Published in final edited form as:

Curr Top Microbiol Immunol. 2009 ; 336: 41–61. doi:10.1007/978-3-642-00549-7_3.

Chapter 3. Toll-like receptors (TLRs) in Brain Abscess

Nilufer Esen¹ and Tammy Kielian^{2,*}

¹Department of Neurology, University of Michigan Medical School, Ann Arbor, MI

²Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE

Abstract

Brain abscesses arise from a localized parenchymal infection, typically elicited by pyogenic bacteria such as *S. aureus*. Despite improvements in detection and treatment strategies, brain abscesses continue to occur, with an increased prevalence in developing countries and immune compromised patients. Adding to the seriousness of these infections is the recent emergence of antibiotic-resistant strains of bacteria, which are becoming more commonly associated with brain abscesses. Recent studies using a mouse experimental brain abscess model have revealed a complex role for Toll-like receptors (TLRs) in disease pathogenesis. Interestingly, TLR2 has limited impact on the innate immune response during the acute stage of brain abscess formation induced by *S. aureus* but influences adaptive immunity. In contrast, mice deficient in MyD88, a central adaptor molecule for the majority of TLRs in addition to the IL-1R and IL-18R, demonstrate severe defects in innate immunity coupled with exaggerated tissue destruction. It is envisioned that understanding the roles for TLRs in both resident CNS glia as well as infiltrating immune cells will provide insights as to how the immune response to bacterial infection can be tailored to achieve effective pathogen destruction without inducing excessive bystander damage of surrounding non-infected brain parenchyma. A discussion of recent findings in this field is presented along with outstanding questions and the concept of a pathogen-necrosis-autoantigen triad for the amplification for TLR signaling is introduced.

3.1 Etiopathogenesis of brain abscess

Brain abscesses develop in response to localized infections in the parenchyma, leading to the formation of an encapsulated suppurative lesion. The main etiologic agents of brain abscess are the *streptococcal* strains and *Staphylococcus aureus* (*S. aureus*), although a variety of organisms including Gram-negative bacteria and fungi have also been reported (Mathisen and Johnson 1997; Townsend and Scheld 1998; Bhand 2004; Prasad et al. 2006; Njambo et al. 2007; Kantarcioglu et al. 2008). Brain abscesses can form by bacterial perforation of the thin bony structures separating the brain from neighboring sites of chronic infection occurring in the paranasal sinuses, middle ear, or upper molars. Other routes include seeding of the brain with bacterial emboli originating from systemic sites of infection (i.e. endocarditis or septicemia) (Mathisen and Johnson 1997) where the frontal and temporal lobes are most commonly affected (McClelland et al. 1978; Carpenter et al. 2007), penetrating trauma to the head, or following neurosurgery (Tenney 1986; Schliamser et al.

*Corresponding Author.

1988). In addition, predisposing conditions such as congenital heart disease, hereditary hemorrhagic telangiectasia, or hemorrhagic or ischemic strokes have been associated with an increased incidence of brain abscess (Chen et al. 1995; Kumar 2000; Emmez et al. 2007; Sell et al. 2008). From a therapeutic standpoint, the diagnosis of brain abscess can often be delayed since patients typically present with non-descript clinical symptoms including fever, poorly localized headache, and nausea. A differential diagnosis that includes brain abscess is often not made until patients begin to demonstrate neurological signs such as seizures, hemiparesis, and/or cognitive decline. By this time, a considerable amount of time has elapsed for the infection to disseminate and destroy a large portion of brain parenchyma. Therefore, if the diagnosis is significantly delayed and/or proper treatment is not administered, brain abscesses represent a significant medical problem, with mortality rates ranging from 30–80%. A large percentage of patients who recover from brain abscesses experience long-term complications including seizures, loss of mental acuity, and focal neurological defects (Mathisen and Johnson 1997). The incidence of brain abscess is one in every 10,000 hospital admissions in the United States (Mathisen and Johnson 1997) and 1.3 per 100,000 persons world-wide (Nicolosi et al. 1991). For unknown reasons, the prevalence of brain abscess in males is 2–3 times more frequent compared to females (McClelland et al. 1978; Nicolosi et al. 1991; Kao et al. 2003). In underdeveloped countries, the disease incidence is higher and the source of infection typically originates from the ear or teeth due to poor sanitary conditions. In developed countries, improvements in health quality, advances in diagnostic tools, and treatment modalities improve infection outcome; however, the recent emergence of multi-drug resistant microorganisms presents a new therapeutic challenge (Krcmery and Fedor-Freybergh 2007). Indeed, several case reports have described the isolation of methacillin-resistant *S. aureus* from brain abscesses, revealing the increased incidence of antibiotic-resistant bacteria in recent years (Smith et al. 1997; Khan et al. 2000; Roche et al. 2003; Jones et al. 2004). Moreover, immunosuppressive therapies employed for the treatment of tumors or following organ/bone marrow transplantation are associated with an increased prevalence of brain abscess formation often caused by commensal microorganisms (Calfee and Wispelwey 2000; Baddley et al. 2002; Hristea et al. 2007). Collectively, these issues highlight the need for further studies to understand host-pathogen interactions during brain abscess development.

3.2 Experimental brain abscess model

Despite the continued incidence of brain abscesses, as evident by the vast number of case reports in the literature, experimental models examining basic science aspects of this disease are limited. Although pioneering data were provided by experimental models of brain abscess in the rabbit (Falconer 1943), dog (Britt et al. 1981), and rat (Winn et al. 1979; Flaris and Hickey 1992), studies describing the nature of the ensuing CNS and peripheral immune responses were rare or not detailed. Our laboratory has developed an experimental brain abscess model to elucidate the importance of host immune factors in disease pathogenesis (Kielian et al. 2001a; Kielian et al. 2001b; Baldwin and Kielian 2004; Kielian et al. 2004b) which was adapted from a previously published model in the rat (Flaris and Hickey 1992) and utilizes *S. aureus*, one of the main etiologic agents of brain abscess in humans (Roche et al. 2003; Jones et al. 2004) (Mathisen and Johnson 1997; Townsend and Scheld 1998). The

mouse brain abscess model accurately reflects the course of disease progression in humans, providing an excellent model system to study immunological pathways influencing abscess pathogenesis and the effects of therapeutic agents on disease outcome.

At the histological level, brain abscess is typified by a sequential series of pathological changes that have been elucidated using the experimental rodent models described in detail below (Flaris and Hickey 1992; Kielian and Hickey 2000; Kielian et al. 2001a; Kielian et al. 2001b; Kielian et al. 2004b). Staging of brain abscess in humans has been based on findings obtained during computed tomography (CT) or magnetic resonance imaging (MRI) scans. The early stage or early cerebritis occurs from days 1–3 and is typified by neutrophil accumulation, tissue necrosis, and edema. Microglial and astrocyte activation is also evident at this stage and persists throughout abscess development. The intermediate, or late cerebritis stage, occurs from days 4–9 and is associated with a predominant macrophage and lymphocyte infiltrate. The final or capsule stage occurs from days 10 onward and is associated with the formation of a well-vascularized abscess wall, in effect sequestering the lesion and protecting the surrounding normal brain parenchyma from additional damage. It should be noted that these intervals represent general time frames that may deviate slightly depending on the colonizing pathogen and other host factors such as immune status or age. In addition to limiting the extent of infection, the immune response that is an essential part of abscess formation also destroys surrounding normal brain tissue. This is supported by findings in experimental models where lesion sites are greatly exaggerated compared to the localized nature of bacterial growth, reminiscent of an over-active immune response (Kielian et al. 2001a; Kielian et al. 2001b; Baldwin and Kielian 2004). This phenomenon is also observed in human brain abscess, where lesions can encompass a large portion of brain tissue, often spreading well beyond the initial focus of infection. Therefore, controlling the intensity and/or duration of anti-bacterial immune responses in the brain may allow for effective elimination of bacteria while minimizing damage to surrounding brain tissue. This may conceivably be achieved by regulating the extent of TLR activation during brain abscess development. For example, upon abscess sterilization the presence of microbial debris such as cell wall fragments and/or bacterial DNA may serve as a continual trigger for TLR-mediated activation of resident glia and infiltrating immune cells. This would effectively culminate in prolonged cytokine/chemokine release that may exacerbate the destruction of normal surrounding brain parenchyma via bystander lysis. In effect, the brain immunologically “senses” an infection in the absence of viable bacteria, resulting in pathological inflammation. In addition, tissue necrosis that accompanies these infections likely liberates endogenous TLR ligands, in a so-called “pathogen-necrosis-autoantigen triad” that effectively amplifies TLR signaling to further exacerbate tissue damage. This concept is introduced in a later section of this Chapter. However, these possibilities remain to be directly tested in the experimental brain abscess model.

