Stroke-Induced Immunodepression
Experimental Evidence and Clinical Relevance

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Abstract—Stroke affects the normally well-balanced interplay of the 2 supersystems: the nervous and the immune system. Recent research elucidated some of the involved signals and mechanisms and, importantly, was able to demonstrate that brain-immune interactions are highly relevant for functional outcome after stroke. Immunodepression after stroke increases the susceptibility to infection, the most relevant complication in stroke patients. However, immunodepression after stroke may also have beneficial effects, for example, by suppressing autoaggressive responses during lesion-induced exposure of central nervous system-specific antigens to the immune system. Until then, anticipating an important consequence of stroke-induced immunodepression, bacterial infection, preventive antibiotic strategies have been proposed. In mouse experiments, preventive antibiotic treatment dramatically improves mortality and outcome. Results of clinical studies on this issue are contradictory at present, and larger trials are needed to settle the question whether (and which) stroke patients should be preventively treated. Nevertheless, clinical evidence is emerging demonstrating that stroke-induced immunodepression in humans not only exists, but has very similar features to those characterized in rodent experiments. (Stroke. 2007;38[part 2]:770-773.)

Key Words: brain infarction ■ brain ischemia ■ experimental ■ focal ischemia ■ immunology ■ infectious disease ■ inflammation ■ treatment

Recently, major advances have been made in our understanding of the roles of both cellular and humoral inflammation in cerebral ischemia. In particular, it is emerging that innate and adaptive immunity play an important role for the outcome after focal cerebral ischemia (stroke). Stroke has dramatic consequences for the normally well-balanced interplay of the two supersystems, the nervous and the immune systems: brain inflammation and immunodepression. We are only beginning to understand that the consequences of brain-immune interactions after stroke are neither “good” nor “bad,” an insight that might imply that targeted therapeutic intervention (“anti-inflammation”; “immunomodulation,” etc) may prove to be less straightforward as initially hoped for. Here we briefly review stroke-induced inflammation, and focus on stroke-induced immunodepression, a recently described phenomenon that may be the key factor underlying susceptibility to infection after stroke.

Stroke Induces Local Inflammation In Situ
After stroke, leukocytes home toward the lesion, and brain parenchymal cells (microglia, astrocytes, endothelia, even neurons) transform to an inflammatory phenotype. Most of the available literature links stroke induced inflammation to the progression of damage. Thus, inhibition of inflammation would seem to be the most straightforward therapeutic strategy. However, inflammation is a key mechanism of any tissue to cope not only with the invasion of pathogens but also with tissue destruction. Inflammation plays an important part in the clearing of damaged tissue, and in the ensuing processes of angiogenesis, tissue remodeling, and regeneration. This is probably best studied in wound healing, which is severely compromised if inflammation is inhibited.1,2 The potential benefits of inflammation after stroke have received relatively little attention so far, but indirect evidence suggests that certain kinds of inflammatory reactions are neuroprotective and neuroregenerative.3,4 In brief, macrophages and microglia produce a host of trophic cytokines when activated, and macrophages or T-cells exposed to certain central nervous system (CNS)-specific antigens ex vivo partake in tissue repair and recovery after nerve transection and spinal cord injury. However, these benefits may be at least partially offset not only by “bystander toxicity” of inflammation but also by
administration of IFN\(\gamma\) at day 1 after focal cerebral ischemia greatly decreases the bacterial burden. Importantly, the IFN\(\gamma\) deficiency and the occurrence of bacterial infections can be prevented by blocking the sympathetic nervous system, but not the hypothalamo-pituitary-adrenal axis. Furthermore, administration of the \(\beta\)-adrenoreceptor blocker propranolol drastically reduces mortality after middle cerebral artery occlusion.\(^9\) Immunodepression after stroke can be detected within a few hours after induction of ischemia, and lasts for several weeks. These and other studies indicate that a catecholamine-mediated defect in early lymphocyte activation is the key factor in the impaired anti-bacterial immune response after stroke.

**Is Stroke-Induced Pneumonia a Result of Aspiration, Immunodepression, or Both?**

Bacterial pneumonia is the most common cause of death in acute stroke patients. Reduction of bulbar reflexes, drowsiness, dysphagia, and subsequent aspiration are considered to be major contributors to the high incidence of bacterial pneumonia after stroke.\(^10\) In a murine model of aspiration pneumonia, we have evaluated whether stroke-induced immunodepression contributes to the development of pneumonia after stroke. We were able to show that intranasal aspiration of only 200 colony-forming units of *Streptococcus pneumoniae* cause severe pneumonia and bacteremia in mice after transient middle cerebral artery occlusion. In contrast, 200,000 colony-forming units are needed to induce pneumonia of similar severity, but fail to induce bacteremia in sham animals. Aspiration pneumonia in animals after focal cerebral ischemia was prevented by \(\beta\)-adrenoceptor blockade, suggesting that immunodepression by sympathetic hyperactivity is essential for poststroke pneumonia.\(^11\) Therefore, the deleterious combination of stroke-facilitated aspiration and stroke-induced immune deficiency drastically increases the susceptibility to infection.

