

## THE TOLL RECEPTOR FAMILY: FROM MICROBIAL RECOGNITION TO SEIZURES

### Toll-like Receptor 4 on Nonhematopoietic Cells Sustains CNS Inflammation during Endotoxemia, Independent of Systemic Cytokines

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Inflammatory agonists such as lipopolysaccharide (LPS) induce robust systemic as well as CNS responses after peripheral administration. Responses in the innate immune system require triggering of toll-like receptor 4 (TLR4), but the origin of CNS sequelae has been controversial. We demonstrate expression of TLR4 transcripts in mouse brain in the meninges, ventricular ependyma, circumventricular organs, along the vasculature, and in parenchymal microglia. The contribution of TLR4 expressed in CNS resident versus hematopoietic cells to the development of CNS inflammation was examined using chimeric mice. Reciprocal bone marrow chimeras between wild-type and TLR4 mutant mice show that TLR4 on CNS resident cells is critically required for sustained inflammation in the brain

after systemic LPS administration. Hematopoietic TLR4 alone supported the systemic release of acute phase cytokines, but transcription of proinflammatory genes in the CNS was reduced in duration. In contrast, TLR4 function in radiation-resistant cells was sufficient for inflammatory progression in the brains of chimeric mice, despite the striking absence of cytokine elevations in serum. Surprisingly, a temporal rise in serum corticosterone was also dependent on TLR4 signaling in nonhematopoietic cells. Our findings demonstrate a requirement for TLR4 function in CNS-resident cells, independent of systemic cytokine effects, for sustained CNS-specific inflammation and corticosterone rise during endotoxemia.

### Innate Immune Reaction in Response to Seizures: Implications for the Neuropathology Associated with Epilepsy

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In the present study, the expression of proinflammatory transcripts was assessed across the brain of mice having undertaken pilocarpine-induced seizures. Pilocarpine-induced marked neurodegeneration and demyelination in multiple regions of the forebrain. The pattern of genes encoding toll-like receptor type 2 (TLR2) and  $I\kappa B\alpha$  (index of NF- $\kappa$ B activation) was associated with the neurodegenerating areas, but this was not the case for the mRNA encoding other inflammatory proteins. Scattered tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )-expressing cells were found across brain, whereas the signals for monocyte-chemoattractant protein-1 and microsomal prostaglandin synthase were robust in thalamus and cerebral cortex and weak in the hippocampus and amygdala.

TLR2 and TNF- $\alpha$  transcripts were expressed mainly in microglia/macrophages. Cyclooxygenase-2 was induced specifically in the hippocampus and piriform cortex. A low increase in interleukin-12 mRNA was detected in the brain, but the signal for interferon- $\gamma$  (IFN- $\gamma$ ) remained undetectable. Although proinflammatory markers were induced in a different manner across the CNS, their patterns were not characteristic of those caused by other inflammatory challenges, such as endotoxin. These data suggest a different mechanism involved in regulating the innate immune reaction in response to seizures and could have direct implications for the neuropathology associated with epilepsy.

## COMMENTARY

The CNS is considered an immuno-privileged site because of the presence of a blood–brain barrier, graft acceptance, lack of conventional lymphatic drainage, and an apparently low level of monocyte and lymphocyte trafficking. However, it may be more appropriate to define the CNS as an immunologically specialized site (1), since it is becoming clear that immune and inflammatory reactions do occur in the CNS, where they appear to originate either intrinsically (thus, constituting part of the innate immunity) or in the peripheral tissues, imported by competent immune cells into the CNS (as part of the acquired immunity). The transition between innate and adaptive immunity is mediated by a large variety of inflammatory mediators, among which cytokines and toll-like receptors (TLRs) play a key role (2).

TLRs are transmembrane proteins that are evolutionarily conserved between insects and humans. The first member of the toll family was identified in *Drosophila* as an essential molecule for embryonic patterning and subsequently shown to be a key element in antifungal immunity. A homologous family of TLRs exist in mammals: 10 members of this family have been described and their genes are dispersed throughout the genome. TLRs have a crucial role in mammalian immune recognition because they enable the innate immune system to detect the presence of infectious agents and to initiate a set of endogenous signals (e.g., inflammatory cytokines and chemokines) that, in turn, recruit and activate antigen-specific lymphocytes and related adaptive immune reactions. TLRs are expressed by monocytes and other myeloid cells as well as by vascular endothelial cells, adipocytes, cardiac myocytes, and intestinal epithelial cells. Recently, TLRs also have been described in various brain compartments.

The ligands for most TLRs are currently unknown, although toll-like receptor 2 (TLR2) and toll-like receptor 4 (TLR4) have been implicated in the recognition of various microbial products, including components of the gram-positive or gram-negative (for TLR2 or TLR4, respectively) bacterial wall, zymosan, bacterial DNA, and bacterial lipoproteins and peptidoglycans. Activation of TLRs also may be involved in protecting the host from viruses and fungi. TLRs can recognize structures called pathogen-associated molecular patterns, which display specific characteristics (2). First, pathogen-associated molecular patterns often represent a molecular signature of a class of pathogens, thus allowing the body to recognize the type of infecting pathogen. Second, they are not produced by host cells and, therefore, can allow the immune system to distinguish self from nonself molecules. However, increasing evidence suggests that endogenous ligands can stimulate TLRs and trigger an immune response in the absence of infection. For exam-

ple, signals from damaged cells undergoing necrosis can initiate changes in the lipid or carbohydrate moieties expressed on the cell surface or can lead to expression of proteins not normally found in tissue. In this respect, fibronectin fragments (3) and hsp60 (4) as well as components of the degradation of the extracellular matrix produced during an inflammatory response, appear to activate TLRs.

