. Thus, neuronal activity is one of the major factors influencing cerebral blood vessels.[9] Conversely, cerebral blood vessels exert powerful effects on neurons and glia by controlling the microenvironment of these cells through blood flow delivery and formation of a blood-brain barrier.[9] Furthermore, bidirectional cell trafficking between the brain and blood is needed for immune function, injury, and repair. The cells of the neurovascular units share common mediators and signaling systems. For example, nitric oxide and tissue plasminogen activator participate in both brain parenchymal cells and vascular function.[9,10] In brain diseases, the close interaction between the cells of the neurovascular unit becomes altered, resulting in dysfunction that may lead to ischemic brain injury, neuroinflammation, or neurodegeneration.[8,9,11] The imbalance between substrate delivery and energy utilization that results from insufficient increases in blood flow during brain activity (impaired functional hyperemia) has deleterious effects on brain cell functions, such as protein synthesis, that are sensitive to insufficient blood flow.[9] Therefore, the brain and its vessels need to be considered as a single entity, the fundamental constituent of which is the neurovascular unit.

During early ischemia, vascular and extravascular matrices are degraded simultaneously, with the loss of matrix ligands and integrin counterreceptors bringing instability to the neurovascular unit. Importantly, matrix proteases (including several pro-matrix metalloproteinases, their activation systems, and urokinase and its receptor), which are known to degrade the target extracellular matrix proteins, are generated in microvessels and neurons in concert. During focal cerebral ischemia, endothelial cells and astrocytes initiate both humoral (cytokine, chemokine) and cellular inflammatory responses. Astrocytes are also a major source of proteases that can be released in the immediate vicinity of the endothelial cells. Microglia provide a rich source of proteases and also free radicals that may directly act on the blood vessels or activate the proteases. Studies of immune system responses and immunomodulation of those responses in cerebrovascular disease can be expected to help bridge the artificial dichotomy between focusing only on vascular injury mechanisms and focusing only on parenchymal injury mechanisms in the stroke research field; ie, the studies will tend to cross the blood-brain barrier in both directions.

Vascular disease of the brain (and heart) is characterized by neurovascular remodeling, an adaptive response to chronic (eg, hypertension) or acute (eg, ischemia) insults. Neurovascular remodeling is manifested by changes in the anatomic organization of vessels (inward hypertrophic remodeling, intimal hyperplasia), formation of new vessels (angiogenesis), and functional changes (eg, stiffness, blood-brain barrier disruption, etc). Neurovascular remodeling is important for the outcome of stroke. For example, hypertension-induced vascular stiffness and impaired endothelial function characteristic of vascular ageing exacerbate stroke damage; on the other hand, angiogenesis after stroke could be a component of the "recovery" response. Inflammatory mediators and extracellular matrix reorganization are important components of neurovascular remodeling and could be "targeted" to modulate the process.

It is becoming increasingly clear that vascular beds in different tissues can exhibit markedly different endothelial phenotypes, which in turn can result in differential modulation of the blood clotting and fibrinolytic systems.[12] Genetic models have demonstrated that vascular hemostasis is regulated by organ-specific endothelial pathways that include tissue-type plasminogen activator– and thrombomodulin-dependent function in the vasculature of the heart and brain.[13] The regulation of vascularly mediated inflammatory pathways also operates in an organ-specific manner. Moreover, the shifts in these pathways can contribute to age-related increases in vascular pathophysiology in older persons. Specifically, changes in receptors for tumor necrosis factor-$\alpha$ and brain-derived neurotrophic factor underlie a senescent enhancement in proinflammatory, proapoptotic signal transduc-

tion pathways and loss of cardioprotection in the ageing heart.[14,15] Hemostatic, anticoagulant, and fibrinolytic cascades may play important signaling roles in the cerebral vasculature that can be neuroprotective under some circumstances or lead to neurotoxicity under other circumstances.[16,17] The goal of developing effective interventions/treatments for ischemic stroke necessitates that the act of restoring vessel patency does not subsequently lead to either reocclusion or hemorrhage into the CNS. Thus, it is extremely important to gain a much better understanding of hemostatic control mechanisms in the vasculature of the CNS.