**What Causes Stroke-Induced Immunodepression?**

Although the phenomenon of stroke-induced immunodepression is well-established, it remains unclear which signals and mechanisms trigger the sympathetic nervous system and the hypothalamic–pituitary axis to downregulate immune responses after brain ischemia. Several lines of clinical and experimental evidence indicate that pro-inflammatory cytokines produced by damaged brain tissue can directly lead to hypothalamic–pituitary axis and CNS activation. Increased levels of cytokines, such as interleukin-1\(\beta\), tumor necrosis factor-\(\alpha\), and interleukin-6, have been measured after stroke in brain parenchyma and cerebrospinal fluid. Because the autonomic system of the CNS is “hard-wired” with secondary lymphoid organs, interruption of these circuits can result in immune dysfunction. Stroke can lead to direct damage of sympathetic CNS structures involved in vegetative neuroimmunomodulation. Further support for the concept of an at least partially neurogenic nature of CIDS comes from studies on the lateralization of the autonomic nervous system in the brain. Lateralization of the structures of the vegetative nervous system might also serve to explain some localization-

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**Figure 1.** Experimental stroke induces immunodepression via the hypothalamic pituitary axis (HPA) and the sympathetic nervous system (SNS). SNS plays a key role because \(\beta\)-receptor blockade exerts comprehensive effects on stroke induced alterations of the immune system and outcome (box). Blockade of the HPA axis with the glucocorticoid receptor antagonist RU486 only improves lymphopenia and monocyte deactivation.\(^9\)

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scar formation, which in peripheral tissues is key to wound closure, but in the brain is a major impediment of regeneration and plasticity.\(^3\) A recent example for the dual nature and complexities of ischemia-induced inflammation has been the uncovering of a regenerative role for matrix metalloproteinases; of which, infection is the most frequent (23% to 65%). Infection also is the most relevant complication during rehabilitation, and infection is the number one cause of death in stroke after day 1. It has become clear that CNS injury is an independent risk factor, which specifically and significantly increases susceptibility to infection. Only recently it has been realized that CNS injury, including stroke, induces immunodepression, and that this is the mechanism by which stroke downregulates peripheral immunity (Figure 1). In brief, within 3 days after focal cerebral ischemia, mice develop spontaneous pneumonia and septicaemia. Focal cerebral ischemia induces an extensive apoptotic loss of lymphocytes and a shift from T helper cell (Th)1 to Th2 cytokine production. Secondary lymphatic organs like spleen and thymus atrophy after focal cerebral ischemia.\(^8\) In parallel to the changes in the adaptive immune system, monocyte counts and function are compromised as well. Adoptive transfer of T and natural killer cells from wild-type mice, but not from interferon (IFN)-\(\gamma\)-deficient mice, or...
dependent effects of neural-immune interactions subsequent to stroke. Therefore, multiple causes—“unspecific” stress response, CNS injury-specific neurogenic signaling, and local CNS inflammation—have to be considered as triggers of systemic immunodepression.

**Does Immunodepression After Stroke Protect the Brain?**

At least in animal experiments it has been demonstrated that stroke-induced immunodepression underlies the increased rate of infection and unfavorable outcome after focal cerebral ischemia. In principal, immunomodulatory therapy (eg, IFNγ, granulocyte colony-stimulating factor, thymopentin) to reverse the immunodepression after stroke might seem to be a straightforward strategy to reduce the negative consequences of CIDS. However, too little is known about CIDS to rule out that it is an adaptive response, which is CNS protective at the price of an increased risk of infection. For example, it has been demonstrated that T lymphocytes and IFNγ contribute to the inflammatory and thrombogenic brain injury that results from cerebral ischemia. Therefore, depletion of circulating T-cell populations and suppression of IFNγ expression, which is the key mechanisms of stroke-induced immunodepression, might counteract the inflammatory brain after stroke. Indeed, recent evidence from our laboratory indicates that stroke-induced immunodepression suppresses autoaggressive Th1 responses. The blood–brain barrier disruption and tissue destruction after stroke expose brain epitopes that are normally “invisible” to the immune system. This may induce autoregulatory, anti-inflammatory, and potentially regenerative T-cell responses, or rather prime the immune system to attack the CNS that may be a particular problem in the context of systemic inflammatory response or a second ischemic event.

