Genetic Regulation of Neuroinflammation after Infection and Injury

Nada Omar Abdelmagid

Stockholm 2012
“wa qul rabbi zidni ilma”

and say, "My Lord, increase me in knowledge."

*Holy Qur’an; Chapter 20, Surat Ţāhâ verse 114*
ABSTRACT

Neuroinflammation is a common theme in a spectrum of central nervous system (CNS) diseases resulting in neuronal damage and degeneration. The exact mechanisms regulating inflammation in the CNS or its consequences in terms of nerve injury are still not known in detail. However, it is known that many inflammatory conditions at least in part are regulated by genetic factors. Exact definition of these factors will provide a better understanding of underlying mechanisms. The focus of this thesis has been to explore the genetic regulation of neuroinflammation in viral infections and mechanical nerve injuries using experimental models.

The genetic regulation of susceptibility to Herpes simplex type-1 encephalitis (HSE), a devastating condition that affects humans, was investigated in different inbred rat strains. Using a series of different experimental approaches we succeeded in defining two different candidate genes that regulate susceptibility to HSE. Interestingly, these genetic influences act at two different stages of neurotropic HSV-1 virus CNS entry. Thus, the calcitonin receptor (Calcr) gene was identified as a candidate for peripheral neuronal infection and propagation to the CNS, while the von Willebrand factor (Vwf) gene was identified as a candidate for disease progression in the CNS and blood-brain barrier (BBB) dysfunction. The latter has previously been associated to cerebral malaria infection by Plasmodium falciparum and endothelial cell activation, suggesting that this gene is important in several human infectious conditions. More detailed histopathological and molecular studies of HSV-1 propagation, immune cell recruitment and inflammatory changes was the focus of another study, which provides further support for the notion that entry of virus into the perineurium is an important step regulating susceptibility for virus propagation into the CNS. Also, the differences found between the studied strains in relation to immune activation and responses in the peripheral nervous system (PNS) and CNS clearly demonstrate that genetic factors regulate virus-host interactions. Thus, our studies on HSE have provided several new perspectives of how susceptibility to HSV-1 virus can be regulated at different levels, as well as identifying two different candidate genes, all of which can serve as basis for further studies of human HSE.

In a fourth study, the genetic regulation of the response to a mechanical nerve injury was explored in order to identify regulatory pathways for innate immune responses occurring without an infectious trigger. We identified two quantitative trait loci (QTLs) on chromosome 1 (Neuinflam4) and 7 (Neuinflam5), respectively, regulating major histocompatibility complex class II (MHC II) expression after ventral root avulsion. Additionally, another QTL (Neuinflam9) on chromosome 10 regulated the expression of several important innate immune genes, including C1q, Il1β, Tlr2 and Irf7. The chromosome 10 region containing Neuinflam9 overlaps with Toxo1, which regulates resistance to Toxoplasma gondii infection. Interestingly, a part of this QTL conferred resistance to experimental autoimmune encephalomyelitis (EAE), an animal model for multiple sclerosis (MS) indicating that this QTL could regulate susceptibility both to infectious and autoimmune conditions.

In conclusion, the identification of several genetically regulated pathways presented herein provides a basis for further molecular exploration of different conditions characterized by neuroinflammation. This will be a necessary prerequisite for the formulation of new targeted therapeutic interventions that can prevent permanent damage to the nervous system.
I. The Calcitonin receptor gene is a candidate for regulation of susceptibility to Herpes simplex type 1 neuronal infection leading to encephalitis in rat.
   Nada Abdelmagid, Biborka Bereczky-Veress, André Ortlieb Guerreiro-Cacais, Petra Bergman, Katarina Luhr, Tomas Bergström, Birgit Sköldenberg, Fredrik Piehl, Tomas Olsson*, Margarita Diez*
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II. Influence of perineurial cells and Toll-like receptors 2 and 9 on Herpes simplex type 1 entry to the central nervous system in rat encephalitis.

III. Genetic variant of Vwf identified as a candidate gene regulating CNS immune activation and outcome in experimental Herpes simplex encephalitis.
   Nada Abdelmagid*, Biborka Bereczky-Veress*, Santosh Atanur, Alena Musilová, Václav Zidek, Mohsen Khademi, Laura Saba, Cécile Denis, Ana Garcia-Diaz, Tomas Bergström, Birgit Sköldenberg, Timothy Aitman, Norbert Hübner, Tomas Olsson*, Michal Pravenec*, Margarita Diez*.
   Manuscript.

IV. Identification of gene regions regulating inflammatory microglial response in the rat CNS after nerve injury.
   Margarita Diez, Nada Abdelmagid*, Karin Harnesk*, Mikael Ström, Olle Lidman, Maria Swanberg, Rickard Lindblom, Faiez Al-Nimer, Maja Jagodic, Tomas Olsson, Fredrik Piehl.

(*These authors contributed equally).
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>Ab</td>
<td>Antibody</td>
</tr>
<tr>
<td>ACI</td>
<td>August Copenhagen Irish</td>
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<tr>
<td>AD</td>
<td>Alzheimer’s disease</td>
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<tr>
<td>AIL</td>
<td>Advanced Intercross Line</td>
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<tr>
<td>APC</td>
<td>Antigen Presenting Cell</td>
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<tr>
<td>BB</td>
<td>Bio Breeding</td>
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<td>BBB</td>
<td>Blood Brain Barrier</td>
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<td>BN</td>
<td>Brown Norway</td>
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<td>C1q</td>
<td>Complement Factor 1q</td>
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<td>C3</td>
<td>Complement Factor 3</td>
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<tr>
<td>CalcR</td>
<td>Calcitonin Receptor</td>
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<tr>
<td>Calcr</td>
<td>Calcitonin Receptor Gene</td>
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<tr>
<td>CD</td>
<td>Cluster of Differentiation</td>
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<td>CD11b/c</td>
<td>Complement Receptor 3</td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
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<tr>
<td>CIS</td>
<td>Clinically Isolated Syndrome</td>
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<tr>
<td>CNS</td>
<td>Central Nervous System</td>
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<tr>
<td>CSF</td>
<td>Cerebrospinal Fluid</td>
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<tr>
<td>DA</td>
<td>Dark Agouti</td>
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<tr>
<td>DAMP</td>
<td>Danger/Damage Associated Molecular Pattern</td>
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<tr>
<td>DC</td>
<td>Dendritic Cell</td>
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<tr>
<td>DNA</td>
<td>Deoxyribonucleic Acid</td>
</tr>
<tr>
<td>dpi</td>
<td>Days Post-Infection</td>
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<tr>
<td>E3</td>
<td>Fawn-Hooded</td>
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<tr>
<td>EAE</td>
<td>Experimental Autoimmune Encephalomyelitis</td>
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<tr>
<td>EBV</td>
<td>Epstein-Barr Virus</td>
</tr>
<tr>
<td>ED1</td>
<td>Cellular marker for the phago-lysosomal membrane</td>
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<tr>
<td>F2</td>
<td>Intercross Generation 2</td>
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<tr>
<td>F&lt;sub&gt;344&lt;/sub&gt;</td>
<td>Fisher 344</td>
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<tr>
<td>GAPDH, Gapdh</td>
<td>Glyceraldehyde 3-Phosphate Dehydrogenase</td>
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<tr>
<td>GFAP</td>
<td>Glial Fibrillary Acidic Protein</td>
</tr>
<tr>
<td>gp</td>
<td>Glycoprotein</td>
</tr>
<tr>
<td>GWAS</td>
<td>Genome-Wide Association Study</td>
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<tr>
<td>hpi</td>
<td>Hours Post-Infection</td>
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<td>HSE</td>
<td>Herpes Simplex Encephalitis</td>
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<td>HSV</td>
<td>Herpes simplex virus</td>
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<tr>
<td>IFN, Ifn</td>
<td>Interferon</td>
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<td>Interferon</td>
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</table>
IHC  Immunohistochemistry
IL, Il  Interleukin
IRF, Irf  Interferon Regulatory Factor
LEW  Lewis
LOD  Logarithm of Odds
mAb  Monoclonal Antibody
MHC I  Major Histocompatibility complex class I
MHC II  Major histocompatibility complex class II
MHC2TA  Major histocompatibility complex Class 2 Transactivator
mRNA  Messenger Ribonucleic Acid
miRNA  Micro Ribonucleic Acid
MOG  Myelin Oligodendrocyte Glycoprotein
MRI  Magnetic Resonance Imaging
MS  Multiple sclerosis
MYD88, Myd88  Myeloid differentiation primary response factor 88
MΦ  Macrophages
NFkB, NfkB  Nuclear Factor kappa B
NLRP1, Nlrp1  NOD-like Receptor Family, Pyrin domain containing 1
NK  Natural Killer
PAMP  Pathogen Associated Molecular Pattern
pAb  Polyclonal Antibody
PCR  Polymerase Chain Reaction
PFU  Plaque Forming Unit
PNS  Peripheral Nervous System
PRR  Pattern Recognition Receptor
PVG  Piebald Virol Glaxo
qRT-PCR  Quantitative Real Time Polymerase Chain Reaction
QTL  Quantitative Trait Locus
RIL  Recombinant Inbred Line
SHR  Spontaneously Hypertensive Rat
SNP  Single Nucleotide Polymorphism
TBI  Traumatic Brain Injury
TLR, Tlr  Toll-Like Receptor
TNF, Tnf  Tumor Necrosis Factor
Tuj1  Mouse Monoclonal Anti-Neuronal Class III β-tubulin
VRA  Ventral Root Avulsion
Vwf  Von Willebrand Factor Gene
WF  Wistar Furth
IMMUNE RESPONSES IN THE CNS