3.3 Importance of TLRs in Glial Recognition of *S. aureus*

As previously mentioned, microglial and astrocyte activation ensues immediately following bacterial infection and persists throughout the various stages of brain abscess development. Both glial cell types are important for providing a first line of defense against invading microbes into the CNS parenchyma. Astrocytes are strategically located at the blood-brain

barrier interface, providing a mechanism to immediately sense pathogenic motifs. Based on its prevalence in human brain abscesses, we have focused our attention to characterize the receptors responsible for eliciting glial activation and subsequent pro-inflammatory mediator production in response to *S. aureus*. We have primarily focused on the roles of TLR2 and the central adaptor molecule MyD88 in these processes using primary glia isolated from TLR2 and MyD88 KO mice. A summary of the findings obtained with microglia and astrocytes is provided below.

3.3.1 TLRs and Microglial Responses to *S. aureus*

Microglia are the resident professional mononuclear phagocyte population in the brain parenchyma and function as the principal innate immune effector cells in the CNS (Aloisi 2001; Rivest 2003). Upon pathogen recognition, resting microglia transform into activated cells that migrate and accumulate at sites of injury (Gonzalez-Scarano and Baltuch 1999; Gonzalez-Scarano and Martin-Garcia 2005). Activated microglia express a range of genes related to inflammation such as pro-inflammatory cytokines, pro-inflammatory enzymes, and adhesion molecules, which are dictated by the nature of the inflammatory stimulus (Gonzalez-Scarano and Baltuch 1999; Aloisi 2001; Block et al. 2007). The pattern of inflammatory molecule production can dictate leukocyte migration across the blood–brain barrier (Persidsky et al. 1999) and promote the effector functions of these newly recruited cells. Recent studies have demonstrated the presence of mRNA and/or protein expression of TLRs1-9 in microglia (Dalpke et al. 2002; Rasley et al. 2002a; Kielian 2002b) (Olson and Miller 2004) and have shown that TLR levels are modulated following exposure to bacterial pathogens (Rasley et al. 2002a; Kielian 2002b; Olson and Miller 2004; Esen and Kielian 2005). These findings are, perhaps, not surprising considering that microglia share the same myeloid lineage as macrophages and dendritic cells – important anti-bacterial effector and antigen presenting cells.

One well characterized PAMP of *S. aureus* is peptidoglycan (PGN), a component of the outer bacterial cell wall (Dziarski 2003; Weber et al. 2003). Although recently scrutinized, PGN is still considered a potent TLR2 agonist (Dziarski and Gupta 2005). With regard to brain abscess, PGN is released during normal bacterial growth as well as from dying organisms within the necrotic environment that is typical of these infections. In addition, many antibiotics that are used to treat CNS Gram-positive infections enhance PGN release from the bacterial cell wall (van der Flier et al. 2003; Weber et al. 2003), liberating additional antigen to engage PRRs such as TLR2. Collectively, these findings indicate that PGN represents a PAMP of significant biological importance in brain abscess as well as other CNS Gram-positive infections. Therefore, our *in vitro* studies have centered on the responses of glial cells to PGN as well as intact *S. aureus*, the latter of which presents glia with a more complex antigenic milieu and multiple TLR ligands (i.e. lipoproteins, bacterial DNA).

Our results have demonstrated that microglia respond to both intact *S. aureus* and its cell wall component PGN robustly. Specifically, exposure to both stimuli led to a dose- and time-dependent induction of the pro-inflammatory cytokines IL-1 β , TNF- α , IL-12 p40, and several chemokines including CXCL1 (KC), CXCL2 (MIP-2), CCL2 (MCP-1), CCL3

(MIP-1 α), and CCL4 (MIP-1 β) (Kielian 2002b; Kielian et al. 2004a; Kielian et al. 2005a). The importance of microglia in the early host response to infection in brain abscess is suggested by the fact that pro-inflammatory mediator production is detected within 1 to 3 hours following the initial *S. aureus* infection, well before the significant accumulation of peripheral immune cell infiltrates (Kielian and Hickey 2000; Esen et al. 2004). Moreover, both heat-inactivated *S. aureus* and PGN are capable of inducing microglial MHC Class II, CD40, CD80, and CD86 expression (Kielian 2002b; Kielian et al. 2004a; Esen and Kielian 2007), similar to what has been described for microglia in response to the Gram-negative bacterial cell wall product lipopolysaccharide (LPS) and interferon- γ (IFN- γ) (Frei et al. 1994; Xu and Ling 1995; Menendez Iglesias et al. 1997; Aloisi 1998; O'Keefe et al. 2001). The ability of *S. aureus* to augment the expression of receptors that are important for antigen presentation suggests that the ability of microglia to present bacterial peptides to antigen-specific T cells may be greatly enhanced following *S. aureus* exposure. The effects of *S. aureus* and PGN on microglial CD40, CD80, CD86, and MHC Class II expression may either be a direct consequence of bacterial stimulation or indirect via the autocrine/paracrine action of cytokines produced by activated microglia.

An analysis of the importance of TLR2 in mediating microglial responses to *S. aureus* revealed some interesting findings. Namely, primary microglia isolated from TLR2 KO mice were non-responsive to *S. aureus*-derived PGN, demonstrating a pivotal role for TLR2-dependent signals in eliciting pro-inflammatory mediator release from microglia in response to this PAMP (Kielian et al. 2005a). In contrast, microglial activation following treatment with intact *S. aureus* was only marginally affected by TLR2 loss, with the majority of mediators expressed at roughly equivalent levels between TLR2 KO and WT primary microglia. Interestingly, we consistently observed elevated IL-12 p40 production by TLR2 KO microglia in response to intact *S. aureus*, suggesting dysregulated signaling pathways (Kielian et al. 2005a). Heightened IL-12 p40 expression was never detected in TLR2 KO microglia following PGN treatment, indicating antigenic specificity in cytokine regulation. The mechanism(s) responsible for this phenomenon are not known but are under investigation in our laboratory.

Unlike what was observed with TLR2, MyD88 expression was essential for eliciting pro-inflammatory mediator production from microglia in response to both intact *S. aureus* as well as PGN. This finding strongly suggests the involvement of alternative TLRs that recognize additional PAMPs present in *S. aureus* and/or amplification of signaling networks through the autocrine/paracrine actions of cytokines that utilize MyD88, such as the IL-1R and IL-18R. The importance of MyD88 in CNS resident cells has recently been demonstrated using radiation bone marrow chimera mice (Nichols et al., manuscript in preparation). Specifically, cytokine expression was restored to wild type levels during brain abscess development only when MyD88 was present in the CNS compartment. The absence of MyD88 in the CNS compartment, with expression restricted to infiltrating immune cells, was not sufficient to restore cytokine levels to that observed in wild type mice. Although we cannot ascertain whether MyD88 is pivotal in microglia, astrocytes, and/or other CNS resident cell types this data does indicate that CNS MyD88 is essential for generating a potent inflammatory response during brain abscess development.

