Inflammation and the Emerging Role of the Toll-Like Receptor System in Acute Brain Ischemia

Brenda J. Marsh, BS; Susan L. Stevens, BS; Brian Hunter, BS; Mary P. Stenzel-Poore, PhD

Background and Purpose—Systemic administration of cytosine-guanine (CpG) oligodeoxynucleotides provides neuroprotection against subsequent cerebral ischemic injury. We examined the genomic response of leukocytes and brain cells after ischemia in the context of CpG preconditioning.

Methods—RNA was isolated from circulating leukocytes and ischemic cortex 3 and 24 hours after middle cerebral artery occlusion after CpG or saline pretreatment and subjected to microarray analysis. Genes uniquely upregulated in CpG-pretreated mice were examined for overrepresented transcriptional regulatory elements.

Results—CpG preconditioning induced a novel response to middle cerebral artery occlusion within circulating leukocytes that was dominated by natural killer cell-associated genes and the GATA-3 transcriptional regulatory element. Preconditioning also caused a novel brain response to stroke that was dominated by Type I interferon, interferon-associated genes, and transcriptional regulatory elements.

Conclusion—CpG preconditioning invokes novel leukocyte and brain responses to stroke. In this, CpG may be a unique preconditioning agent, coordinating peripheral and brain responses to protect against ischemic injury. (Stroke. 2009; 40[suppl 1]:S34-S37.)

Key Words: inflammation ■ hypoxia-ischemia ■ stroke

Bacterial nonmethylated cytosine-guanine (CpG) oligodeoxynucleotide motifs alert the body to infection through activation of Toll-like receptor 9 (TLR9). In mice, TLR9 is expressed by B cells, plasmacytoid dendritic cells, macrophages, microglia, and astrocytes. TLR9-activated cells produce the proinflammatory cytokines tumor necrosis factor α, interferon α, and interleukin (IL)-12. These cytokines further activate monocytes, neutrophils, natural killer cells (NK cells), and T cells, facilitating a coordinated inflammatory response to pathogen invasion.

Pre-exposure to CpG reprograms the cellular response to subsequent TLR stimulation. Unlike naïve cells, macrophages pretreated with CpG do not generate tumor necrosis factor α in response to TLR4 stimulation, instead generating IFNβ. Furthermore, systemic administration of CpG increases resistance to polymicrobial sepsis. Hence, pre-exposure to CpG redirects both cellular and systemic responses to subsequent TLR stimulation.

Systemic administration of CpG also protects the brain from subsequent ischemic damage. Such “CpG preconditioning” is time- and dose-dependent and requires tumor necrosis factor α. The precise mechanisms responsible for CpG preconditioning are not well understood but likely involve both direct cellular processes and coordinated systemic responses that minimize ischemic damage.

We hypothesize that CpG preconditioning reprograms the response of the brain and the peripheral immune system to subsequent stroke. We provide evidence for such reprogramming and consider its potential neuroprotective consequences.

Materials and Methods

Mice
C57Bl/6 mice (male, 8 to 10 weeks) were obtained from Jackson Laboratories (West Sacramento, Calif). All mice were housed in a facility approved by the Association for Assessment and Accreditation of Laboratory Animal Care International. The animal protocols met National Institutes of Health guidelines with the approval of the Oregon Health and Science University Institutional Animal Care and Use Committee.

Drug Treatments
CpG oligodeoxynucleotide 1826 (20 to 40 μg; 200 μL; Invivogen; San Diego, Calif) or saline was administered by intraperitoneal injection 72 hours before MCAO.

Surgery
Mice were anesthetized with isoflurane and ischemia was induced by MCAO as published previously. Cerebral blood flow was monitored with laser Doppler flowmetry and temperature was maintained at 37°C. After surgery, mice were kept for 24 hours on a heating pad with access to soft food and water.

RNA Isolation
Mice were anesthetized and blood was obtained through retro-orbital puncture. Animals were perfused with saline and, under RNase-free conditions.
find 223 genes to be differentially regulated in CpG-preconditioned animals relative to saline. We next identified overrepresented TREs in the genes uniquely upregulated in CpG-preconditioned animals. In those genes for which upstream sequence was available for analysis (136), we identified 4 overrepresented TREs with an adjusted probability value <0.1. Notably, each TRE was Type I IFN-associated (IRF, IRF8, ISRE, 3-hydroxy-3-methylglutaryl-1-Y). A network depiction of interactions between the identified TREs and the genes in the CpG-preconditioned cluster is displayed in Figure 2. The IFN-associated TREs are linked to 64% of the genes within this upregulated cluster (88 of 136). A literature review identified 12 of the upregulated genes as Type I IFN-associated: Oas1a, MHC class I (H2-D1, H2-K1, H2-L, H2-Q6), Ifi203, Ifi204, Ifi205, Ifi206, Ifi207, Isg201, Lmp7, and Psmb9). Thus, an altered signaling cascade involving Type I IFNs exists in the brain after stroke in CpG-preconditioned mice.