The innate immune system interacts with the hemostatic system.[18] Inflammatory cytokines stimulate thrombosis and leukocyte adhesion mechanisms in endothelial cells and increase the number and thrombogenicity of platelets. Platelets contain high concentrations of proinflammatory CD40 ligand. Endothelial cells preserve the antithrombotic milieu by activating protein C; synthesizing adenosine and prostacyclin (prostaglandin $I_2$ [$PGI_2$]); secreting plasminogen activators; and suppressing nuclear factor (NF)-$\kappa$B activation, von Willebrand factor release, and tissue factor activation.

The understanding of atherogenesis has advanced considerably by a combined approach in which patient data are used to formulate hypotheses that are tested in genetically modified murine models. Innate and adaptive immune mechanisms are important in atherogenesis.[19] Current thinking regarding coronary and carotid artery atherosclerosis emphasizes the vulnerable plaque, in which the fibrous cap is thinned and weakened and therefore, susceptible to rupture. Recent studies in atherosclerotic arteries have identified a dynamic switch (possibly mediated by cyclooxygenase-2) from PGI synthase and PGD synthase that catalyze the synthesis of protective molecules such as $PGI_2$ and $PGD_2$ (15d-$PGJ_2$) to PGE synthase that catalyzes the synthesis of proinflammatory prostaglandins such as $PGE_2$ and predisposes to plaque rupture.[20] The role of activation of surface endothelium in plaque thrombogenesis remains unclear.

Recently, selected transcription factors have been identified that exhibit inflammatory properties and can modulate the initial cascade of transcriptional activation in response to inflammatory stimuli. The prototypic transcription factor for mediating these responses is NF-$\kappa$B.[21] In addition to NF-$\kappa$B, the so-called "immediate-early genes," including c-*jun* and c-*fos*, mediate early inflammatory responses.[22,23] The peroxisome proliferator–activated receptor (PPAR) nuclear receptors are transcription factors expressed in endothelial cells, vascular smooth muscle cells, and monocytic cells. Activation of PPAR-$\alpha$ and PPAR-$\gamma$ is associated with favorable effects in lipid metabolism and insulin sensitivity that are also beneficial with regard to limiting the development of atherosclerosis.[24] Further validation of the importance of transcription factors in regulating inflammation in patients with atherosclerosis has come in a landmark report of genetic studies linking mutations in the gene for the transcription factor MEF2A to a subset of patients with premature coronary heart disease.[25] Furthermore, the ability to identify non–ligand-dependent small molecules that specifically block transcription factors has also been recently demonstrated.[26]

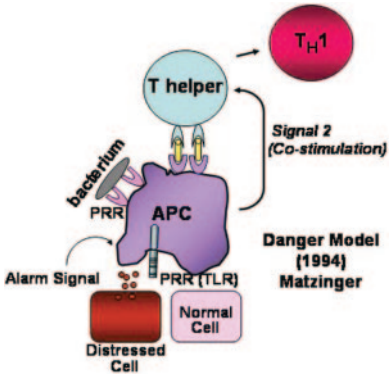


**Figure 2.** Schematic diagram of the danger model. In response to problems that iterations of the self–non-self model for immune system activation had in explaining various findings, a danger model was proposed. According to this model, immune system responses are activated by alarm signals from injured or stressed tissues rather than by recognition of non-self. Cells undergoing necrosis release molecules that signal inflammatory responses. Stressed cells generate signals that include modified lipid or carbohydrate moieties, altered matrix constituents, and proteins not normally found on the outer cell membrane. These signals activate germ line–encoded pattern recognition receptors (PRRs) such as Toll-like receptors (TLRs) that activate quiescent antigen-presenting cells (APCs) to process antigens, upregulate costimulatory signals, and present antigen to T-helper cells that then become $T_H1$ effector cells. Adapted from Matzinger P. The Danger Model: a renewed sense of self. *Science.* 2002;296:301–305.