In a mouse model of focal cerebral ischemia we detected increased numbers of myelin oligodendrocyte glycoprotein (MOG) specific T-cells in the spleen, and an increased influx of MOG-specific T-cells into the brain. After experimental stroke, mice transgenic for a MOG-specific T-cell receptor developed clinical signs of mild experimental autoimmune encephalitis, which is the animal model of multiple sclerosis. Transfer of T-cells of MOG–T-cell receptor transgenic mice into the brain. After experimental stroke, mice transgenic for a MOG-specific T-cell receptor developed clinical signs of mild experimental autoimmune encephalitis, which is the animal model of multiple sclerosis. Transfer of T-cells of MOG–T-cell receptor transgenic mice induced experimental autoimmune encephalitis in naive mice. Adoptive transfer of wild-type splenocytes (which were harvested after focal cerebral ischemia) before induction of focal cerebral ischemia worsens outcome, whereas transfer of naive splenocytes has no effect (manuscript in preparation). These findings indicate that stroke triggers an autoreactive phenotype, against which stroke-induced immunodepression may partially protect (Figure 2). Clearly, further research is needed before immunomodulation should be considered in the treatment of human stroke.

**Cell Therapy and the Immune System in Stroke: A Hypothesis**

Recently, a number of groups have demonstrated that treatment with various types of stem or progenitor cells can favorably improve outcome after experimental stroke. For example, hematopoietic or mesenchymal stem cells, even when given via a systemic route, seem to foster recovery. Most of the research in this field has concentrated on the homing of these cells toward the ischemic lesion, and their putative differentiation to neuronal phenotypes. However, autologous, heterologous, or xenologous transplantation have multiple and complex effects on the immune system. These may be the result of the presentation of foreign epitopes (even in autologous transplantation, because cells are often cultivated in the presence of sera containing xenologous growth factors), the apoptotic death of a substantial number of transplanted cells, or endogenous production of immunomodulators by stem cells (eg, indoleamine).

Because infection, particularly pneumonia, appears to be the clinically most significant consequence of stroke-induced immunodepression, and because immunomodulation at present is not a viable therapeutic option, preventive antibiotic therapy has been proposed as a measure to improve outcome after stroke. In an animal model of stroke, prophylaxis against bacterial infection dramatically improved mortality and reduced infarct sizes. In a recently published randomized clinical trial (ESPIAS trial), the fluoroquinolone levofloxacin did not prevent infections in patients with acute stroke. In the PANTHERIS trial (http://controlled-trials.com/lsrctn/ trial/PANTHERIS/0/74386719.html), we are currently testing whether another fluoroquinolone, moxifloxacin, prevents infections in patients with severe stroke. PANTHERIS is a double-blind, randomized, controlled phase Ib multicenter trial in which 80 patients with severe stroke (National Institutes of Health Stroke Scale >11) of the middle cerebral artery territory were randomized to receive moxifloxacin (400 mg intravenous daily) or placebo for 5 days starting within 36 hours after stroke onset. Seventy-nine patients were analyzed in the intention-to-treat population and 66 in the per-protocol population. In the intention-to-treat analysis, patients receiving moxifloxacin showed a strong tendency toward a lower infection rate compared with patients receiving placebo. Per-protocol analysis revealed a significant
reduction of the cumulative infection rate (mainly pneumonia) in the moxifloxacin compared with the placebo group.

Patients with infectious complications showed strong signs of systemic immunodepression (monocyte deactivation, T-cell dysfunction, etc) before clinical onset of infection. Our data (manuscript in preparation) indicate that preventive antibacterial therapy with moxifloxacin prevents infectious complications in patients with severe stroke. In addition, inhibition of cellular immune function after acute stroke appears to increase the risk of infectious complications. The conflicting results of the ESPIAS and PANTHERIS may be explained by patient selection, differences in antibiotics, dosages, and duration of treatment. Larger trials are needed to characterize stroke-induced immunodepression in humans and to answer the questions whether immunodepression and consequently infection have a clinical impact that can be positively affected by preventive anti-infective therapy.

Conclusions
There is emerging experimental and clinical evidence that brain–immune interactions play an important role for outcome after stroke. Because these interactions may have protective, destructive, or regenerative effects in the brain, and also impact the organism as a whole, development of therapeutic strategies is not straightforward. Further research is needed to understand the signals and mechanisms by which the adaptive and innate immune systems react to brain tissue damage, and to unravel the consequences that this has for the patient.

Sources of Funding
This study was supported by the Hermann and Lilly Schilling Foundation and the Helmholtz-Gemeinschaft.

Disclosures
U.D., C.M., K.P., and A.M. are inventors on a patent application (owner: Charité–Humboldt University) in which stroke-induced immunodepression is claimed.

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Stroke. 2007;38:770-773
doii: 10.1161/01.STR.0000251441.89665.bc

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2007 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/38/2/770

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