3.3.2 Astrocytes and TLR2-dependent Recognition of *S. aureus*

Besides microglia, astrocytes can also participate in the immune response against CNS pathogens, in addition to their traditional roles in influencing CNS development and homeostasis. Indeed, activated astrocytes have been recognized as a major source of inflammatory chemokines in response to diverse stimuli (Dong and Benveniste 2001; Farina et al. 2007). Several reports have provided direct evidence for the presence of TLRs in astrocytes (Bsibsi 2002; Bowman et al. 2003; Esen et al. 2004; Carpentier et al. 2005; Jack et al. 2005); however, the number of TLRs expressed is much lower compared to microglia, which may be explained by the fact that microglia are more classical innate immune effector cells. Primary murine astrocytes constitutively express low levels of TLR2, TLR3, TLR4, TLR5 and TLR9 with the expression of each TLR homolog rapidly up-regulated following exposure to its cognate bacterial ligand (Bowman et al. 2003; Carpentier et al. 2005).

With regard to *S. aureus*, astrocytes appear to rely heavily on TLR2 for signaling cell activation and subsequent pro-inflammatory mediator release. This was evident by the finding that TLR2 KO astrocytes were relatively non-responsive to either PGN or intact *S. aureus* (Esen et al. 2004), the latter observation being a major discriminating factor between astrocytes and microglia that utilize alternative PRRs for maximal recognition of intact bacteria. Specifically, a wide array of pro-inflammatory cytokines and chemokines were attenuated in TLR2 and MyD88 KO astrocytes despite the fact that bacterial internalization was not affected by either molecule (Esen et al. 2004)(Esen and Kielian, manuscript in preparation). As mentioned earlier, MyD88 plays an important role in the CNS compartment to elicit a protective immune response during the acute stage of brain abscess development (Nichols et al., manuscript in preparation). It is quite probable that astrocytes, in concert with microglia, contribute to this process. Since astrocytes represent a more robust source of chemokines compared to microglia, the loss of MyD88 in the former may have drastic implications on the recruitment of immune cells from the periphery into the infected CNS.

3.3.3 Cytokine Regulation of TLR2 Expression in Glia

We previously reported that exposure of microglia to *S. aureus* led to elevated TLR2 expression (Kielian et al. 2005a). However, it remained uncertain as to whether this phenomenon resulted from a direct effect of pathogen exposure or indirectly through the autocrine/paracrine action of cytokines released from activated cells. Indeed, microglia produce several inflammatory mediators following *S. aureus* stimulation including TNF- α and IL-1 β (Kielian 2002b; Kielian et al. 2004a; Kielian et al. 2005a). We recently demonstrated that the pro-inflammatory cytokine TNF- α enhances TLR2 expression in microglia-1 β (IL-1 β) had no significant effect (Syed et al. 2007). Strong evidence to implicate TNF- α as a pivotal mediator for augmenting TLR2 expression was demonstrated by the inability of TNF- α KO microglia to upregulate TLR2 following *S. aureus* stimulation (Syed et al. 2007). Direct *in vivo* evidence to support this concept was provided by the finding that TLR2 expression was significantly attenuated during brain abscess development in TNF- α KO mice. In addition, the TNF- α -dependent induction of TLR2 expression in microglia was mediated by a NF- κ B-dependent pathway, whereas PKC or p38 MAPK signals were not involved (Syed et al. 2007). Similar findings were obtained in astrocytes, where TNF- α was shown to act in an autocrine/paracrine fashion to augment TLR2

expression (Phulwani et al. 2008). The ability of TNF- α to increase TLR2 levels was mediated by NF- κ B signaling since disparate inhibitors of this pathway were capable of interfering with TLR2 induction (Phulwani et al. 2008). Collectively, these results suggest that TNF- α likely plays an important role in amplifying the immune responses of both microglia and astrocytes upon *S. aureus* exposure, in part, by augmenting TLR2 expression.

3.3.4 Potential Contribution of other TLRs for *S. aureus* recognition by Glia

Our findings with TLR2 KO primary microglia suggested that alternative receptors are also involved in maximal *S. aureus* recognition. Indeed, this functional redundancy is not surprising since bacterial pathogens have the potential for devastating consequences in a tissue that has limited regenerative capacity such as the CNS. Possible alternative receptors could be TLR9, which recognizes non-methylated CpG DNA motifs of microbes, or other cytoplasmic PRRs which may encounter PAMPs within phagosomal compartments such as the cytosolic NOD2 protein which binds PGN (Inohara et al. 2003; Girardin et al. 2003a; Girardin et al. 2003b). Alternatively, phagocytic scavenger receptors such as macrophage scavenger receptor type AI/AII (MSR) and lectin-like oxidized low-density lipoprotein receptor (LOX)-1 may play a role. Since the expression of LOX-1 is significantly increased in microglia following *S. aureus* exposure *in vitro* and in brain abscesses *in vivo* (Kielian et al. 2005a; Kielian et al. 2005b), we suggest that scavenger receptors may be utilized by microglia to phagocytize and inactivate bacteria. More supporting evidence came with data showing that bacterial phagocytosis is opsonin-independent and does not require TLRs, since microglia lacking the central adapter molecule MyD88 were still capable of phagocytizing *S. aureus* (Esen and Kielian 2006) and unpublished results).

The implications of glial activation in the context of brain abscess are likely several-fold. First, parenchymal microglia and astrocytes are likely involved in the initial recruitment of professional bactericidal phagocytes into the CNS through their elaboration of chemokines and pro-inflammatory cytokines. Second, microglia exhibit modest *S. aureus* bactericidal activity *in vitro*, suggesting that they may also participate in the initial containment of bacterial replication in the CNS. However, their bactericidal activity *in vitro* is not comparable to that of neutrophils or macrophages, suggesting that this activity may not be a major effector mechanism for microglia during acute infection. Third, activated microglia have the potential to influence the type and extent of anti-bacterial adaptive immune responses through their upregulation of MHC Class II and co-stimulatory molecule expression. Finally, if glial activation persists in the context of ongoing inflammation, the continued release of pro-inflammatory mediators could damage surrounding normal brain parenchyma. Indeed, inappropriate glial activation has been implicated in several CNS diseases including multiple sclerosis and its animal model experimental autoimmune encephalomyelitis as well as Alzheimer's disease (Benveniste 1997; McGeer and McGeer 2002).

3.4 Impact of TLR Signaling on Immune Responses During Brain Abscess Development

TLR2 has been shown to play an important role in the host immune response to Gram-positive bacterial infections in the periphery (Takeuchi et al. 2002) and, to some extent, this receptor dictates the ensuing host anti-bacterial response in *Streptococcus pneumoniae* meningitis (Echchannaoui et al. 2002; Koedel et al. 2003)(see Chapter 2 of this volume). However, prior to our studies, the functional role of TLR2 in the context of a CNS parenchymal infection, such as brain abscess, had not been examined and was thought to differ from that of meningitis based upon the highly focal nature of lesions in the former.

3.4.1 Influence of TLRs on Innate Immunity in Brain Abscesses

We first evaluated the expression of numerous pro-inflammatory mediators previously determined to be pivotal for the host immune response during the acute phase of brain abscess development to ascertain whether defects in CNS bacterial recognition were evident in TLR2-deficient animals. The kinetics of pro-inflammatory mediator production, including TNF- α , IL-1 β , CXCL2 and inducible nitric oxide synthase (iNOS), was delayed in TLR2 KO mice compared to WT animals, with lower levels of mediators detected in the former during the acute stage of disease (Kielian et al. 2005a; Kielian et al. 2005b). However, the reduction in these inflammatory mediators was transient, with levels in TLR2 KO mice recovering to those detected in WT animals over time and as such, no striking phenotype was observed in TLR2 KO mice, much to our surprise. However, one dramatic difference was consistently observed in brain abscesses of TLR2 KO animals, namely elevated IL-17 expression (Kielian et al. 2005b). The potential biological impact of increased IL-17 levels in TLR2 KO mice will be discussed in a later section describing the interface of TLRs with the adaptive immune response. In addition, TLR2 did not play a significant role in controlling the extent of infection in brain abscess, with similar bacterial titers observed between TLR2 KO and WT animals, suggesting receptor redundancy for *S. aureus* neutralization in the CNS (Kielian et al. 2005a; Kielian et al. 2005b). Interestingly, the inflammatory phenotype detected in TLR2 KO mice was nearly identical to that observed in CD14 KO animals (T. Kielian, unpublished observations), strongly suggesting that these two receptors may cooperate in a multi-receptor complex to facilitate pathogen recognition and the subsequent shaping of the inflammatory milieu during the acute stage of infection. These results were in accordance with our *in vitro* studies implying a role for alternative receptor(s) in bacterial recognition and subsequent activation of glial cells (Esen et al. 2004; Kielian et al. 2005a). It is worth noting that our findings with TLR2 KO mice in the experimental brain abscess model (Kielian et al. 2005b) are at odds with a recent report by Stenzel et al. (Stenzel et al. 2008). Specifically, Stenzel et al. noted that brain abscess size was exacerbated in TLR2 KO mice; however, bacterial burdens were only marginally increased (Stenzel et al. 2008). Similar to our earlier report (Kielian et al. 2005b), this group also observed that the induction of proinflammatory mediator expression was delayed in TLR2 KO animals but eventually achieved levels equivalent to WT mice (Stenzel et al. 2008).