The central nervous system (CNS) is part of the nervous system. It is a remarkably complex organ that integrates electrochemical signals, it receives and coordinates activities throughout the entire body. Despite the nonreplicative properties of the nervous system cells, it is proposed that through evolutionary pressures, this compartment has acquired distinct processes and mechanisms to minimize neurodegeneration. One potential source of damage comes from our immune system, which has the capacity to scan the CNS and periphery for the presence of foreign antigens. The immune system is equipped with numerous effectors mechanisms and can greatly alter the homeostasis and function of the CNS. Pathogen infection, autoimmunity and degeneration can all result in an acute and sometimes chronic inflammation within the CNS. Understanding the specialized functionality of innate and adaptive immune cells within the CNS is critical to the design of more efficacious treatments to mitigate CNS inflammatory conditions (Kang and McGavern 2009).

INNATE IMMUNE ACTIVATION IN RESPONSE TO CNS DISEASES

The innate immunity is the first line of defense in the body after invading pathogens or after sterile insults to the host tissue (Akira 2006). The innate immune cells trigger responses that are characterized as being rapid and non-specific that recognize self (released after injury) and non-self antigens. A number of immune cells are responsible for this early immediate reaction, including macrophages (MΦ), DC, NK and CD8 T cells.

In the past, more interest was dedicated into investigating the roles of the adaptive immune system and its functions. It received a lot of attention due to the fact that it is considered to be more specific to eradicating particular disease causing pathogens or molecules, with its long lasting response and memory generation. Meanwhile, the innate immune system remained ambiguous and was considered to be representing simple responses. Thus, it was neglected and fewer studies focused on investigating its possible regulation and pathogenesis. Remarkably, in recent years an amendment to this perception occurred as it became more plausible that the innate immune system is more sophisticated and complexly controlled, in turn being the main regulator of immune responses and the generation of disease outcomes. Up to date, an augmentation in the number of publications focused on different aspects of innate immune response is seen; in particular after the description of the pattern recognition receptors (PRR).

In this thesis, I will only touch upon the recent findings concerning PRR and their revolutionizing of our understanding of innate immunity.
PATTERN RECOGNITION RECEPTORS

Germline-encoded pattern recognition receptors (PRRs) are responsible for sensing the presence of microorganisms. They recognize structures conserved among microbial species, which are called pathogen-associated molecular patterns (PAMPs). Recent evidence indicates that PRRs are also responsible for recognizing endogenous molecules released from damaged cells, termed danger or damage-associated molecular patterns (DAMPS). Currently, four different classes of PRR families have been identified. These families include transmembrane proteins such as the Toll-like receptors (TLRs) and C-type lectin receptors (CLRs), as well as cytoplasmic proteins such as the Retinoic acid-inducible gene (RIG)-I-like receptors (RLRs) and NOD-like receptors (NLRs) (Takeuchi and Akira 2010; Thompson, Kaminski et al. 2011).

These PRRs are expressed in various immune cells including macrophages, DCs and in the CNS by microglia (Walter and Neumann 2009), astrocytes (Farina, Aloisi et al. 2007) and in non-immune cells. PAMPs or DAMPs detection by PRRs upregulates the transcription of genes involved in inflammatory responses. These genes encode proinflammatory cytokines, such as TNFα, IL-1β, IL-6; type I interferons such as IFNα/β and chemokines. These cytokines are pleiotropic proteins with multiple functions regulating the apoptosis of inflammatory tissues, modifying vascular endothelial permeability, recruiting peripheral blood mononuclear cells (PBMCs) to inflamed tissues, and inducing the production of acute-phase proteins.

TOLL-LIKE RECEPTORS (TLRs)

The last decade witnessed an augment in the amount of work focused to explore the roles of Toll-like receptors (TLRs) function. The 2011 Nobel Prize in physiology or medicine was awarded to Jules A. Hoffmann and Bruce A. Beutler for their discoveries concerning the activation of innate immunity. Hoffmann discovered the function of the fruit fly Toll gene in innate immunity (Lemaitre, Nicolas et al. 1996). Its mammalian homologs, the Toll-like receptors, were discovered by Beutler (Poltorak, He et al. 1998). The Toll gene was first described by Hashimoto as a gene critical for the establishment of dorsal-ventral axis in the fruit fly embryo (Hashimoto, Hudson et al. 1988).

A number of TLRs (TLR1 – 11) have been identified in mammals, which trigger innate and modify adaptive immune responses (Blasius and Beutler 2010; Kawai and Akira 2011). TLRs are transmembrane glycoproteins with leucine-rich motifs in the extracellular domain and a cytoplasmic signaling domain, or endolysosomal proteins; containing the Toll/IL-1 receptor (TIR) domain. After binding to its ligand, TLRs dimerize and undergo conformational changes that lead to the induction of intracellular complex signaling cascades (Figure 1).

Each TLR has one or more binding ligand(s) identified (Table 1). TLR1 and TLR2 are located in the plasma membrane of cells. They dimerize to recognize triacylated lipoproteins, derived from bacterial cell wall. TLR2 binds the largest range of PAMPs and DAMPs, including lipoproteins and peptidoglycans from bacteria possessing cell
walls, lipoteichoic acid (LTA) from the cell wall of gram-positive bacteria, zymosan from yeast cell wall and glycoproteins from viral envelope such as HSV-1 (Dasgupta, Chentoufi et al. 2011). TLR3 located in endosomes, recognizes viral double-stranded RNA (dsRNA) and its synthetic analog polyinosine-deoxycytidylic acid (polyI:C). TLR4, TLR5 and TLR6 are located in the cell membrane. TLR4 the receptor for lipopolysaccharide (LPS) derived from the outer cell wall of gram-negative bacteria. TLR5 binds bacterial flagellin, while TLR6 forms heterodimers with TLR2 and recognizes diacylated lipoproteins and LTA. TLR7, TLR8 and TLR9 are located inside the endosomes. TLR7 and TLR8 mediate responses to single-stranded RNA (ssRNA) found in virus-infected cells. TLR9 recognizes bacterial and viral DNA containing unmethylated CpG motifs (Takeda, Miyazaki et al. 2011) (Figure 1). Finally, TLR11 located in plasma membranes, recognize uropathogenic bacteria and a profilin-like protein derived from the parasite Toxoplasma gondii (Pifer and Yarovinsky 2011) (Table 1).

TLRs signal through five adaptor proteins known as myeloid differentiation factor-88 (MyD88), MAL, TRIF, TRAM and SARM. MyD88 is involved in the activation of most TLRs, especially TLR2, TLR4, TLR5, TLR7, TLR9 and IL-1R1 downstream signaling. It has been shown also to be essential for the activation of the transcription factors nuclear factor-κB (NF-κB) and interferon regulatory factor-7 (IRF-7) (O’Neill and Bowie 2007; Lehnardt 2010). TLR3 signals through a MyD88-independent pathway, involving the adaptor molecules TRIF, followed by TRIF-related adaptor molecule (TRAM3). These molecules in turn activate TBK1 which comprises a family of IκB kinase that phosphorylates IRF3 and IRF7 to induce the release of type I IFN (Figure 1).