Discussion

We report the first evidence that CpG preconditioning alters the genomic response to stroke in circulating leukocytes and in the brain. We demonstrate a distinct pattern of NK cell activity in the blood and a clear enhancement of Type I IFN signaling in the brain after MCAO. This pattern of upregulated gene expression underscores a unique response to brain ischemia that may actively protect the brain from injury.

CpG preconditioning induced a novel genomic response in blood leukocytes that was evident 24 hours after stroke. Of those genes uniquely upregulated in preconditioned animals, a majority contained the GATA-3 TRE, which is
required for NK cell development. Additionally, 24 of the upregulated genes were NK cell-related and serum IL-12 was increased at this time, supporting the notion of increased NK cell activity.

This unique systemic response may play a role in neuroprotection because NK cells have been shown to limit damaging neuroinflammation in experimental autoimmune encephalomyelitis. Interestingly, administration of CpG oligodeoxynucleotides before experimental autoimmune encephalomyelitis induction also reduces disease severity. Furthermore, treatment with CpG inhibits inflammatory arthritis in an IL-12- and NK cell-dependent manner. Hence, CpG may also initiate a protective NK cell response to cerebral ischemia.

CpG preconditioning induced a novel genomic response in the brain that was evident 24 hours after stroke. Of those genes uniquely upregulated in preconditioned animals, a majority contained one or more Type I IFN-associated TREs. Moreover, 12 of the upregulated genes were associated with Type I IFN signaling, further supporting a role for IFNs after stroke in preconditioned animals.

Microglial, astrocytes, endothelial cells, and neurons all produce the Type I IFN IFNβ. IFNβ can stabilize the blood–brain barrier, suppress inflammatory cytokines, and protect neurons from cytotoxic microglia. Systemic administration of IFNβ reduces infarct damage in several models of ischemic stroke. Hence, an increase in Type I IFN signaling within the brain has the potential to be neuroprotective.

Our data support a shift toward Type I IFN signaling after stroke in CpG-pretreated animals. How might this shift occur? Mice lacking TLR4 incur significantly less damage from MCAO than wild-type controls, indicating a damaging role for this receptor in ischemic injury. Pretreatment with CpG shifts the cellular response to subsequent stimulation of TLR4, leading to a suppression of tumor necrosis factor α and an increase in IFNβ. A similar series of events might occur after CpG preconditioning wherein pretreatment with CpG shifts the response of TLR4 to subsequent stimulation with endogenous ligands such as HSP60, released after stroke, and potentially leads to suppressed cytotoxic tumor necrosis factor α and enhanced neuroprotective IFNβ.

Alternatively, the systemic increase in NK cell activity may explain the Type I IFN shift in the brain. NK cells promote the release of IFNα from plasmacytoid dendritic cells in a CpG- or IL-12-dependent manner. Hence, pretreatment with CpG may activate dendritic cells to produce IL-12, thereby activating NK cells which, in turn, induce plasmacytoid dendritic cells to produce IFNα.

We have shown that CpG preconditioning reprograms the peripheral and central responses to stroke. The appearance of a novel NK cell and IFN genomic “fingerprints” after ischemia indicates that CpG preconditioning fundamentally changes the body’s inflammatory response to stroke. This is consistent with our previous reports of reprogramming in which ischemic and lipopolysaccharide preconditioning induce novel, protective sets of gene transcripts after stroke.

CpG appears to be a unique preconditioning agent, coordinating both systemic and central immune components to actively protect the body from ischemic injury.

Source of Funding
Microarray assays were performed in the Affymetrix Microarray Core of the OHSU Gene Microarray Shared Resource. This work was supported by National Institutes of Health grant R01 NS050567 (M.P.S.-P.).

Disclosures
OHSU, Dr Stenzel-Poore, and Ms Stevens have a financial interest in Neuroprotect, Inc. This potential conflict of interest has been reviewed and managed by OHSU and the Integrity Program Oversight Council. There are no other conflicts to report.

References
Inflammation and the Emerging Role of the Toll-Like Receptor System in Acute Brain Ischemia

Brenda J. Marsh, Susan L. Stevens, Brian Hunter and Mary P. Stenzel-Poore

Stroke. 2009;40:S34-S37; originally published online December 8, 2008; doi: 10.1161/STROKEAHA.108.534917

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 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/40/3_suppl_1/S34

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Stroke can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Stroke is online at:
http://stroke.ahajournals.org//subscriptions/