In contrast to studies with TLR2 KO mice, MyD88 plays an essential role during the early phase of brain abscess. In particular, MyD88 loss led to a significant increase in mortality rates, which correlated with the complete disruption of normal brain tissue architecture in the infected parenchyma (Kielian et al. 2007). Concomitant with these outcomes was the inability to recruit significant numbers of neutrophils and macrophages into the brain parenchyma of MyD88 KO animals, the former representing a leukocyte population that we had previously demonstrated to be essential for *S. aureus* containment during the acute stage of brain abscess (Kielian et al. 2001a). Finally, MyD88 KO mice displayed significant decreases in the expression of numerous pro-inflammatory cytokines and chemokines, which likely contributed, in part, to the impaired leukocyte recruitment observed in KO animals (Kielian et al. 2007). The dramatic phenotype observed during the early stage of brain abscess formation in MyD88 KO mice is likely influenced by the loss of multiple TLRs as well as signaling via the IL-1R and/or IL-18R. Indeed, earlier studies by our group demonstrated that IL-1 KO mice displayed more severe infection as typified by increased bacterial burdens and alterations in inflammatory mediator production during brain abscess development (Kielian et al. 2004b). The relative role of IL-18 signaling is uncertain since no one has yet investigated the importance of IL-18 in the experimental brain abscess model.

MyD88-dependent signals also played an important role in regulating the extent of parenchymal damage during the acute stage of brain abscess development. Indeed, brain abscesses were grossly disseminated in MyD88 KO mice and often encompassed the entire injected hemisphere, whereas lesions were contained and well-demarcated in WT animals at all time points examined (Kielian et al. 2007). A recent study by Stenzel et al. has demonstrated that the astrocytic intermediate filament protein GFAP is important for providing a barrier to restrict brain abscess expansion throughout the parenchyma (Stenzel et al. 2004). Of note is the finding that in our studies, GFAP immunoreactivity was enhanced in MyD88 KO mice (Kielian, unpublished observations), yet these animals had diffuse lesions with ill-defined borders. This result may reflect an attempt by astrocytes to sequester the lesion for effective bacterial containment; however, in the face of the dramatic defects in CNS innate immunity in MyD88 KO mice, this attempt is futile. The dramatic pathology observed in brain abscesses of MyD88 KO mice presents an excellent model system to test the efficacy of various novel anti-microbial therapies since it is reminiscent of a worst-case scenario clinically.

Neutrophil and macrophage accumulation into brain abscesses was significantly attenuated in MyD88 KO mice compared to WT animals (Kielian et al. 2007). Importantly, this correlated with a dramatic decrease in the production of numerous chemokines in brain abscesses of the former. The number of abscess-associated neutrophils in MyD88 KO mice was more significantly affected compared to macrophages, which agreed with the finding that the expression of neutrophil chemokines (i.e. CXCL1, CXCL2) was more depressed compared to macrophage chemoattractants (i.e. CCL2, CCL3). Not only were the numbers of abscess-associated neutrophils and macrophages reduced in brain abscesses of MyD88 KO mice, but their activation status was also impaired. This was reflected by lower levels of several cytokines and chemokines including IL-6, TNF- α , CCL3, and CXCL10 produced by these cell types recovered from lesions of MyD88 KO mice. Collectively, these findings

highlight the importance of MyD88-dependent signals in inducing the host innate immune response during *S. aureus* infection in the brain.

One intriguing and unexpected finding that surfaced during the course of our studies with MyD88 KO mice was the fact that bacterial burdens were equivalent in MyD88 KO and WT animals despite the dramatic inhibition of CNS innate immune responses in the former. This finding suggests that alternative mechanisms are responsible for controlling bacterial replication in the CNS parenchyma since no role for TLRs was evident in our studies. Since TLRs are not phagocytic receptors it may be expected that alternative PRRs are responsible for pathogen uptake and neutralization (Henneke et al. 2002; Underhill and Gantner 2004). Some candidate receptors that may participate in regulating *S. aureus* burdens in the CNS parenchyma include members of the scavenger receptor family as well as opsonic receptors including Fc and complement receptors (Husemann et al. 2002; Peiser et al. 2002). Another possibility is that the extensive tissue damage observed in MyD88 KO mice leads to cell death via a mechanism that is independent of bacterial burdens *per se*. Perhaps with a longer infection period differences in bacterial burdens between MyD88 KO and WT mice would have become evident; however, this analysis was not feasible in our studies since the majority of KO animals did not survive beyond 48 h post-infection. An alternative explanation may be that the defective host immune response in MyD88 KO mice led to altered selective pressures on *S. aureus* that could conceivably modify the organism's virulence factor expression profile. In this case, bacteria may have switched on a cohort of genes that favored invasion and dissemination. We are currently investigating this possibility by performing transcriptional profiling of genes expressed by *S. aureus* in MyD88 KO and WT mice using Affymetrix GeneChips®.

3.4.2 Relationship Between TLR2 and Adaptive Immunity in Brain Abscesses

Innate and adaptive immunity are linked and recent evidence indicates that TLR-dependent signaling leads to the initiation of adaptive immune responses (Hoebe et al. 2004; Pasare and Medzhitov 2005). Indeed, our earlier studies demonstrating lymphocyte influx and generation of *S. aureus*-reactive lymphocytes in the brain abscess model (Kielian and Hickey 2000; Baldwin and Kielian 2004) as well as the induction of molecules involved in antigen presentation (Kielian 2002b; Kielian et al. 2004a; Esen and Kielian 2007), have provided clues to the connection between innate and adaptive immunity in brain abscesses. One apparent link between the two processes was discovered when we detected elevated IL-17 levels in TLR2 KO mice during the early stages of primary infection (Kielian et al. 2005b). In this scenario, attributing cytokine production to memory T cells appeared unusual since animals had not been infected earlier with *S. aureus*. However, we have previously reported the presence of a population of *S. aureus*-reactive T cells in the spleens of "naïve" mice under non-SPF conditions (Baldwin and Kielian 2004). The origin of these *S. aureus*-specific lymphocytes is not known but may have arisen from routine pathogen exposure, since *S. aureus* is ubiquitous in nature, or from the re-stimulation of cross-reactive lymphocytes that are specific for a highly conserved epitope expressed on another bacterial pathogen. Therefore, it is conceivable that "endogenous" *S. aureus*-reactive memory T cells are recruited into the CNS during the early stages of brain abscess development and are responsible for subsequent IL-17 production. Indeed, we have recently demonstrated that the

numbers of both CD4⁺ and CD8⁺ T-cell infiltrates are enhanced in brain abscesses of TLR2 KO mice compared to WT animals (Nichols J. et al. submitted). These findings are in agreement with a recent report by Stenzel et al. in the experimental brain abscess model (Stenzel et al. 2008), providing further evidence that T cell dynamics are indeed altered in the infected CNS of mice lacking TLR2. It is worth noting that elevated Th17 influx in TLR2 KO animals appears to represent a non-traditional pathway since although Th17 cells are present in the CNS of infected WT mice, they do not predominate the adaptive response since Th1 cells producing IFN- γ are also a significant component of the T cell infiltrate (Nichols J. et al., submitted).