Figure 1. Schematic illustration of the complex TLRs signaling pathways, including the main adaptor molecules and transcription factors involved in cytokines release. Adapted from (Kawai and Akira 2011).
The different TLRs activated signaling pathways lead to the upregulation of the proinflammatory cytokines, chemokines, nitric oxides which are necessary for DC maturation, NK cells activation, Ab production and cytotoxic T cells differentiation. Thus, TLR signaling is essential for innate immune response initiation and in turn a key player of adaptive immune system activation (Akira 2006; Takeuchi and Akira 2010).

**RIG-I-LIKE RECEPTORS (RLRs)**

The RLR family is composed of RIG-I, melanoma differentiation-associated gene 5 (MDA5), and LGP2. RLRs are composed of two N-terminal caspase recruitment domains (CARDs), a central DEAD box helicase/ATPase domain, and a C-terminal regulatory domain. They are localized in the cytoplasm and recognize the genomic RNA of dsRNA viruses and dsRNA generated as the replication intermediate of ssRNA viruses. The expression of RLRs is greatly enhanced in response to type I IFN stimulation or virus infection (Takeuchi and Akira 2010) (Table 1).

**NOD-LIKE RECEPTORS (NLRs)**

The Nucleotide-Binding Oligomerization Domain-Like Receptors (NLR) family consists of cytoplasmic pathogen sensors that are composed of a central nucleotide-binding domain and C-terminal leucine-rich repeats. The N-terminal portions of most NLRs harbor protein-binding motifs, such as CARDs, a pyrin domain, and a baculovirus inhibitor of apoptosis protein repeat (BIR) domain (Takeuchi and Akira 2010). NLRs harboring a pyrin domain or a BIR domain in their N terminus are not involved in the transcriptional activation of inflammatory mediators and are components of the inflammasome (Hanamsagar, Hanke et al. 2012) that regulates caspase-1 activation.

The NLR family include five main members; NLRA, NLRB, NLRC, NLRX and NLRP. The activated different NLRs together with the adapter molecule ASC trigger the assembly of large caspase-1 activating complexes termed inflammasomes (Martinon, Mayor et al. 2009; Kersse, Bertrand et al. 2011).

The NLRA (CIITA; MHC II trasactivator) regulates MHC II expression, which was associated with several autoimmune conditions (Swanberg, Lidman et al. 2005); NLRB has apoptotic functions. NLRC includes NOD1 and NOD2, which harbor CARDs in addition to NOD and LRR domains; they activate NF-κB via an adaptor, RIP2/RICK. NOD1 and NOD2 induce transcriptional upregulation of proinflammatory cytokine genes. NOD1 and NOD2 recognize the structures of bacterial peptidoglycans (Table 1) and were shown to influence the progression of EAE, experimental MS model (Shaw, Barr et al. 2011).

NLRP family consists of 14 genes (NLRP1 – 14) which are involved in NF-κB pathways inhibition, pyroptosis and autophagy. NLRP1 and NLRP5 are implicated in CNS injury (Frederick Lo, Ning et al. 2008), while NLRP1 is associated with Alzheimer’s disease (Pontillo, Catamo et al. 2011) and autoimmune diseases such as Addison’s disease and type I diabetes (Magitta, Boe Wolff et al. 2009). NLRP3 is associated with autoimmune diseases (Mason, Beck et al. 2012). NLRs are involved in the induction of the proinflammatory cytokines IL-1β and IL-18 release.
C-TYPE LECTIN RECEPTORS (CLRs)

CLRs comprise a transmembrane receptor family characterized by the presence of a carbohydrate-binding domain. CLRs recognize carbohydrates on microorganisms such as viruses, bacteria, and fungi. CLRs induce an immune response either by stimulating the production of proinflammatory cytokines or inhibiting the TLR-mediated immune complexes.

CLRs genes have been described to be associated with the regulation of autoimmune diseases such as rheumatoid arthritis in rats and humans (Backdahl, Guo et al. 2009; Guo, Verdrengh et al. 2009).

Dectin-1 and dectin-2 are immunoreceptor tyrosine-based activation motif (ITAM)-coupled CLRs responsible for sensing β-glucans from fungi. DCs activated by dectin-1 or dectin-2 are able to instruct T cells to confer protective immunity against *Candida albicans*. The macrophage C-type lectin MINCLE (also known as Clec4e and Clecsf9) senses infection by fungi such as Malassezia and Candida. In addition, MINCLE is responsible for the detection of an endogenous protein, spliceosome-associated protein 130 (SAP130), which is a component of U2 snRNP from necrotic host cells (Takeuchi and Akira 2010) (Table 1).

<table>
<thead>
<tr>
<th>PRR</th>
<th>Localization</th>
<th>Ligand</th>
<th>Origin of the Ligand</th>
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<tr>
<td><strong>Toll-Like Receptors</strong></td>
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<tr>
<td>TLR1</td>
<td>Plasma membrane</td>
<td>Triacyl lipoprotein</td>
<td>Bacteria</td>
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<td>TLR2</td>
<td>Plasma membrane</td>
<td>Lipoprotein</td>
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<td>TLR3</td>
<td>Endolysosome</td>
<td>dsRNA</td>
<td>Virus</td>
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<td>LPS</td>
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<td>Plasma membrane</td>
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<td>CpG-DNA</td>
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<td>Plasma membrane</td>
<td>Profilin-like molecule</td>
<td>Protozoa</td>
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<td>RIG-I</td>
<td>Cytoplasm</td>
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<td>MDA5</td>
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<td>iE-DAP</td>
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<td>NOD2</td>
<td>Cytoplasm</td>
<td>MDP</td>
<td>Bacteria</td>
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<td><strong>C-type Lectin Receptors</strong></td>
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<tr>
<td>Dectin-1</td>
<td>Plasma membrane</td>
<td>β-Glucan</td>
<td>Fungi</td>
</tr>
<tr>
<td>Dectin-2</td>
<td>Plasma membrane</td>
<td>B-Glucan</td>
<td>Fungi</td>
</tr>
<tr>
<td>MINCLE</td>
<td>Plasma membrane</td>
<td>SAP130</td>
<td>Self, fungi</td>
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</table>

Table 1. Summary of the different PRR and their associated ligands.
INFECTIONS OF THE NERVOUS SYSTEM

Different infecting pathogens have preferential susceptibility to infect different host organs, such as in the nervous system certain pathogens target the PNS while others target the CNS. Despite the immune-privileged status of the CNS and tight segregation of vascular components through the blood brain barrier (BBB) (Kang and McGavern 2010), many pathogens can still infect the nervous system through the blood stream, or the direct extension from the cranial nerves, ears, nasopharyngeal tract or in immune-compromised individuals. Many pathogens invade host cells through binding to extracellular matrix proteins (Singh, Fleury et al. 2012). An infectious disease depends on the complex interaction between the virulence of the infectious agent, the susceptibility of the host and the nature of their shared environment (Davies and Thwaites 2011). Thus, to prevent devastating effects on the CNS caused by these pathogens, early diagnosis of the causative agents through blood and CSF examination together with imaging are essential to provide early effective treatments.

INFECTIONS OF THE PERIPHERAL NERVOUS SYSTEM

The most common infections of sensory peripheral nerves are caused by viruses. Common Herpes virus infecting the PNS include Varicella zoster virus (VZV) and HSV latent infections (Schmidbauer, Budka et al. 1992). In a recent study, it was suggested that the downregulation of IRF3 pathway secreting type I IFN are responsible for VZV pathogenesis (Zhu, Zheng et al. 2011).

In addition, PNS infection can originate from mycobacterial infection caused by Mycobacterium leprae causing Leprosy (Hansen’s disease) which is a chronic disease currently associated with poverty (Duthie, Gillis et al. 2011). The initial symptoms of leprosy include hypopigmented skin lesions with loss of sensation and inflamed nerves. M. leprae infects Schwann cells of peripheral neurons and TLR2 recognition triggers apoptosis (Walsh, Portaels et al. 2010). TLR1/2, TLR4 and TLR9 signaling are important for leprosy control. Genetic polymorphisms in some TLR genes were found to be associated with leprosy and tuberculosis (TB) infection (Hart and Tapping 2012).