## Immune System Interactions in the CNS and Heart

As discussed earlier, the local endothelium integrates innate immune system signals from the blood, blood vessel wall, and surrounding parenchymal tissue in a tissue-specific manner and correspondingly changes the properties of its luminal surface.[27] Endothelial surface expression of anti-inflammatory/antithrombotic and proinflammatory/prothrombotic mediators changes, such that the local hemostatic potential within individual vessel segments varies according to local conditions.[28] This provides the opportunity to target immunomodulation to a marker of vessel activation such as the endothelial leukocyte adhesion molecule, E-selectin, to locally suppress hemostatic potential.[29,30] Other preclinical studies suggest that innate and/or adaptive immune responses participate in most aspects of the stroke spectrum, including endothelial activation, development of atherosclerosis, initiation of stroke, progression of ischemic brain damage, ischemic tolerance, and adult neurogenesis.[3,31–40] In addition, small molecules such as statins can induce immune system deviation of T cells, such that their cytokine profiles shift from proinflammatory to immunomodulatory.[41] Immune system activation and participation in organ injury or ischemia may accord with the danger model of immune system regulation proposed by Matzinger[42] (Figure 2).

In animal models of stroke, interfering with the inflammatory response that occurs after ischemic brain injury can improve outcome.[43,44] In the small number of prospective trials so far conducted, strategies that could interfere with polymorphonuclear leukocyte adhesion either have not met with success or have not been fully tested. They point out that

unexplored variables contribute to the complexity of translating such anti-inflammatory approaches to the clinical context. Those studies emphasize that ill-timed delivery, prolonged administration, and inappropriate formulations can undermine the hypothesis test in clinical studies.

Immunomodulation as a strategy to limit ischemic brain injury may have merit. Preclinical evidence demonstrates that immune system tolerance to CNS antigens in animal models is associated with improved outcome from stroke.[30,36,37] Also, an inflammatory stimulus during an ischemic insult predisposes animals toward developing an autoimmune response to brain tissue; animals with an autoimmune response demonstrate worse behavioral outcome than those without an autoimmune response.[40] Infections are a leading cause of death as well as poor outcome in patients suffering from acute CNS injury, such as stroke, traumatic brain injury, or spinal cord injury. Only recently has it been realized that CNS injury induces a disturbance of the normally well-balanced interplay between the immune system and the CNS.[45] Indeed, CNS injury can lead to secondary immunodeficiency (CNS injury–induced immunodepression), and consequently infection.[46] This insight has led to novel therapeutic concepts, which include immunomodulation as well as preventive antibacterial therapy, some of which are currently being tested in clinical trials. However, a suppression of immunity during states in which CNS epitopes are exposed to the adaptive immune system may also have beneficial effects, as it may prevent the development of autoimmunity. Thus, a deeper understanding of the mechanisms and functions of brain–immune system interaction after CNS injury is required for the development of successful therapeutic strategies against CNS injury–induced infections. In concert, these findings suggest that modulation of the immune response after stroke can improve outcome from ischemic brain injury.

There is evidence that both CNS antigens and dendritic cells containing CNS antigen can move from the CNS to secondary lymphoid organs such as the deep cervical lymph nodes and spleen by several routes.[47]Dendritic cells appear able to direct the T cells that they activate in lymph nodes to the organ in which the dendritic cell originated, but the molecular mechanisms are not fully understood. A major question to resolve is whether it is necessary for dendritic cells to migrate from the CNS to secondary lymphoid organs to activate CNS antigen-specific $T_{effector}$ cells and $T_{reg}$ cells or whether microglia, perivascular macrophages, pericytes, and local endothelial cells can activate T cells in situ. Vascular endothelial cells can be considered as active participants in adaptive immune responses. In humans, these cells express both class I and class II major histocompatibility complex molecules and can effectively present antigens to circulating memory T cells, resulting in cytokine production (eg, interleukin-2 and interferon [IFN]-$\gamma$) and proliferation. Human endothelial cells, activated by tumor necrosis factor to express adhesion molecules, are also able to effectively present inflammatory chemokines, such as interferon–inducible protein–10 (IP-10), to effector memory T cells and, in combination with venous levels of shear stress, initiate transendothelial migration. Once activated, T cells and their cytokines, especially IFN-$\gamma$, can contribute to endothelial dysfunction (eg, reduced NO production) and cell death, leading to ischemic tissue injury. There is evidence that accumulations of immunocompetent and antigen-presenting cells in the arterial wall form a vascular-associated lymphoid tissue analogous to mucosa-associated lymphoid tissue.[48] Vascular-associated lymphoid tissue may be related to aneurysm formation, but its other functions remain to be clarified.