We have recently found that primary microglia and astrocytes express mRNA for two IL-17R isoforms, namely IL-17RA and IL-17RC; however not at equivalent levels (Nichols J. et al. submitted). Specifically, IL-17RA was more highly expressed in microglia compared to IL-17RC, whereas astrocytes expressed roughly equivalent levels of both receptors. The reason for preferential IL-17RA expression in microglia is currently not known. Evidence to support the idea that increased IL-17 production in TLR2 KO animals represents a compensatory mechanism to counteract the observed delay in the production of neutrophil-attracting chemokines, came with the observation that IL-17 induced the expression of the neutrophil chemoattractant CXCL2 in astrocytes (Nichols J et al. submitted) in accordance with several studies which report IL-17 as a potent stimulus for the production of neutrophil chemokines (Witowski et al. 2000; Maertzdorf et al. 2002; Ruddy et al. 2004a). In addition, IL-17 has been shown to be pivotal in the establishment of antimicrobial immunity since IL-17 KO mice are more susceptible to systemic bacterial infections (Ye et al. 2001; Chung et al. 2002; Chung et al. 2003).

Since IL-17 has been reported to synergize with other proinflammatory cytokines such as TNF- α and IL-1 β in various cell types (Chabaud et al. 2001; Maertzdorf et al. 2002; Ruddy et al. 2004b; Dong 2008), we examined whether this relationship would hold true for glia. Although TNF- α and IL-1 β alone were capable of eliciting the production of a few inflammatory mediators by glia, the addition of IL-17 did not significantly alter the release of these factors (Nichols et al., submitted). Collectively, the limited response of glial cells to IL-17 stimulation suggests that the primary target of increased IL-17 levels in brain abscesses of TLR2 KO mice may be stimulation of alternative parenchymal cells and/or peripheral immune cells infiltrating the CNS. Evidence to support the latter has been provided by recent studies from our laboratory demonstrating synergistic effects of IL-17 in combination with TNF- α in primary macrophages. These cells are also relevant to brain abscess pathogenesis since macrophages represent a significant constituent of the inflammatory infiltrate in response to infection (Kielian 2004c). Importantly, only a select number of mediators was potentiated by IL-17 in the presence of TNF- α (i.e. IL-4, IL-6, IL-10, CXCL2), whereas others were not affected (i.e. IL-1 β , IL-12, CCL2). Interestingly, we failed to detect any synergy with IL-17 and IL-1 β regardless of the concentrations examined, revealing specificity in the responses obtained. We cannot discount additional roles for IL-17 on other effector responses of microglia and astrocytes including phagocytosis, chemotaxis and/or increased responsiveness to other inflammatory signals. These remain areas of continued investigation in our laboratory.

3.5 Concept of a “Pathogen-necrosis-autoantigen triad”

During brain abscess development, the resultant immune response (mediated, in part, via TLRs) not only leads to pathogen destruction but also bystander damage to surrounding CNS parenchyma by necrotic cell death. Further complicating the issue is the fact that bacteria express numerous virulence factors that can directly lead to cell death/lysis of mammalian cells. One example is the hemolysins produced by *S. aureus* that form transmembrane pores in mammalian cells leading to cell destruction by osmotic lysis. The end result of this necrotic damage is the release of self antigens, many of which would not typically be exposed to the extracellular milieu and available for uptake by antigen presenting cells. Therefore, it is also possible that during brain abscess evolution TLRs play dual roles in ligand recognition: one is to facilitate the initial response to the inciting pathogen and upon tissue destruction TLRs may also recognize newly liberated self-antigens as a result of necrosis, a series of events we have coined a “pathogen-necrosis-autoantigen triad” (Figure 1). In this case, newly liberated self antigens may serve as direct triggers for TLRs and/or internalized by antigen presenting cells in the vicinity of the abscess (i.e. macrophages, dendritic cells, and microglia) whereupon they could conceivably stimulate autoimmune reactions. This “pathogen-necrosis-autoantigen triad” shares similarities to the proposed “fertile field” concept for autoimmunity involving molecular mimicry in concert with bystander activation (von Herrath et al. 2003). However, it is imperative to point out that there is no evidence to date, either experimental or clinical, to indicate that patients who have recovered from brain abscess exhibit increased prevalence of subsequent autoimmune disease, although it is likely that no one has investigated this relationship with retrospective studies. Although it is clear that self antigens are liberated during brain abscess development as a result of necrosis, what is not certain is whether this primes an individual for downstream autoimmunity. It could very well be that the liberation of self antigen during disease serves as a trigger for further TLR activation (via endogenous TLR agonists), which merely contributes to the inflammatory foci without inducing long-lasting overt consequences.

3.6 Summary

Despite recent studies investigating the importance of TLR2 and MyD88 in regulating host immune responses during brain abscess development, several outstanding questions remain. For example, studies with TLR2 KO microglia and in the experimental brain abscess model have clearly demonstrated the presence of alternative redundant pathways for bacterial recognition. This was initially unexpected given the importance of TLR2 in *S. aureus* sepsis (Takeuchi et al. 2000); however, upon closer examination this may be a dose-dependent effect since severe pathology was not observed systemically unless TLR2 KO mice were challenged with high numbers of bacteria (Takeuchi et al. 2000). Nonetheless, the identity of alternative receptor(s) involved in *S. aureus* recognition in the CNS remains to be defined. Another unresolved issue is the functional importance of IL-17 during brain abscess development and most importantly, whether IL-17 neutralization will reverse the proposed compensatory effect of elevated cytokine expression in brain abscesses of TLR2 KO mice. These are questions that are currently under investigation in our laboratory. Collectively, the TLR family of molecules likely influences a wide array of inflammatory responses during

the course of brain abscess development, impacting both innate and adaptive arms of the immune system.

Acknowledgments

This work was supported by the NIH National Institute of Neurological Disorders and Stroke (RO1s NS055385, NS40730, and NS053487) to T.K.

List of Abbreviations

CNS	Central nervous system
CT	Computed tomography
GFAP	Glial fibrillary acidic protein
IFN-γ	Interferon-gamma
IL-1β	Interleukin-1 beta
IL-1R	Interleukin-1 receptor
IL-17	Interleukin-17
IL-17RA	IL-17 receptor A
IL-17RC	IL-17 receptor C
IL-18R	Interleukin-18 receptor
iNOS	Inducible nitric oxide synthase
KC	Keratinocyte chemoattractant
KO	Knockout
LOX-1	Lectin-like oxidized low-density lipoprotein receptor-1
LPS	Lipopolysaccharide
MAPK	Mitogen-activated protein kinase
MCP-1	Macrophage chemoattractant protein-1
MHC	Major histocompatibility complex
MIP-1α	Macrophage inflammatory protein-1 alpha
MIP-1β	Macrophage inflammatory protein-1 beta
MIP-2	Macrophage inflammatory protein-2
MRI	Magnetic resonance imaging
MSR	Macrophage scavenger receptor
MyD88	Myeloid differentiation primary-response protein 88
NF-κB	Nuclear factor-kappa B
NOD2	Nucleotide-binding oligomerization domain containing 2

PAMP	Pathogen-associated molecular pattern
PGN	Peptidoglycan
PKC	Protein kinase C
PRR	Pattern recognition receptor
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SPF	Specific pathogen-free
Th1	T helper 1 cells
Th17	T helper 17 cells
TLR	Toll-like receptor
TNF-α	Tumor necrosis factor-alpha
WT	Wild type