Another infection caused by mycobacterial infection is Buruli ulcer. It is caused by Mycobacterium ulcerans which is a neglected tropical disease characterized by painless necrotizing skin ulcers found in the limbs that could reach the bones and joints causing serious deformities. Mycolactone toxin secreted by the mycobacterium inhibits immune activation through macrophages, monocytes, B cells, and T cells by inhibiting production of IL-1, IL-2, IL-6, IL-10, TNF-a, interferon-γ (IFN-γ), and the chemokine IL-8 (Walsh, Portaels et al. 2010). Thus the patients have no fever or pain around the lesions. It is hypothesized that mycolactone toxin released could possibly also affect peripheral neurons leading to the loss of sensation; this will be of interest to be explored in depth to understand the exact mechanism. In
a recent study, it is suggested that UV light can degrade the effects of the toxin released by the mycobacterium, thus the wounds should be exposed to light to heal (Marion, Prado et al. 2012).

INFECTIONS OF THE CENTRAL NERVOUS SYSTEM

Acute infections and inflammation of the meninges and brain tissue are referred to as meningitis and encephalitis, respectively. A diverse spectrum of pathogens are capable of causing CNS disease, such as bacteria, viruses, fungi and parasites.

Bacterial CNS infections mostly cause meningitis, such as Streptococcous pneumonia (Hoffmann, Becker et al. 2007), Neisseria meningitides, Staphylococcous aureus leading to neuronal damage and loss through TLR2 dependent microglia activation and bacterial hydrogen peroxide release causing apoptosis (Echchannaoui, Frei et al. 2002; Koedel, Angele et al. 2003; Hoffmann, Braun et al. 2007; Lehnardt, Wennekamp et al. 2007; Weber and Tuomanen 2007).

Mycobacterium tuberculosis causes brain granulomas (tuberculoma), abscess and TB meningitis, which is the most severe form of tuberculosis infection (Cherian and Thomas 2011). Immune activation through NF-κB signaling and inducible nitric oxide synthase (iNOS) in lesions is detected in a murine model of TB (Zucchi, Pelegrini-da-Silva et al. 2012). CNS-TB infection is currently more associated with HIV infection in immunocompromised individuals (Nelson and Zunt 2011) and epileptic seizures occurrence (AlSemari, Baz et al. 2012). Mendelian mycobacterial susceptibility genes have been identified by positional cloning including the immune related genes IFNGR1, IFNGR2, STAT1 and IL-12B (Casanova and Abel 2002).

Borrelia burgdorferi infection is transmitted through ticks and is more common in the northern latitudes (Markeljevic, Sarac et al. 2011). The neurological symptoms range
from lymphocytic meningoradiculoneuritis, cranial neuritis, encephalitis, transverse myelitis. In the chronic stage after the initial infection, other neurologic complications may occur, such as encephalomyelitis, epileptic seizures, cognitive impairment, peripheral neuropathy and psychiatric disturbances. IL-1β and caspase-1 secretion by monocytes take part in control of the infection (Bachmann, Horn et al. 2010) and elevated levels of CXCL13 were measured in CSF of patients, which could be used as a diagnostic biomarker (Khademi, Kockum et al. 2010; Schmidt, Plate et al. 2011).

Extracellular and intracellular parasites can cross the BBB and cause severe life threatening infections of the CNS (Masocha and Kristensson 2012) which are common infections of the Tropics. These include the protozoan infections by Trypanosoma brucei causing disruption of sleeping patterns (Malvy and Chappuis 2011) and elevated proinflammatory cytokines release (Rodgers 2010). Toxoplastic encephalitis is caused by Toxoplasma gondii, where the NOD-like receptor (NALP1) influences susceptibility to infection and proinflammatory cytokines release (Witola, Mui et al. 2011).

Plasmodium falciparum infection causes cerebral malaria (CM) in the CNS, which is characterized by cognitive impairment and epileptic seizures. Recently, polymorphisms in the ADAMTS13 gene were found to be associated with cerebral malaria (Kraisin, Naka et al. 2011). ADAMTS13 (a disintegrin and metalloproteinase with a thrombospondin type 1 motif, member 13) is known to cleave and regulate platelet-decorated ultra-large von Willebrand factor (ULVWF) strings. Increased levels of plasma VWF have been reported to be released in cerebral malaria patients after endothelial activation after Plasmodium falciparum infection (Hollestelle, Donkor et al. 2006; de Mast, Groot et al. 2007; Larkin, de Laat et al. 2009; Bridges, Bunn et al. 2010). Despite the controversy on the influence of multiple TLRs signaling on CM pathogenesis (Carty and Bowie 2011), a polymorphism in TLR9 has been associated with altered levels of IFNγ in children with CM (Sam-Agudu, Greene et al. 2010).

CNS VIRAL INFECTIONS

Viral infections are a common cause of human diseases, and can lead to several CNS acute or persistent conditions (McGavern and Kang 2011). A number of neurotropic virus genera can induce severe neuronal dysfunction, degeneration often leading to life threatening consequences to the host. Many RNA and DNA viruses enter the CNS by evading the BBB due to axonal transport from the periphery (Denizot, Neal et al. 2012). They induce CNS injury by direct replication and lysis of host cells; by activation of innate PRRs pathways and adaptive response leading to apoptosis or autophagy induction (Amor, Puentes et al. 2010).

The most common viruses causing encephalitis belong to the Flaviviridae family including West Nile virus (WNV) (Denizot, Neal et al. 2012), Japanese encephalitis virus (Ghosal, Das et al. 2007) and Tick-borne encephalitis virus (TBEV) (Hubalek and Rudolf 2012) that lead to an increase in proinflammatory cytokines release resulting in neuronal death (Bardina and Lim 2012). WNV can also lead to autoimmune
diseases, presenting with various neuromuscular conditions such as Guillain-Barré syndrome (GBS) and other demyelinating neuropathies (Leis and Stokic 2012). These viruses belong to the arbovirus genus of RNA viruses that are transmitted by arthropods such as mosquitoes and ticks.

Enteroviruses are a genus of ssRNA viruses which belong to the Picornaviridae family. They include the coxsackie virus that causes CNS disorders (Denizot, Neal et al. 2012). In addition, polio virus, which enters the CNS, infects motor neurons and activates immune reactions causing apoptosis, leading to flaccid paralysis (Ohka, Nihei et al. 2012). Poliomyelitis cases are still prevalent in some parts of the developing world despite the effective oral polio vaccine (OPV) given to all children below 5 years of age (Thompson and Tebbens 2012).

In rabies viral infection there is evidence for TLR3 activation in the cerebral cortex that causes neuronal destruction and cognitive changes (Jackson, Rossiter et al. 2006). However, another report indicates the involvement of mainly TLR7 in rabies pathogenesis in mice (Li, Faber et al. 2011).

The common childhood disease due to infection with measles virus (MV) can cause the complication of CNS impairment. A possibility of an autoimmune cause leading to the condition known as acute measles encephalomyelitis due to myelin damage is proposed (Liebert 1997).

CNS latent infections include the infection with JC virus. It is a polyomavirus which is associated with the risk of developing progressive multifocal leukoencephalopathy (PML), which occurs mostly as a complication after Multiple sclerosis (MS) treatment with natalizumab or in immunocompromised individuals such as AIDS patients (Iacobaeus, Ryschkewitsch et al. 2009; Major 2010; Sorensen, Bertolotto et al. 2012).

In addition, a complication of lentivirus infection by Human immune-deficiency virus (HIV) is neurodegeneration of brain parenchyma causing the condition known as HIV-associated dementia (HAD) (Gonzalez-Scarano and Martin-Garcia 2005). Infected macrophages invade the CNS and induce chemokine receptors and inflammatory mediators release causing neuronal deficits (Kaul and Lipton 2006).

**HERPES VIRUSES**

*Herpesviridae* is a dsDNA (125-250 kbp) virus family characterized mainly by causing latent, recurrent infections. Their virions conform to a common architecture. The genome is confined at high density within a thick-walled icosahedral capsid. The envelope consists of a membrane in which 11 different viral glycoproteins are implanted. Between the capsid and the envelope is a capacious compartment called the tegument that accommodates ~20-40 different viral proteins destined for delivery into a host cell (Cardone, Heymann et al. 2012).
*Herpesviridae* is subdivided into different genera including *α-herpesvirinae* which remain latent in neurons and includes HSV-1, HSV-2 and VZV; *β-herpesvirinae* which remain latent in mononuclear cells and includes *Cytomegalovirus* (CMV) and *Human Herpesvirus 6* (HHV-6) and finally *γ-herpesvirinae* which remain latent in B cells such and *Epstein-Barr virus* (EBV) and *Kaposi sarcoma associated herpesvirus* or (HHV-8).