Inflammatory mediators may stimulate changes in cellular or adaptive immunity. For example, angiotensin II (Ang II) leads to T-cell recruitment and activation. In particular, Ang II promotes Th1 responses that are associated with an increase in IFN-$\gamma$ expression and a decrease in Th2-mediated interleukin-4 expression.[49] Ang II–mediated Th1 responses are associated with increased plaque vulnerability in apolipoprotein E–deficient mice.[50] In the setting of atherosclerosis, activated T cells were recently shown to express the death receptor, TRAIL, and promote apoptosis of vascular smooth muscle cells.[51] A novel role for selective T cell–specific transcription factor, T-bet, in the development of atherosclerosis has recently been shown. T-bet deficiency reduces the development of atherosclerosis in LDL receptor–deficient mice.[52] T-bet deficiency is associated with a Th2 switch in response to the atherosclerosis-associated heat shock protein-60. The E26 transformation–specific (ETS) transcription factor Ets-1 has similarly been shown to mediate cellular immune responses. Ets-1–deficient mice exhibit abnormalities in T-cell function that are associated with diminished Th1 responses, including a decrease in IFN-$\gamma$ production.[53] In response to Ang II infusion, marked reductions in expression of the inflammatory genes and in vascular inflammation and remodeling occur in Ets-1–deficient mice.[54]

T cells play a role in CNS maintenance and repair. For example, animals immunized with brain antigens and animals that receive lymphocytes from donors sensitized to CNS antigens experience improved outcomes from spinal cord and optic nerve injury.[55] "Protective autoimmunity" in these studies is attributed to a T cell–mediated neuroprotective response and upregulation of glutamate uptake by microglia (Figure 3). There is no doubt that uncontrolled autoimmunity leading to autoimmune disease is destructive. Nevertheless, T cells directed to specific CNS proteins (self-antigens) were recently shown to be essential for brain homeostasis. Experiments have demonstrated that naive rodents experiencing immune deficiency show cognitive deficits. Likewise, immune-deficient rodents show significantly less ability than their normal counterparts to withstand any adverse conditions in the CNS.[56] The unexpected discovery that T cells directed to specific CNS self-antigens are part of the body's own defense mechanism has led to a paradigm shift in the perception of autoimmunity and of the role of proinflammatory cytokines. Whereas uncontrolled autoimmune "proinflammatory" T cells are destructive, they are defensive and protective when properly controlled. Likewise, whereas IFN-$\gamma$, (a proinflammatory cytokine associated with disease conditions) is cytotoxic, it is neuroprotective and restorative at low concentrations; suppressing it altogether, therefore, can have a negative effect. Moreover, autoimmune T cells of the anti-inflammatory phenotype directed to self-antigens contribute to tissue protection. Taken together, T cells have a