3.7 References

- Aloisi F. Immune function of microglia. *Glia*. 2001; 36:165–179. [PubMed: 11596125]
- Aloisi F, Ria F, Penna G, Adorini L. Microglia are more efficient than astrocytes in antigen processing and in Th1 but not Th2 cell activation. *J Immunol*. 1998; 160(10):4671–4680. [PubMed: 9590212]
- Baddley JW, Salzman D, Pappas PG. Fungal brain abscess in transplant recipients: epidemiologic, microbiologic, and clinical features. *Clin Transplant*. 2002; 16:419–424. [PubMed: 12437621]
- Baldwin AC, Kielian T. Persistent immune activation associated with a mouse model of *Staphylococcus aureus*-induced experimental brain abscess. *J Neuroimmunol*. 2004; 151:24–32. [PubMed: 15145600]
- Benveniste EN. Role of macrophages/microglia in multiple sclerosis and experimental allergic encephalomyelitis. *J Mol Med*. 1997; 75:165–173. [PubMed: 9106073]
- Bhand AA. Brain abscess--diagnosis and management. *J Coll Physicians Surg Pak*. 2004; 14:407–410. [PubMed: 15279742]
- Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci*. 2007; 8:57–69. [PubMed: 17180163]
- Bowman CC, Rasley A, Tranguch SL, Marriott I. Cultured astrocytes express toll-like receptors for bacterial products. *Glia*. 2003; 43:281–291. [PubMed: 12898707]
- Britt RH, Enzmann DR, Yeager AS. Neuropathological and computerized tomographic findings in experimental brain abscess. *J Neurosurg*. 1981; 55:590–603. [PubMed: 6168748]
- Bsibsi M, Ravid R, Gveric D, van Noort JM. Broad expression of Toll-like receptors in the human central nervous system. *J Neuropathol Exp Neurol*. 2002; 61(11):1013–1021. [PubMed: 12430718]
- Calfee DP, Wispelwey B. Brain abscess. *Semin Neurol*. 2000; 20:353–360. [PubMed: 11051299]
- Carpenter J, Stapleton S, Holliman R. Retrospective analysis of 49 cases of brain abscess and review of the literature. *Eur J Clin Microbiol Infect Dis*. 2007; 26:1–11. [PubMed: 17180609]
- Carpentier PA, Begolka WS, Olson JK, Elhofy A, Karpus WJ, Miller SD. Differential activation of astrocytes by innate and adaptive immune stimuli. *Glia*. 2005; 49:360–374. [PubMed: 15538753]
- Chabaud M, Page G, Miossec P. Enhancing effect of IL-1, IL-17, and TNF-alpha on macrophage inflammatory protein-3alpha production in rheumatoid arthritis: regulation by soluble receptors and Th2 cytokines. *J Immunol*. 2001; 167:6015–6020. [PubMed: 11698482]
- Chen ST, Tang LM, Ro LS. Brain abscess as a complication of stroke. *Stroke*. 1995; 26:696–698. [PubMed: 7709419]

- Chung DR, Chitnis T, Panzo RJ, Kasper DL, Sayegh MH, Tzianabos AO. CD4+ T cells regulate surgical and postinfectious adhesion formation. *J Exp Med*. 2002; 195:1471–1478. [PubMed: 12045245]
- Chung DR, Kasper DL, Panzo RJ, Chitnis T, Grusby MJ, Sayegh MH, Tzianabos AO. CD4+ T cells mediate abscess formation in intra-abdominal sepsis by an IL-17-dependent mechanism. *J Immunol*. 2003; 170:1958–1963. [PubMed: 12574364]
- Dalpke AH, Schafer MK, Frey M, Zimmermann S, Tebbe J, Weihe E, Heeg K. Immunostimulatory CpG-DNA activates murine microglia. *J Immunol*. 2002; 168:4854–4863. [PubMed: 11994434]
- Dong C. TH17 cells in development: an updated view of their molecular identity and genetic programming. *Nat Rev Immunol*. 2008; 8:337–348. [PubMed: 18408735]
- Dong Y, Benveniste EN. Immune function of astrocytes. *Glia*. 2001; 36:180–190. [PubMed: 11596126]
- Dziarski R. Recognition of bacterial peptidoglycan by the innate immune system. *Cell Mol Life Sci*. 2003; 60:1793–1804. [PubMed: 14523544]
- Dziarski R, Gupta D. Staphylococcus aureus peptidoglycan is a toll-like receptor 2 activator: a reevaluation. *Infect Immun*. 2005; 73:5212–5216. [PubMed: 16041042]
- Echchannaoui H, Frei K, Schnell C, Leib SL, Zimmerli W, Landmann R. Toll-like receptor 2-deficient mice are highly susceptible to Streptococcus pneumoniae meningitis because of reduced bacterial clearing and enhanced inflammation. *J Infect Dis*. 2002; 186:798–806. [PubMed: 12198614]
- Emmez H, Borcek AO, Dogulu F, Ceviker N. Ischemic stroke complicated by a brain abscess: a case report and review of the literature. *Turk Neurosurg*. 2007; 17:48–54. [PubMed: 17918680]
- Esen N, Kielian T. Recognition of Staphylococcus aureus-derived peptidoglycan (PGN) but not intact bacteria is mediated by CD14 in microglia. *J Neuroimmunol*. 2005; 170:93–104. [PubMed: 16229899]
- Esen N, Kielian T. Central role for MyD88 in the responses of microglia to pathogen-associated molecular patterns. *J Immunol*. 2006; 176:6802–6811. [PubMed: 16709840]
- Esen N, Kielian T. Effects of low dose GM-CSF on microglial inflammatory profiles to diverse pathogen-associated molecular patterns (PAMPs). *J Neuroinflammation*. 2007; 4:10. [PubMed: 17374157]
- Esen N, Tanga FY, DeLeo JA, Kielian T. Toll-like receptor 2 (TLR2) mediates astrocyte activation in response to the Gram-positive bacterium Staphylococcus aureus. *J Neurochem*. 2004; 88:746–758. [PubMed: 14720224]
- Falconer MA, McFarlan AM, Russell DS. Experimental brain abscesses in the rabbit. *British Journal of Surgery*. 1943; 30:245–260.
- Farina C, Aloisi F, Meinel E. Astrocytes are active players in cerebral innate immunity. *Trends Immunol*. 2007; 28:138–145. [PubMed: 17276138]
- Flaris NA, Hickey WF. Development and characterization of an experimental model of brain abscess in the rat. *Am J Pathol*. 1992; 141:1299–1307. [PubMed: 1281616]
- Frei K, Lins H, Schwerdel C, Fontana A. Antigen presentation in the central nervous system. The inhibitory effect of IL-10 on MHC class II expression and production of cytokines depends on the inducing signals and the type of cell analyzed. *J Immunol*. 1994; 152:2720–2728. [PubMed: 8144879]
- Girardin SE, Boneca IG, Viala J, Chamaillard M, Labigne A, Thomas G, Philpott DJ, Sansonetti PJ. Nod2 is a general sensor of peptidoglycan through muramyl dipeptide (MDP) detection. *J Biol Chem*. 2003b; 278:8869–8872. [PubMed: 12527755]
- Girardin SE, Travassos LH, Herve M, Blanot D, Boneca IG, Philpott DJ, Sansonetti PJ, Mengin-Lecreulx D. Peptidoglycan molecular requirements allowing detection by Nod1 and Nod2. *J Biol Chem*. 2003a; 278:41702–41708. [PubMed: 12871942]
- Gonzalez-Scarano F, Baltuch G. Microglia as mediators of inflammatory and degenerative diseases. *Annu Rev Neurosci*. 1999; 22:219–240. [PubMed: 10202538]
- Gonzalez-Scarano F, Martin-Garcia J. The neuropathogenesis of AIDS. *Nat Rev Immunol*. 2005; 5:69–81. [PubMed: 15630430]
- Henneke P, Takeuchi O, Malley R, Lien E, Ingalls RR, Freeman MW, Mayadas T, Nizet V, Akira S, Kasper DL, Golenbock DT. Cellular activation, phagocytosis, and bactericidal activity against