Herpes viruses’ infections have been implicated in a number of neurological and autoimmune disorders, such as encephalitis and MS. Herpes viruses lead to MΦ activation through TLR dependant (Bowie and Haga 2005) or independent pathways (Malmgaard, Melchjorsen et al. 2004).

Encephalitis is most commonly caused by HSV-1 infection (Kimberlin 2003). It is the commonest cause of encephalitis in the western world (Aurelius, Forsgren et al. 1993), while Herpes simplex meningitis is mostly caused by HSV-2, but can cause a few cases of encephalitis as well. A number of studies have associated the HSV-1 persistence in brain cells to the risk of developing Alzheimer’s disease (Itzhaki, Cosby et al. 2008; Mori 2010), as HSV-1 is proposed to induce amyloid beta (Aβ) accumulation, tau phosphorylation in neurons and autophagy disruption (Miklossy 2011). A recent study showed that TLR3 deficiency in astrocytes rendered infection with HSV-2 in the CNS in mice (Reinert, Harder et al. 2012).

*CMV infection* (Cannon, Schmid et al. 2010; Varani and Landini 2011; Waubant, Mowry et al. 2011) is associated with autoimmune diseases as well as malignant glioblastoma and medulloblastoma tumours development in the brain (Soderberg-Naucler 2008; Baryawno, Rahbar et al. 2011). HHV-6 is associated with neurological disorders such as epilepsy, encephalitis and MS (Fotheringham, Donati et al. 2007; Hammarstedt, Ahlqvist et al. 2007; Karatas, Gur et al. 2008).

While, EBV infection has been associated with MS risk in recent years (Ascherio and Munger 2007; Serafini, Rosicarelli et al. 2007; Salvetti, Giovannoni et al. 2009; Lauer 2010; Sundqvist, Sundstrom et al. 2012).

**Focus on Herpes Simplex Virus Type -1**

In this thesis, we only conducted our studies using a neurovirulent strain of HSV-1. HSV-1 infects the majority of the population inducing cold sores in affected individuals. Human necropsy studies suggest that viral DNA can be isolated from nearly all post-mortem brains, implying that with time most individuals are infected. However, in younger people (between 20 to 49 years of age) HSV-1 serology suggests that only 50 – 60% have been infected, as reported by independent studies in different populations (Malvy, Ezzedine et al. 2007; Tunback, Bergstrom et al. 2007; Karjala, Neal et al. 2011). These studies also demonstrate an increasing prevalence of HSV-1 infection with age. Thus, different susceptibility patterns to establish HSV-1 infection are possible in the human population.
It is known that entry of HSV-1 into the host cell depends on the interaction of several glycoproteins on the surface of the enveloped virus with receptors on the surface of the host cell. The viral envelope glycoprotein C (gC) binds to heparan sulfate, present on the cell surface and glycoprotein D (gD) binds specifically to at least one of three known entry receptors. These include herpes virus entry mediator (HVEM), nectin-1 and 3-O sulfated heparan sulfate. Once bound to the HVEM, gD changes its conformation and interacts with viral glycoproteins H (gH) and L (gL), which form a complex. Subsequently, glycoprotein B (gB) interacts with the gH/gL complex and creates an entry pore for the viral capsid (Subramanian and Geraghty 2007).

PRRs are essential for recognition of HSV-1 and induction of type I interferon release (Rasmussen, Jensen et al. 2009), especially TLR2 and TLR9 (Dasgupta, Chentoufi et al. 2011; Takeda, Miyazaki et al. 2011).

**Herpes Simplex Encephalitis**

HSE is a rare complication of HSV-1 infection, with an incidence of 2 to 3 individuals per million per year, yet it still remains the commonest cause of sporadic encephalitis in the western world (Hjalmarsson, Blomqvist et al. 2007; Sancho-Shimizu, Zhang et al. 2007; Hjalmarsson, Granath et al. 2009). HSE may result from a primary, but more commonly from an earlier, reactivated HSV-1 infection. The age distribution of HSE differs from that of primary HSV-1 infection (Hjalmarsson, Blomqvist et al. 2007). Since the 1960s the pathogenesis of HSE has been hypothesized that the virus either crosses the BBB or through neural pathways of infection (Johnson 1964).

The clinical presentation of HSE is initiated by acute onset of fever, seizures, altered state of consciousness, behavioral/personality change and confusion. The early diagnosis is essential to determine the prognosis and outcome of disease. Imaging by MRI or CT scan show temporal lobe oedema and haemorrhage. Examination of CSF for HSV DNA is the definite confirmation of diagnosis and serological analysis of HSV antibodies. Increased cell count, pleocytosis and red blood cells can be found in CSF from HSE patients. Antiviral treatment by acyclovir is the treatment of choice (Sköldenberg 1991; Skoldenberg 1996; Whitley 2006). The disease has a tendency to relapse or to have a progressive course (De Tiege, Rozenberg et al. 2006; Sköldenberg, Aurelius et al. 2006).

A number of studies have shown that microglial immune responses directed towards HSV-1 infection are dependent on TLR2 and TLR9 signaling to produce a range of cytokines (IFNα/β, TNFα, IL-1β and IL-6) and chemokines causing neuronal damage through oxidative stress and apoptosis (Dvorak, Martinez-Torres et al. 2004; Kurt-Jones, Chan et al. 2004; Aravalli, Hu et al. 2005; Wickham, Lu et al. 2005; Aravalli, Hu et al. 2007; Lundberg, Welander et al. 2007; Sergerie, Rivest et al. 2007; Lundberg, Ramakrishna et al. 2008; Marques, Cheenan et al. 2008; Sorensen, Reinert et al. 2008; Vilela, Mansur et al. 2008; Schachtele, Hu et al. 2010).
Host Genetics Regulating HSE

In spite of the scarce number of HSE cases per year, the host factors determining disease susceptibility remain elusive. An important question remaining is why certain individuals are prone to developing HSE and what are the determining factors leading to the condition.

Genetic susceptibility to HSE in mice

Animal studies using HSE mice models through forward and reverse genetics revealed a variety of host immune genes regulating protection against HSV-1, especially IFNα and –β (Sancho-Shimizu, Zhang et al. 2007). A variety of models were established using different routes of infection and virus strains, which could explain the discrepancies behind the findings from separate groups. Infection routes varied from ocular (Cleator, Klapper et al. 1987), intranasal, cutaneous, intracerebral (Ben-Hur, Itzik et al. 2004), intraperitoneal or intravenous infections.

In addition, strain-dependent susceptibility was determined by works from Lopez in the mid 70s, where HSV-1 infection led to lethal encephalitis in susceptible A/J and BALB/c and resistance in C57BL/6J mice (Lopez 1975). The major histocompatibility complex (MHC) did not influence resistance or susceptibility to HSV-1 infection in inbred and congenic mice strains (Lopez 1980; Kastrukoff, Lau et al. 1986). In an experimental mouse model for HSE, a natural killer (NK) complex-linked locus, Rhs1 (resistance to Herpes simplex virus 1), on chromosome 6 has been identified to control resistance to acute and latent HSV-1 infections resulting in HSE (Pereira, Scalzo et al. 2001). However, in another mouse model of corneal HSV-1 infection an additional locus on chromosome 6, Hrl (Herpes resistance locus) influencing survival after HSV-1 infection in C57BL/6J mice and the HSE development in 129S6SvEv/Tac mice (Lundberg, Welander et al. 2003). Hrl is closely linked to the Tnfrsf1a gene encoding the p55 TNF receptor, and controls survival in male mice. A strong sex bias was detected, which lead to the identification of Sml (sex modifier locus) that enhances female resistance.

Genetic susceptibility to HSE in humans

Most of the identified innate immune related genes regulating susceptibility of HSE in humans come from Casanova’s group body of work in sequencing pedigrees of paediatrics cases. Deficiencies in STAT1 (Dupuis, Jouanguy et al. 2003), which is a downstream regulator of IFN response genes and NEMO (Puel, Reichenbach et al. 2006), which is a NF-κB regulator are reported to increase susceptibility to several infections including TB and HSE. However, polymorphisms in the UNC-93B and TLR3 genes were shown to regulate specifically susceptibility to HSE in small human pedigrees, in which the production of IFN-α/β and -λ dependent on UNC-93B protein expression controls HSV-1 by TLR3-dependent and/or TLR-independent pathways (Casrouge, Zhang et al. 2006; Zhang, Jouanguy et al. 2007; Guo, Audry et al. 2011).
UNC-93B interacts with TLR3, TLR7 and TLR9 in cells. An autosomal dominant deficiency in the adaptor molecule TRAF3 (TNF receptor associated factor 3) which is a downstream signaling molecule of TLR3 has also been reported in 1 human case (Perez de Diego, Sancho-Shimizu et al. 2010). In addition, recently autosomal dominant and recessive deficiencies in TRIF, an adaptor molecule involved in downstream signaling of TLRs, have been reported in a few children with HSE (Sancho-Shimizu, Perez de Diego et al. 2011). It is important to realise that these are rare variants in few cases of paediatric HSE.