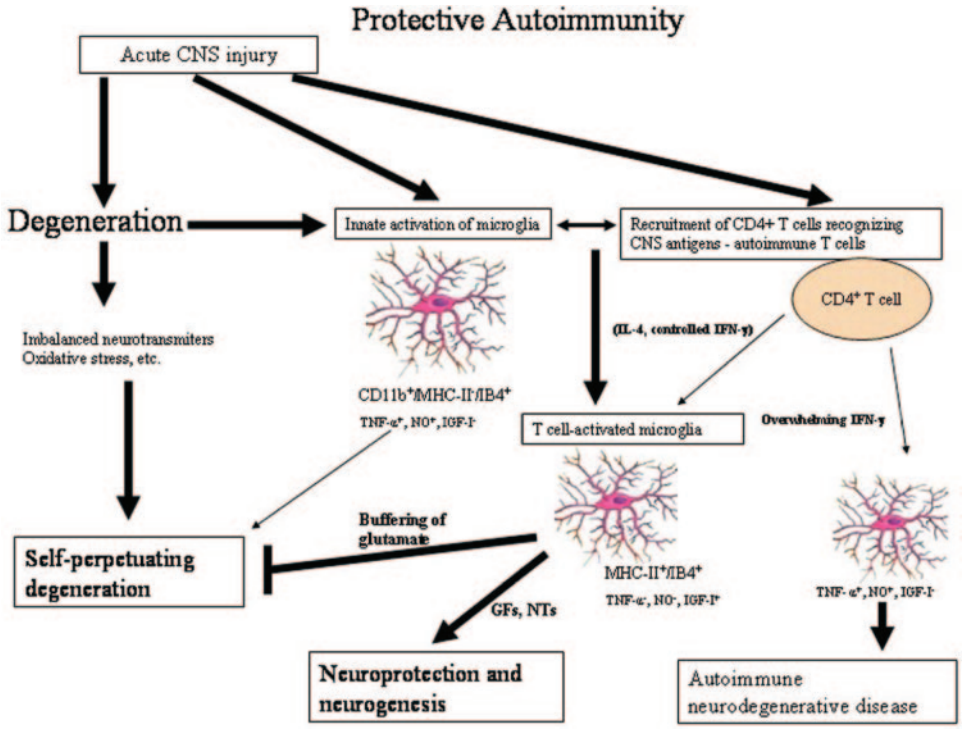


**Figure 3.** Schematic diagram of protective autoimmunity. See text for discussion. GFs indicate growth factors; NTs, neurotrophins. Contributed by Michal Schwartz, PhD, Weizmann Institute of Science, Israel.

role in brain homeostasis, and interference with T-cell function could potentially render the brain vulnerable to viral and other types of infections. This is not meant to imply that drugs based on antibodies that block infiltration of T cells should not be developed; indeed they might have powerful therapeutic effects in acute conditions. If they are to be used for chronic conditions, however, they should be used with caution and with careful monitoring.

## Translation of Immune System Modulation in the Brain and Heart

Strategies for immunomodulation are being considered for translation into clinical trials for the prevention and treatment of stroke and acute coronary syndromes. There is reproducible evidence that both mucosal tolerization and immunization to various endogenous antigens can provide beneficial effects in several different facets of vascular disease (eg, stroke prevention[29] and suppression of atherosclerosis[34,35]). In addition, there are convincing studies showing that small molecules such as Ang II receptor blockers and statins exert beneficial effects in cardiovascular disease, partly as anti-inflammatory and immunomodulatory agents.[57,58] Efforts to move immunomodulatory therapies into clinical trials of atherosclerosis and vascular disease affecting the brain or heart need to address several potential adverse effects by preclinical toxicology and immunotoxicology studies and by appropriate clinical trial design. As shown by the multiorgan failure that occurred in volunteers given TGN1412, a CD28 agonist designed to mitigate autoimmune and immunodeficiency diseases, the first-time administration of novel agents to humans should begin with observation of a single dose in a single individual with careful monitoring[59,60] Recent experience with Natalizumab, a humanized murine $\alpha 4\beta 1$ integrin (leukocyte adhesion molecule) blocker, emphasizes the potential for long-term reduction of resistance to infection by its association with progressive multifocal leukoencephalopathy.[61] Susceptibility to infection and effects on growth and survival of transplanted tumors must be examined in animals subjected to protracted immunomodulation. Studies should examine the duration of immunological tolerance after mucosal tolerization procedures have been terminated and whether a rebound immune system activation can occur at some point after a series of tolerization procedures has stopped. Other questions include whether tolerization procedures can ever sensitize or immunize the host to the presented antigen and whether there are risks to long-term immunosuppression of vessel activation. The available in vivo data present an opportunity for early translation toward human studies for proof of concept and potential efficacy biomarkers. There is no conflict between the need to better understand cellular and molecular mechanisms and a concurrent process of carefully designing human studies.