- group B streptococcus involve parallel myeloid differentiation factor 88-dependent and independent signaling pathways. *J Immunol.* 2002; 169:3970–3977. [PubMed: 12244198]
- Hoebe K, Janssen E, Beutler B. The interface between innate and adaptive immunity. *Nat Immunol.* 2004; 5:971–974. [PubMed: 15454919]
- Hristea I, Bunnapradist S, Peng A, Puliyanda D, Vo A, Jordan SC. The onset of rapidly progressive neurologic deterioration after a brief gastrointestinal illness in a renal allograft recipient. *Transpl Infect Dis.* 2007; 9:142–147. [PubMed: 17462001]
- Husemann J, Loike JD, Anankov R, Febbraio M, Silverstein SC. Scavenger receptors in neurobiology and neuropathology: their role on microglia and other cells of the nervous system. *Glia.* 2002; 40:195–205. [PubMed: 12379907]
- Inohara N, Ogura Y, Fontalba A, Gutierrez O, Pons F, Crespo J, Fukase K, Inamura S, Kusumoto S, Hashimoto M, Foster SJ, Moran AP, Fernandez-Luna JL, Nunez G. Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem.* 2003; 278:5509–5512. [PubMed: 12514169]
- Jack CS, Arbour N, Manusow J, Montgrain V, Blain M, McCrea E, Shapiro A, Antel JP. TLR signaling tailors innate immune responses in human microglia and astrocytes. *J Immunol.* 2005; 175:4320–4330. [PubMed: 16177072]
- Jones ME, Draghi DC, Karlowsky JA, Sahm DF, Bradley JS. Prevalence of antimicrobial resistance in bacteria isolated from central nervous system specimens as reported by U.S. hospital laboratories from 2000 to 2002. *Ann Clin Microbiol Antimicrob.* 2004; 3:3. [PubMed: 15043754]
- Kantarcioglu AS, Guarro J, de Hoog GS. Central nervous system infections by members of the *Pseudallescheria boydii* species complex in healthy and immunocompromised hosts: epidemiology, clinical characteristics and outcome. *Mycoses.* 2008
- Kao PT, Tseng HK, Liu CP, Su SC, Lee CM. Brain abscess: clinical analysis of 53 cases. *J Microbiol Immunol Infect.* 2003; 36:129–136. [PubMed: 12886965]
- Khan MA, Greig JR, Jayamohan J. Community-acquired methicillin-resistant *Staphylococcus aureus* brain abscess in an immunocompetent individual. *Scand J Infect Dis.* 2000; 32:423–424. [PubMed: 10959655]
- Kielian T. Immunopathogenesis of brain abscess. *J Neuroinflammation.* 2004c; 1:16. [PubMed: 15315708]
- Kielian T, Barry B, Hickey WF. CXC chemokine receptor-2 ligands are required for neutrophil-mediated host defense in experimental brain abscesses. *J Immunol.* 2001a; 166:4634–4643. [PubMed: 11254722]
- Kielian T, Bearden ED, Baldwin AC, Esen N. IL-1 and TNF- α play a pivotal role in the host immune response in a mouse model of *Staphylococcus aureus*-induced experimental brain abscess. *J Neuropathol Exp Neurol.* 2004b; 63:381–396. [PubMed: 15099027]
- Kielian T, Cheung A, Hickey WF. Diminished virulence of an α -toxin mutant of *Staphylococcus aureus* in experimental brain abscesses. *Infect Immun.* 2001b; 69:6902–6911. [PubMed: 11598065]
- Kielian T, Esen N, Bearden ED. Toll-like receptor 2 (TLR2) is pivotal for recognition of *S. aureus* peptidoglycan but not intact bacteria by microglia. *Glia.* 2005a; 49:567–576. [PubMed: 15593098]
- Kielian T, Haney A, Mayes PM, Garg S, Esen N. Toll-like receptor 2 modulates the proinflammatory milieu in *Staphylococcus aureus*-induced brain abscess. *Infect Immun.* 2005b; 73:7428–7435. [PubMed: 16239543]
- Kielian T, Hickey WF. Proinflammatory cytokine, chemokine, and cellular adhesion molecule expression during the acute phase of experimental brain abscess development. *Am J Pathol.* 2000; 157:647–658. [PubMed: 10934167]
- Kielian T, Mayes P, Kielian M. Characterization of microglial responses to *Staphylococcus aureus*: effects on cytokine, costimulatory molecule, and Toll-like receptor expression. *J Neuroimmunol.* 2002b; 130(1–2):86–99. [PubMed: 12225891]
- Kielian T, McMahon M, Bearden ED, Baldwin AC, Drew PD, Esen N. *S. aureus*-dependent microglial activation is selectively attenuated by the cyclopentenone prostaglandin 15-deoxy-Delta^{12,14}-prostaglandin J₂ (15d-PGJ₂). *J Neurochem.* 2004a; 90:1163–1172. [PubMed: 15312171]

- Kielian T, Phulwani NK, Esen N, Syed MM, Haney AC, McCastlain K, Johnson J. MyD88-dependent signals are essential for the host immune response in experimental brain abscess. *J Immunol.* 2007; 178:4528–4537. [PubMed: 17372011]
- Koedel U, Angele B, Rupprecht T, Wagner H, Roggenkamp A, Pfister HW, Kirschning CJ. Toll-like receptor 2 participates in mediation of immune response in experimental pneumococcal meningitis. *J Immunol.* 2003; 170:438–444. [PubMed: 12496429]
- Kremer V, Fedor-Freybergh PG. Neuroinfections in developed versus developing countries. *Neuro Endocrinol Lett.* 2007; 28(Suppl 2):5–6. [PubMed: 17558364]
- Kumar K. Neurological complications of congenital heart disease. *Indian J Pediatr.* 2000; 67:S15–19. [PubMed: 11129914]
- Maertzdorf J, Osterhaus AD, Verjans GM. IL-17 expression in human herpetic stromal keratitis: modulatory effects on chemokine production by corneal fibroblasts. *J Immunol.* 2002; 169:5897–5903. [PubMed: 12421973]
- Mathisen GE, Johnson JP. Brain abscess. *Clin Infect Dis.* 1997; 25:763–779. quiz 780–761. [PubMed: 9356788]
- McClelland CJ, Craig BF, Crockard HA. Brain abscesses in Northern Ireland: a 30 year community review. *J Neurol Neurosurg Psychiatry.* 1978; 41:1043–1047. [PubMed: 712373]
- McGeer PL, McGeer EG. Local neuroinflammation and the progression of Alzheimer's disease. *J Neurovirol.* 2002; 8:529–538. [PubMed: 12476347]
- Menendez Iglesias B, Cerase J, Ceracchini C, Levi G, Aloisi F. Analysis of B7-1 and B7-2 costimulatory ligands in cultured mouse microglia: upregulation by interferon-gamma and lipopolysaccharide and downregulation by interleukin-10, prostaglandin E2 and cyclic AMP-elevating agents. *J Neuroimmunol.* 1997; 72:83–93. [PubMed: 9003248]
- Nicolosi A, Hauser WA, Musicco M, Kurland LT. Incidence and prognosis of brain abscess in a defined population: Olmsted County, Minnesota, 1935–1981. *Neuroepidemiology.* 1991; 10:122–131. [PubMed: 1922645]
- Njambi S, Huttova M, Kovac M, Freybergh PF, Bauer F, Muli JM. Fungal neuroinfections: rare disease but unacceptably high mortality. *Neuro Endocrinol Lett.* 2007; 28(Suppl 2):25–26. [PubMed: 17558372]
- O'Keefe GM, Nguyen VT, Ping Tang LL, Benveniste EN. IFN-gamma regulation of class II transactivator promoter IV in macrophages and microglia: involvement of the suppressors of cytokine signaling-1 protein. *J Immunol.* 2001; 166:2260–2269. [PubMed: 11160280]
- Olson JK, Miller SD. Microglia initiate central nervous system innate and adaptive immune responses through multiple TLRs. *J Immunol.* 2004; 173:3916–3924. [PubMed: 15356140]
- Pasare C, Medzhitov R. Toll-like receptors: linking innate and adaptive immunity. *Adv Exp Med Biol.* 2005; 560:11–18. [PubMed: 15932016]
- Peiser L, Mukhopadhyay S, Gordon S. Scavenger receptors in innate immunity. *Curr Opin Immunol.* 2002; 14:123–128. [PubMed: 11790542]
- Persidsky Y, Ghorpade A, Rasmussen J, Limoges J, Liu XJ, Stins M, Fiala M, Way D, Kim KS, Witte MH, Weinand M, Carhart L, Gendelman HE. Microglial and astrocyte chemokines regulate monocyte migration through the blood-brain barrier in human immunodeficiency virus-1 encephalitis. *Am J Pathol.* 1999; 155:1599–1611. [PubMed: 10550317]
- Phulwani NK, Esen N, Syed MM, Kielian T. TLR2 expression in astrocytes is induced by TNF-alpha and NF-kappaB-dependent pathways. *J Immunol.* 2008; 181:3841–3849. [PubMed: 18768838]
- Prasad KN, Mishra AM, Gupta D, Husain N, Husain M, Gupta RK. Analysis of microbial etiology and mortality in patients with brain abscess. *J Infect.* 2006; 53:221–227. [PubMed: 16436297]
- Rasley A, Anguita J, Marriott I. *Borrelia burgdorferi* induces inflammatory mediator production by murine microglia. *J Neuroimmunol.* 2002a; 130:22–31. [PubMed: 12225885]
- Rivest S. Molecular insights on the cerebral innate immune system. *Brain Behav Immun.* 2003; 17:13–19. [PubMed: 12615045]
- Roche M, Humphreys H, Smyth E, Phillips J, Cunney R, McNamara E, O'Brien D, McArdle O. A twelve-year review of central nervous system bacterial abscesses; presentation and aetiology. *Clin Microbiol Infect.* 2003; 9:803–809. [PubMed: 14616700]