In the future more genes will be reported by the use of genome wide association studies (GWAS) and exon sequencing, including larger cohorts of HSE cases, which will requires collaborative efforts from different groups worldwide.
NEUROINFLAMMATION AFTER CNS INJURIES

The acute CNS inflammatory responses following sterile mechanical or molecular injuries and insults contribute to secondary injury which results in the further expansion of lesions leading to neurological loss of function and neurodegeneration.

Pattern recognition receptors (PRR), especially TLRs (Carpentier, Duncan et al. 2008; Amor, Puentes et al. 2010) mediated signaling cascade of events after CNS injuries lead to the activation of innate immune responses including the migration of microglia and active recruitment of circulating leukocytes (Finsen and Owens 2011; Fung, Vizcaychipi et al. 2012). More evidence of involvement of CNS resident cells such as astrocytes, neurons and oligodendrocytes, can also express multiple TLRs upon activation (Bsibsi, Ravid et al. 2002; van Noort and Bsibsi 2009; Ransohoff and Brown 2012).

Intriguingly, the contribution of the local immune cells such as microglia (Marin-Teva, Cuadros et al. 2012) compared to infiltrating macrophages in the CNS damage is still equivocal. In a recent study, it was demonstrated that two subpopulations of macrophages are present in the lesion after spinal cord injury in transgenic mice (Mawhinney, Thawer et al. 2012). Thus, still the contribution of CNS inherent microglia compared to infiltration of monocytes in cellular infiltrations occurring after nerve injuries is unclear.

Microglia activation in CNS conditions has been the focus of several groups to determine the role in degeneration and regeneration (Neumann, Kotter et al. 2009; Walter and Neumann 2009; Amor, Puentes et al. 2010; Lehnardt 2010). Moreover, the role of TLRs in the CNS is starting to be unraveled and gain more interest (Rivest 2009; van Noort and Bsibsi 2009; Carty and Bowie 2011; Wang, Lin et al. 2011).

In sterile CNS injury, a number of danger associated molecular patterns (DAMPs) are proposed as TLR ligands. These endogenous molecules include heat shock proteins (HSP), high-motility group box 1 protein (HMGB1), surfactant protein A and D, hyaluronan and fibrinogen (Beg 2002), uric acid, chromatin, adenosine and ATP. Other DAMPs include modified or misfolded proteins such as amyloid-beta (Aβ) (Amor, Puentes et al. 2010). The chaperone HSP60 released for dying CNS cells activates microglia through TLR4 and MyD88 pathway (Lehnardt, Schott et al. 2008). This could be a common neuronal injury signaling pathway that could link neuroinflammation with neurodegeneration.

In neurodegenerative disorders such as Alzheimer’s disease (AD), Parkinson’s disease (PD) and Amyotrophic lateral sclerosis (ALS) microglial activation through TLR2 and TLR3 signaling are suggested to induce neuronal damage progression (Jackson, Rossiter et al. 2006; Letiembre, Liu et al. 2009). TLR4 is also increased in microglia in AD brains (Walter, Letiembre et al. 2007) and PD (Panaro, Lofrumento et al. 2008). Interestingly, a genetic variant of the NOD-Like receptor, NLRP1 was found to be associated with AD (Pontillo, Catamo et al. 2011).
In stroke and cerebral ischaemia up-regulation of TLR2 (Lehnardt, Lehmann et al. 2007), TLR3 and TLR4 were detected (Jackson, Rossiter et al. 2006; Letiembre, Liu et al. 2009), whereas in traumatic brain injury (TBI) (Koedel, Merbt et al. 2007), MyD88 activation of inflammatory processes independent of TLR2/TLR4 involvement.

Multiple sclerosis (MS) is an autoimmune disease in which genetic and environmental factors are involved in the pathogenesis. Microglial activation is mediated through TLR4, TLR9 and MyD88 signaling (Prinz, Garbe et al. 2006; Marta, Andersson et al. 2008). NLRs are highly expressed in EAE (MS model), although their exact role is still not defined. In one EAE study, it was shown the NLRP3 deficient mice had a delayed onset to EAE compared to their wild-type counterparts (Gris, Ye et al. 2010). In contrast, another study suggests that EAE induction depends on ASC and caspase-1, but not NLRP3 (Shaw, Lukens et al. 2010). However, association of NLRP1 to autoimmune diseases was suggested, although it was less obvious in MS (Magitta, Boe Wolff et al. 2009).

Even though, most immune responses within the CNS could lead to progressive neuropathology which ends with irreversible neurodegeneration, the neuroprotective roles of innate immunity and neuronal repair needs to be further elucidated. It will be of vital importance to define the common CNS disorders regulatory pathways involved in the initiation of glial activation. A better understanding of these pathways regulating pathogenesis will play a key role in development of effective therapeutics to prevent neurodegeneration.
USE OF EXPERIMENTAL ANIMAL POPULATIONS TO STUDY GENETIC DISORDERS

A main focus in understanding the human genome is to discover and interpret all functional elements encoded within the sequence. Protein encoding sequence represents 1.5% of the human genome only. Comparative genomics analysis with several mammalian species including the mouse (Waterston, Lindblad-Toh et al. 2002), rat (Gibbs, Weinstock et al. 2004) and other species (Karlsson and Lindblad-Toh 2008) genomes showed that at least 5% is under purifying selection and thus probably functional, of which, 3.5% consists of non-coding elements with probable regulatory roles (Lindblad-Toh, Garber et al. 2011).

Complex diseases result from an interaction between a number of genetic and environmental factors. However, the predisposing alleles of common complex disorders are heterogeneous and rare; only functionally related and thus more difficult to detect (Holmberg and Lejon 2001).

In recent decades the use of experimental models resembling human diseases in genetic studies enhanced our knowledge about the causative genes and revealed the different molecular mechanisms responsible for the disease phenotypes. It also enabled the identification of better diagnostic and prognostic markers as well as the development of targeted therapies.

A number of strategies could be followed in the genetic screening for the identification of genes regulating different diseases. One approach is positional cloning or ‘forward genetics’ in which the approximate genomic regions regulating a disease phenotype are identified. The exact gene or candidate gene can be investigated further to prove the specific role in the phenotype. The advantage of this technique is the use of an unbiased hypothesis free approach. Another tool used in genetic screening would be ‘reverse genetics’ which is a hypothesis driven approach, in which genes of known functions are manipulated or modulated to interfere with their function. This allows the study of the exact functions of these genes with regard to the disease model being studied, both in-vivo and/or in-vitro.

The advantage of using inbred strains in genetic search is the large size of experimental population that could be used, the minimized heterogeneity, controlled environmental influences, as well as the possibility of investigating the pathogenesis mechanisms through functional studies.

INBRED STRAINS

The breeding of mouse and rat strains for over twenty generations results in a panel of inbred strains that are homozygous throughout their genome. All animals within an inbred strain are genetically identical in all alleles. However, each inbred strain is unique, presenting strain specific characteristics and phenotypes, allowing the study of various traits.
The majority of genetic studies in experimental setups were conducted in mice. Mice and rats share more than 90% of conserved coding sequences and regulatory regions with humans which make them relevant to study human disease. In this thesis, all studies were conducted using inbred rat strains.

WHOLE GENOME SCANS

The intercross breeding between two different inbred strains presenting a different disease phenotype will generate a heterozygous F\textsubscript{1} generation. The F\textsubscript{1} intercross will generate a heterogenous F\textsubscript{2} population used for whole genome scan with several unique recombinations. Genotyping a whole F\textsubscript{2} population and performing linkage analysis of the generated results from all animals allows the identification of new genomic regions, named quantitative trait loci (QTLs) regulating phenotypes. Linkage analysis is based on correlating a certain genotype to a disease phenotype (Figure 2).