## Opportunities for Future Research on Immune System Mechanisms and Immunomodulation in Vascular Disease Affecting the Brain and Heart

Studies aimed at enriching our understanding of molecular and cellular mechanisms of innate and adaptive immune system participation in vascular disease of the brain and heart, as well as efforts to identify novel ways to achieve immunomodulation, should be vigorously promoted. Identification of the critical transcriptional factors that regulate vascular inflammation, immune responses, and remodeling not only may

further our basic understanding of the molecular mechanisms of vascular inflammation but also may provide novel therapeutic targets for drug discovery in patients with vascular diseases such as hypertension and atherosclerosis involving the cardiovascular system and CNS. We should determine whether, during ischemia, dendritic cells, macrophages, and brain microglia regulate activation of $T_{effector}$ cells by responding to endogenous ligands from stressed cells in accord with the Matzinger "danger model" of immune system regulation.[42]

Therapeutic approaches to immunomodulation and control of inflammatory processes in cerebrovascular and cardiovascular diseases should be pursued with appropriate preclinical toxicology and immunotoxicology studies. Because preclinical models do not fully reflect the clinical situation, efforts should be made to move innovations in this area into clinical trials as expeditiously as reasonable. In addition to assessment of safety, early human studies potentially lead to proof of concept and identification of efficacy biomarkers. The clinical trials need to be designed to detect safety concerns at an early stage.

Although some important differences in the cell biological properties of the brain and heart exist that affect the evolution of local ischemic injury (eg, phenotypic differences in endothelium and differing biology of parenchymal cells), collaboration between scientists studying cardiovascular and cerebrovascular disease can be expected to accelerate progress in the understanding of vascular biology and vascular disease.

It is essential to develop tissue culture systems that reliably predict responses in the in vivo environment. Once the systems are validated, molecular and cellular aspects of endothelial responses to relevant immunological and proinflammatory mediators should be comprehensively investigated by cell biological, molecular genetic, biochemical, pharmacological, and immunological imaging (eg, bioluminescence for T-cell trafficking, annexin V for apoptosis, etc) approaches. Ideally, the culture systems should be able to examine not only endothelial function but also the interaction between endothelial cells and organ-specific parenchymal cells. Establishing good in vitro models to study a complexity of cellular interactions of the neurovascular unit is important, but translation will be difficult without good in vivo models assessed by multiple outcome measures (including imaging, neurological/behavioral, histological, and molecular outcomes). Study of neurovascular integration should also include integrated modeling that embraces a combination of in vitro and in vivo models and molecular dissection with evaluation of system responses. Similarities and differences in immune system responses during ischemia among preclinical animal models and humans need to be identified to develop these high-fidelity models. Whether endothelial progenitor cells are detectable in brain vessels and whether they play a role in vessel repair should be addressed. Are endothelial progenitor cells a marker of vessel disease? Are they involved in protecting the brain and heart from tissue injury by immunological and proinflammatory mediators?

The temporal relation between Th cell, cytotoxic T lymphocyte, and natural killer cell invasion and injury to the brain and heart should be further investigated. We should learn whether the immune system cells play a role in initiating the injury process or whether they play a more persistent role in parenchymal injury and how they produce injury. The interplay of $T_{reg}$ and $T_{effector}$ cells during ischemia of the brain and heart in relation to the degree and duration of ischemic injury is a central issue about which little is known. The effect of age and sex on this interplay should also be examined. The role of T-cell subsets in the various phases of ischemic injury in the heart and brain should be examined and clarified. The targets of acute and chronic immune system responses in ischemic brain and heart should also be studied. Similarities and differences between immunomodulation in the CNS and heart should be identified. Molecular mechanisms of immune system participation in induction and maintenance of tolerance to ischemia of the brain and heart should be studied.

We need to determine what roles protective autoimmune phenomena might play in prevention of vascular disease. Whether immunomodulation by regulatory T cells and protective autoimmunity are related in any way should be investigated. The conditions under which IFN-$\gamma$ can be good or interleukin-10 and transforming growth factor-$\beta$ can be bad should be clarified. The relevance of protective autoimmunity to myocardial ischemia should be investigated.