Lipopolysaccharide (LPS) is a glycolipid component of the outer membranes of gram-negative bacteria, which can cause endotoxic shock in gram-negative septicemia. LPS binding to a serum protein, called LPS-binding protein, initiates signaling through the membrane-bound or soluble costimulatory receptor, CD14. Because the TLRs share sequence similarity with the interleukin-1 receptor family in their cytoplasmic region, it is not unexpected that downstream signaling events share common components.

The recent work by Chakravarty and Herkenham demonstrates the expression of TLR4 in the CNS by measuring its transcript in mouse brain in physiological conditions. They demonstrate the presence of TLR4 in the meninges, ventricular ependyma, circumventricular organs, microvasculature, and in parenchymal microglia. Colocalization experiments with specific cell markers showed lack of expression of TLRs in neurons and astrocytes. This cellular analysis along with previous studies showing the expression of TLR2 and TLR4 in the CNS (5,6) strongly suggest that the brain may sustain an inflammatory response to infection independently from the peripheral immune system. The work by Chakravarty and Herkenham provides a convincing demonstration of this possibility. These authors used bone marrow chimeras between wild-type and TLR4 mutant mice, which are hyporesponsive to LPS, to show that TLR4 on CNS resident cells is “critically required for sustained inflammation in the brain after systemic LPS administration,” and is independent of systemic cytokine effects. The strategy used in this study allowed the investigators to restrict LPS responsiveness to either the hematopoietic or nonhematopoietic derived tissue (e.g., brain) by exposing recipient mice to a single-dose radiation of 9 grays. Bone marrow from donor mice (either wild-type or hyporesponsive to TLR4) was then injected in irradiated recipient mice (either wild-type or hyporesponsive to TLR4), and 12 weeks after reconstitution, the four different combinations of chimeric mice were injected with LPS. The results of this study show that: (i) hematopoietic TLR4 function is required to elicit peripheral inflammation after systemic LPS administration, and (ii) TLR4 function in nonhematopoietic brain cells *alone* is sufficient for initiation and progression of inflammatory reactions in the CNS. In addition, corticosterone elevation in serum after LPS exposure, resulting from increased activity of hypothalamic–pituitary axis by endotoxemia, appears also to be dependent on brain resident TLR4-bearing cells.

The notion that the brain may initiate and self-sustain a long-lasting inflammatory response, even after a systemic challenge, is a novel concept that is increasingly supported by experimental evidence and one that can be extended to seizures models. In this regard, the work by Turrin and Rivest shows that seizures, induced in mice by systemic pilocarpine application, upregulate the expression of TLR2 and various proinflammatory mediators in the brain. TLR2 was overexpressed, mainly by microglia and macrophages, lasting for at least 72 hours after the onset of seizures in areas of brain damage. Notable differences exist with the pattern of TLR2 brought about by endotoxemia. LPS induces a first wave of TLR2 upregulation in the microvasculature, leptomeninges, choroid plexus, and circumventricular organs. In these districts upregulation is transient lasting as long as 6–12 hours. Microglia are recruited, with a delay of several hours after LPS exposure, and express TLR2 for longer time.

The activation of TLR2 is accompanied by the upregulation of NF $\kappa$ B signaling, which leads to the transcriptional activation of genes of various inflammatory mediators, including cytokine, chemokine, and cyclooxygenase-2. Accordingly, activation of TLRs in microglia has been demonstrated to result in local production of various inflammatory mediators (7). Moreover, TLR4 appears to play a pivotal role in the migration of leukocytes to inflamed tissue (8), thus representing a possible crucial link between innate inflammatory reactions and subsequent recruitment of adaptive immunity. Although it is clear that the TLR family plays a central role in innate immune recognition, an obvious, still unanswered question relates to the possible endogenous ligands for these receptors when their upregulation is determined by seizures.

The available evidence indicates that the innate immune response that takes place in the CNS during infection is unlikely to be detrimental to brain tissue, since it is rapid and reversible. However, sustained microglia reactivity, possibly mediated by TLR activation from endogenous ligands, which are produced during seizures or tissue injury, can overproduce inflammatory molecules and alter blood–brain barrier permeability (1,9,10). In this context, the chronic production of innate immune proteins and the presence of cells of the adaptive immune system

may represent essential features of tissue hyperexcitability and of neurodegeneration. Thus, various proinflammatory mediators (and LPS administration) have been shown to lower the threshold for seizure induction in rodents, prolong epileptic-like activity, and increase neuron susceptibility to excitotoxic damage (9,10). In sum, the emerging evidence indicates that TLRs represent “sensors” of the innate immune system, which because of their position at the intersection of several critical immune pathways, may exert a key influence on the balance between health and disease in the brain.

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