![Image of genetic mapping strategies](image)

Figure 2. Schematic illustration for different breeding strategies used in genetic mapping in experimental populations.
BREEDING STRATEGIES

Congenic Strains

To isolate and confirm the influence of an identified QTL and its effect on the phenotype, a congenic strain is usually established. A genomic region of interest is transferred from one donor strain to the background of another recipient strain by backcrossing for 10 generations or more. A congenic strain could be used to dissect the effects of the QTL by functional studies. The effect of interacting alleles will be lost in the congenic and only the influence from the recipient strain alleles.

The breeding of a congenic is time-consuming, thus a faster technique could be applied through the speed congenic approach with marker assisted selection (Wakeland, Morel et al. 1997). When producing a speed congenic strain, the whole genome of offspring generated after each backcross is genotyped. Animals with less genotype from the donor background, while still carrying the region of interest are selected for breeding of the next generations (Figure 2). After selective backcross breeding for N5 to N8 generations, a congenic strain can be generated. In this thesis, we used the speed congenic approach to generate our congenics.

Advanced Intercross Lines

The breeding of inbred strains for more than 7–10 or 12 generations results in more recombinations across the genome between the parental strains background resulting in a better resolution. Using these populations (G7 to G12) allows the narrowing done of the confidence interval (CI) of QTL regions containing several genes (Figure 2). This allows the identification of possible candidate genes.

Recombinant Inbred Lines

Another breeding strategy used for genomic research which proved to be efficient and cost-effective, is the recombinant inbred strains or lines. RILs are made by crossing two inbred strains to obtain an F1 generation, which are then crossed to generate an F2 generation. Breeding pairs from the F2 generation are kept separate and breed consecutively with siblings mating for 20 generations. Each line will have a unique set of recombinations separating them from the other lines (Figure 3).

Thus, rodent RI panels are powerful and permanent resources for genetic mapping that offer the opportunity to accumulate genetic and physiological data over time. A further advantage of RI strains, as with chromosome substitution strains is the ability to study genetically identical biological replicates, which increases trait heritability by reducing environmental variance. Since each RI line is nearly homozygous, its genotype is reproducible and individual genetic variation is minimized. Furthermore,
once a line has been genotyped, this information can be used over and over (Crow 2007).

In the early 1980s, the SHR strain was crossed with the normotensive BN strain for over 60 generations to generate the BXH/HXB panel of RILs (Pravenec, Klir et al. 1989). The BXH/HXB set of RILs were used in this thesis (Figure 3).

Figure 3. Schematic illustration of breeding scheme to generate unique RILs for genetic studies e.g BXH/HXB RILs.
AIMS OF THE THESIS

The overall aim of this thesis was to investigate the influence of host genetics on susceptibility to CNS infections and inflammation using experimental models.

- To perform whole genome linkage analysis in different animal populations in search for genetic regions that regulate viral propagation into the CNS, causing encephalitis.

- To fine map the identified QTL regions and identify candidate genes regulating viral encephalitis.

- To study the host immune responses in different inbred rat strains after CNS viral infection.

- To investigate the host genetic regulation of early innate immune molecules after CNS injuries.
METHODOLOGICAL CONSIDERATIONS

The materials and methods used for generating this thesis are detailed in the respective papers. I here briefly summarize the different techniques used.

THE EXPERIMENTAL MODELS

Herpes Simplex Encephalitis (HSE)

A previously established HSE model was used in paper I - III. HSE was induced by injecting $2 \times 10^6$ PFU of HSV-1 neurovirulent strain subcutaneously into the right-side whiskers area of 45 days rats (Bereczky-Veress, Lidman et al. 2008). Using this model, susceptible inbred strains developed severe clinical HSE symptoms including coordination/balance disturbance, paralysis and death around 5 dpi. All rats were monitored daily for clinical HSE symptoms development.

This *in vivo* model for HSE mimics in several aspects the viral spread seen in human HSE, where the virus starts spreading from the whiskers area of the rats (the labio-facial area in humans), through the trigeminal nerve to the ipsilateral side of the brain stem spreading both to the contralateral side and towards the thalamus, causing severe immune activation and lethal encephalitis at 5 dpi (Bereczky-Veress, Lidman et al. 2008). The HSE model was used in paper I, II and III in this thesis.

Ventral Root Avulsion (VRA)

In paper IV (and preliminary results) rats were subjected to unilateral avulsion of the left lumbar ventral roots (L3- L5) under anaesthesia. Five days after the surgery, rats were euthanized with CO$_2$ and perfused with cold PBS. The meninges of the spinal cord were removed and the L3 segment was dissected out and the ipsilateral ventral quadrant was isolated for expression analysis. The L4- L5 segments were kept intact at -80°C for histological analysis.

Figure 4. (A) A picture of a rat spinal cord at the lumbar level 3-5. (B) A schematic illustration of the unilateral avulsion of L3-L5 in the spinal cord of rats used in VRA model.
The injury results in a lesion between the CNS and PNS boundary, causing local immune activation and leukocytes infiltration leading to neurodegeneration. VRA is a reproducible mechanical injury model that represents the local neuroinflammatory processes and provides the chance to study them in depth.

**Traumatic Brain Injury (TBI)**

TBI model was used in the preliminary results study. Rats underwent traumatic brain contusion as previously described (Feeney, Boyeson et al. 1981; Al Nimer, Beyeen et al. 2011). Briefly, the rats were placed in a stereotactic frame and an anaesthetic was injected subcutaneously in the sagittal midline of the skull prior to the skin incision. A 2 mm craniotomy was drilled 3 mm posterior and 2.3 mm lateral to the bregma and a standardized parietal contusion was made by allowing the 24g weight fall onto a rod with a flat end diameter of 1.8 mm from a 7 cm height (Feeney, Boyeson et al. 1981). The rod compressed the tissue for 3 mm maximum. All animals were euthanized at 6 days after the trauma.

![Figure 5. Schematic illustration of the device used to perform the cortical contusion in rodents. Adapted from (Feeney, Boyeson et al. 1981).](image)

**Experimental Autoimmune Encephalomyelitis (EAE)**

MOG was used for the induction of EAE in the preliminary results study. MOG was emulsified with PBS and Freund’s adjuvant, a 200μl of the emulsion was injected at the tail base subcutaneously in anaesthetized rats. Rats were weighed and followed daily for development of EAE clinical signs and symptoms from 8 days post immunization until the end of the experiment at 35 days. The clinical EAE scoring was used daily to monitor the rats for the development of clinical symptoms, starting with ascending paralysis, reaching complete paralysis and death in susceptible inbred strains.
ANIMAL POPULATIONS

In this thesis, a number of different inbred rat strains and intercrosses have been utilized to conduct the different papers to study genetic regulation and immune responses in viral infection and CNS injury models.

In paper I, inbred rats DA and MHC-congenic PVG\textsuperscript{1AV1} have been used to establish an F\textsubscript{2} cross (DAxPVG\textsubscript{1AV1}). In addition a panel of other inbred rat strains LEW, F\textsubscript{344}, SHR, ACI, WF, E3, BB and BN were used to generate a haplotype map. Moreover, congenic strains DA.PVG\textsuperscript{-Hse1} and DA.PVG\textsuperscript{-Hse1}-R1 with PVG fragments transferred onto DA background were generated and used during this study together with previously generated congenics R21, R11, R2 (Marta, Stridh et al. 2010) and R17 (Guo, Verderengh et al. 2009).

In paper II, the parental strains DA and PVG\textsubscript{1AV1} were used to study the host differences and immune regulation at different time points after infection.

Paper III was conducted using parental SHR and BN inbred rat strains, as well as 29 RILs established from SHR and BN. This is the only study in which we used inbred mice BALB/c, C57BL/6 and C57BL/6\textsuperscript{VWF-/-} knock-out mice (Denis, Methia et al. 1998).

In paper IV and the follow-up study (preliminary results) the parental strains BN and MHC-congenic LEW\textsuperscript{1N} together with an F\textsubscript{2} (BNxLEW\textsuperscript{1N}) population were used. Additionally, in paper IV the F\textsubscript{12} (DAxPVG\textsuperscript{1AV1}) and F\textsubscript{2} (DAxPVG.C) (Lidman, Swanberg et al. 2003) were used. The congenic lines BN.LEWc10-B and BN.LEWc10-E (Lagrange and Fournie 2010) were used in the preliminary results study.