## Recommendations

1. Promote interdisciplinary and interinstitutional crosstalk and collaboration among groups with a stake in basic research and the development of therapeutic approaches to ischemic and hemorrhagic stroke and/or acute coronary syndromes.
2. Develop high-fidelity in vitro and in vivo models of human vascular disease responses in the brain and heart.
3. Advance fundamental understanding of immune system (innate and adaptive) interactions with the progression of ischemic injury of both CNS and cardiac model systems; variables include depth of injury, timing of cellular responses, species- and age-dependent immune response pathways, and sex differences in immune responses.
4. Study molecular and cellular mechanisms that regulate innate and adaptive immune system participation in vascular disease of the brain (neurovascular unit) and heart and the effects of immunomodulation on these mechanisms. Exploit existing molecular imaging techniques and develop new tracers and new cell lines to serially image regulatory processes.
5. Determine the impact of immune system modulation on local and regional vascular flow characteristics and neurovascular reactivity in the CNS.
6. Define the effects of immunomodulation on endothelial and other progenitor cell responses in vascular diseases of the brain and heart.
7. Promote clear understanding of interactions between vascular hemostasis and immune system responses in the CNS and heart.
8. Define the role of T-cell subsets in vascular diseases of the brain and heart.

9. Define the molecular mechanisms of immune system participation in the induction and maintenance of tolerance to ischemia of the brain and heart.
10. Examine the effects of protective autoimmunity in cerebrovascular and cardiovascular diseases and determine whether those effects have any relation to other forms of induced tolerance to ischemia.
11. Move promising innovations in immunomodulation and control of inflammatory processes in cerebrovascular and cardiovascular diseases into clinical trials expeditiously.

## Appendix

### Participants

Participants included the following individuals: Kyra J. Becker, MD, University of Washington; Ellen L. Berg, PhD, Bioseek, Inc; Bruce M. Coull, MD, University of Arizona; Thomas J. DeGraba, MD, Uniformed Services University of the Health Sciences; Gregory del Zoppo, MD, Scripps Institute; Ranil DeSilva, MD, NHLBI; Ulrich Dirnagl, MD, Humboldt University Berlin; Jay M. Edelberg, MD, PhD, Weill Medical College, Cornell University; Giora Z. Feuerstein, MD, MSc, Wyeth Research; Jeffrey Friedman, MD, Boehringer Ingelheim Pharmaceuticals, Inc; Stephen Goldman, PhD, NHLBI; Vladimir Hachinski, MD, DSc, London Health Sciences Centre, London, Ontario; Antoine Hakim, MD, PhD, Canadian Stroke Network; John Hallenbeck, MD, NINDS; Constantino Iadecola, MD, Weill Medical College, Cornell University; Thomas Jacobs, PhD, NINDS; Robert J. Lederman, MD, NHLBI; David J. Lefer, PhD, Louisiana State University Health Sciences Center; Brian A. MacVicar, PhD, University of British Columbia; Victor Marder, MD, University of California, Los Angeles; Ruth Maron, PhD, Harvard Medical School; Roland Martin, MD, NINDS; James Morrissey, PhD, University of Illinois at Urbana-Champaign; J. Peter Oettgen, MD, Harvard University; Jordan S. Pober, MD, PhD, Yale University School of Medicine; Michael K. Racke, MD, University of Texas Southwestern Medical Center; Gary A. Rosenberg, MD, University of New Mexico; Lyanne C. Schlichter, PhD, Toronto Western Research Institute, Toronto, Ontario; Michal Schwartz, PhD, Weizmann Institute of Science, Israel; Danica Stanimirovic, MD, PhD, Institute for Biological Sciences, National Research Council Canada, Ottawa; Mary P. Stenzel-Poore, PhD, Oregon Health and Science University; Kenneth K. Wu, MD, PhD, University of Texas Health Science Center at Houston; Scott Zamvil, MD, PhD, University of California, San Francisco

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## Disclosures

None.

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