- Ruddy MJ, Shen F, Smith JB, Sharma A, Gaffen SL. Interleukin-17 regulates expression of the CXC chemokine LIX/CXCL5 in osteoblasts: implications for inflammation and neutrophil recruitment. *J Leukoc Biol.* 2004a; 76:135–144. [PubMed: 15107456]
- Ruddy MJ, Wong GC, Liu XK, Yamamoto H, Kasayama S, Kirkwood KL, Gaffen SL. Functional cooperation between interleukin-17 and tumor necrosis factor- α is mediated by CCAAT/enhancer-binding protein family members. *J Biol Chem.* 2004b; 279:2559–2567. [PubMed: 14600152]
- Schliamser SE, Backman K, Norrby SR. Intracranial abscesses in adults: an analysis of 54 consecutive cases. *Scand J Infect Dis.* 1988; 20:1–9. [PubMed: 3363298]
- Sell B, Evans J, Horn D. Brain abscess and hereditary hemorrhagic telangiectasia. *South Med J.* 2008; 101:618–625. [PubMed: 18475224]
- Smith NP, Nelson MR, Moore D, Gazzard BG. Cerebral abscesses in a patient with AIDS caused by methicillin-resistant *Staphylococcus aureus* (MRSA). *Int J STD AIDS.* 1997; 8:459–460. [PubMed: 9228595]
- Stenzel W, Soltek S, Sanchez-Ruiz M, Akira S, Miletic H, Schluter D, Deckert M. Both TLR2 and TLR4 are required for the effective immune response in *Staphylococcus aureus*-induced experimental murine brain abscess. *Am J Pathol.* 2008; 172:132–145. [PubMed: 18165267]
- Stenzel W, Soltek S, Schluter D, Deckert M. The intermediate filament GFAP is important for the control of experimental murine *Staphylococcus aureus*-induced brain abscess and *Toxoplasma* encephalitis. *J Neuropathol Exp Neurol.* 2004; 63:631–640. [PubMed: 15217091]
- Syed MM, Phulwani NK, Kielian T. Tumor necrosis factor- α (TNF- α) regulates Toll-like receptor 2 (TLR2) expression in microglia. *J Neurochem.* 2007; 103:1461–1471. [PubMed: 17961202]
- Takeuchi O, Hoshino K, Akira S. Cutting edge: TLR2-deficient and MyD88-deficient mice are highly susceptible to *Staphylococcus aureus* infection. *J Immunol.* 2000; 165:5392–5396. [PubMed: 11067888]
- Takeuchi O, Sato S, Horiuchi T, Hoshino K, Takeda K, Dong Z, Modlin RL, Akira S. Cutting edge: role of toll-like receptor 1 in mediating immune response to microbial lipoproteins. *J Immunol.* 2002; 169:10–14. [PubMed: 12077222]
- Tenney JH. Bacterial infections of the central nervous system in neurosurgery. *Neurol Clin.* 1986; 4:91–114. [PubMed: 3523205]
- Townsend GC, Scheld WM. Infections of the central nervous system. *Adv Intern Med.* 1998; 43:403–447. [PubMed: 9506189]
- Underhill DM, Gantner B. Integration of Toll-like receptor and phagocytic signaling for tailored immunity. *Microbes Infect.* 2004; 6:1368–1373. [PubMed: 15596122]
- van der Flier M, Geelen SP, Kimpen JL, Hoepelman IM, Tuomanen EI. Reprogramming the host response in bacterial meningitis: how best to improve outcome? *Clin Microbiol Rev.* 2003; 16:415–429. [PubMed: 12857775]
- von Herrath MG, Fujinami RS, Whitton JL. Microorganisms and autoimmunity: making the barren field fertile? *Nat Rev Microbiol.* 2003; 1:151–157. [PubMed: 15035044]
- Weber JR, Moreillon P, Tuomanen EI. Innate sensors for Gram-positive bacteria. *Curr Opin Immunol.* 2003; 15:408–415. [PubMed: 12900272]
- Winn HR, Mendes M, Moore P, Wheeler C, Rodeheaver G. Production of experimental brain abscess in the rat. *J Neurosurg.* 1979; 51:685–690. [PubMed: 387920]
- Witowski J, Pawlaczyk K, Breborowicz A, Scheuren A, Kuzlan-Pawlaczyk M, Wisniewska J, Polubinska A, Friess H, Gahl GM, Frei U, Jorres A. IL-17 stimulates intraperitoneal neutrophil infiltration through the release of GRO α chemokine from mesothelial cells. *J Immunol.* 2000; 165:5814–5821. [PubMed: 11067941]
- Xu J, Ling EA. Induction of major histocompatibility complex class II antigen on amoeboid microglial cells in early postnatal rats following intraperitoneal injections of lipopolysaccharide or interferon- γ . *Neurosci Lett.* 1995; 189:97–100. [PubMed: 7609927]
- Ye P, Rodriguez FH, Kanaly S, Stocking KL, Schurr J, Schwarzenberger P, Oliver P, Huang W, Zhang P, Zhang J, Shellito JE, Bagby GJ, Nelson S, Charrier K, Peschon JJ, Kolls JK. Requirement of interleukin 17 receptor signaling for lung CXC chemokine and granulocyte colony-stimulating

factor expression, neutrophil recruitment, and host defense. *J Exp Med.* 2001; 194:519–527. [PubMed: 11514607]

Figure 1. Proposed pathogen-necrosis-autoantigen triad

With regard to brain abscesses induced by *S. aureus*, the pathogen induces microglia and astrocyte activation via engagement of several Toll-like receptors (TLRs) in addition to other receptors to elicit pro-inflammatory mediator release to combat the infection. However, this response also leads to extensive necrosis of the infected parenchyma, which is also facilitated by virulence factors produced by the bacterium. Necrosis leads to the release of self antigens (autoantigens) that would not typically be encountered in the extracellular milieu. It is plausible that some of these autoantigens (as of yet to be defined) serve as further triggers for TLR activation, effectively exacerbating inflammation and further bystander destruction to surrounding uninfected brain parenchyma.