Virus

We used HSV-1 virus strain I-2762 in paper I, II and III which was isolated from a biopsy taken from a male patient on day 2 after onset of the first clinical symptoms of HSE. The patient died 2 days later as a consequence of the encephalitis. The virus was propagated in green monkey kidney (GMK-AH1) cells for maximum two passages, aliquoted and stored at −80°C. The isolate was typed as HSV-1 by ELISA using type-specific mAbs. In previous experimental studies, using this strain showed a high degree of neurovirulence and neuroinvasiveness both in vivo and in vitro (Bergström, Alestig et al. 1990) (Bergström and Lycke 1990).

DNA Isolation and Genotyping

In paper I and IV, genomic DNA was extracted from tail tips using a standard protocol (Laird, Zijderveld et al. 1991). Polymorphic microsatellite markers were selected from available Internet databases: Rat Genome Database, Ensembl and The National
Center for Biotechnology Information and have been used to genotype experimental crosses.

Genotyping was performed using radioactive and fluorescent methods. Radioactive PCR amplification was performed as previously described (Jacob, Brown et al. 1995) with [γ-33P] ATP end-labeled forward primers. The PCR products were size fractioned on 6% polyacrylamide gels and visualized by autoradiography.

Fluorescent PCR amplification was also performed using a standard protocol and PCR products were separated using the electrophoresis capillary sequence and analyzed in the GeneMapper software.

In paper III, all rats from RILs were previously whole genome SNP genotyped.

**Linkage Analysis**

Different diseases or conditions can be regulated by host factors positioned in different genomic regions or QTLs. To study these disease regulating regions (QTLs), the positional cloning approach was used to identify the candidate gene region. Thus, the concept of linkage analysis relies upon linking the phenotypic traits or disease symptoms to the genotype values of the DNA markers to position the QTLs responsible for the disease using different intercrosses. Linkage analysis in paper I, IV and preliminary results was performed using R/qtl package (Broman, Wu et al. 2003).

The logarithm of odds (LOD) represents the level of significance of linkage. Thus the higher the LOD score, the higher the likelihood that the disease regulating gene lies within the QTL region. The confidence interval (CI) was defined by a LOD drop of 1.5 for the nearest markers for the peak marker (Manichaikul, Dupuis et al. 2006).

In paper III, linkage analysis for RILs (Williams, Gu et al. 2001) was performed using WebQTL and the bootstrap analysis was used to evaluate the approximate confidence intervals of the QTL peaks. The QTLs were presented in likelihood ratio statistic (LRS) which can be converted to LOD values by dividing the LRS value by 4.6.

**Sequencing**

Genomic sequencing was performed for the two identified QTLs in paper I and III. Sequencing of *Hse1* region on chromosome 4 was performed for both DA and PVG genomes. In addition, sequencing was performed for *Hse6* region towards the end of chromosome 4 in SHR and BN rat strains. All sequences were generated from genome-wide sequencing.
EXPRESSİON ANALYSIS

qRT-PCR

All rats were perfused transcardially with 50 ml phosphate buffer saline (PBS) or saline containing heparin. Tissues from the whiskers area, trigeminal ganglia, brain stem and spinal cord (L3 segments) were dissected and then disrupted using standard methods for RNA production and then cDNA synthesis, described in details in the respective studies.

qRT-PCR was performed using a three-step PCR protocol with SYBR green as fluorophore. Primers were designed using the Beacon designer 5.0 and the Primer Express of ABI Beacon software. Primer specificity was assessed by product evaluation on silver gel and by running a dissociation curve for each sample.

Relative expression quantification of the mRNA levels was performed using the standard curve method using serial five-fold dilutions from a pool of undiluted samples as standard. Relative amounts of mRNA were calculated as the ratio between expression of the specific target and the expression of the housekeeping genes Gapdh and Hprt. The reference genes referred to as “housekeeping genes” should be expressed in a stable pattern in all cells, not influenced by inflammatory or disease conditions.

Immunohistochemistry

Rats were anaesthesized and transcardially perfused with 50 ml PBS containing heparin followed by 200 ml fixative containing 4% paraformaldehyde (Pease 1962) (Zamboni L. 1967). In paper I – III, the whiskers’ pad and the trigeminal ganglion from the ipsilateral side and the brain stem were dissected.

Paper IV and the preliminary results section focused on the spinal cord. Rats were euthanized by CO₂ and transcardially perfused with 50 ml PBS containing heparin, and spinal cord segments from L4 and L5 were collected and kept at -80°C until use.

To obtain tissue sections for immunohistochemistry, 14µm thick coronal/transversal sections were cut from the different tissues collected in a cryostat and thawed onto microscope glass slides. The slides were subjected to the indirect immunofluorescence staining (Coons 1958) and incubated overnight in humidifying chambers at 4°C with primary antibodies specific for molecules of interest, diluted in PBS containing Triton and sodium azide. Fluorophore labeled secondary antibodies were subsequently used to visualize the protein distribution in brain tissue (brain stem; spinal cord), trigeminal ganglia and whiskers area. After incubation with primary antisera/antibodies, sections were rinsed and incubated with secondary antibodies.
RESULTS AND DISCUSSION

GENETIC REGULATION OF HERPES SIMPLEX ENCEPHALITIS IN RAT

HSE is a rare complication of HSV-1 infection which could be fatal in some cases. Up to date no genes regulating HSE were identified using experimental animal models, mainly mice. Few immune related genes were identified in human pedigree cases.

In our laboratory, in an earlier study a rat model for HSE was established in the inbred DA rat resembling the human HSE disease in several clinical aspects (Bereczky-Veress, Lidman et al. 2008). Interestingly, in this model the inbred PVG rat was completely resistant to the disease displaying reduced or no uptake of viral particles into the peripheral and central nerve compartments respectively.

HSE genetic regulation in F2 (DAxPVG^{1ov1})

In order to identify the gene(s) regulating HSE pathogenesis, in paper I we crossed the susceptible DA and the resistant PVG^{1ov1} rats for two generations and infected 239 rats of the F2 (DAxPVG^{1ov1}) cohort with HSV-1. The total HSE incidence was 15\% in the F2 population; however, a slight difference in the incidence was noticed between the sexes. Females had a lower incidence than males; a similar observation was previously reported in a HSE mouse study where a strong sex bias was detected in the C57BL/6J and 129S6SvEv/Tac cross leading to the identification of Sml (sex modifier locus), which enhanced resistance in females (Lundberg, Welander et al. 2003).

Then, using microsatellite markers we genotyped 180 animals and performed a genome-wide linkage scan which identified a strong QTL, on rat chromosome 4 regulating disease susceptibility, named Hse1. The QTL, Hse1 mainly regulated the HSE incidence as well as onset of disease and correlated with weight changes after infection.

Hse1, is located between the markers D4Kini1 and D4Arb25 (24.3 – 31.1 Mb), with a LOD score of 29.5 and the peak marker is D4Kini3 (27.8 Mb). When stratifying the linkage according to sex, the LOD score of Hse1 in females was found to be lower in females (Figure 6).

The influence of Hse1 was also confirmed by infecting different congenic strains. Hse1 is different from the antigen-presenting lectin-like receptor gene complex (APLEC) located towards the end of chromosome 4, previously reported to have a disease regulatory effect in a different model of HSV-1-induced encephalitis in the DA strain (Guo, Verderench et al. 2009).
Fine mapping of the confidence interval (CI) using more F₂ (DAXPVG) animals to detect recombinant rats in the QTL region as well as haplotype mapping through a panel of different inbred rat strains were performed to narrow down the CI region. The CI through these approaches was narrowed to a region containing about 15 genes.

Sequencing and expression analysis of the genes in the Hse1 interval collectively support the underlying genetic variation to be located in, or adjacent to the calcitonin receptor gene (Calcr). The sequencing of the genes in the narrowed CI showed synonymous SNP variations in PVG rats, mainly in Ccdc132, Calcr, and Tfpi2 genes. Even though these variations do not change the protein sequence, nevertheless, they could influence the transcriptional or translational stability of the genes. However, measuring the mRNA expression levels of these genes, only detected the main differences in Calcr in the whiskers area of the resistant PVG strain. Calcr had low or no expression in the trigeminal ganglia. Thus, the variations present in Calcr are most likely to be influencing the virus cellular infectivity and replication in the periphery.

Further support for the peripheral role of CalcR in regulating HSV-1 replication and propagation was provided by strain-dependent differences in the calcitonin receptor protein tissue localization and in functional studies.

In tissue sections from the whiskers area of naïve and infected rats after 5 dpi, the CalcR staining visualized by IHC was more pronounced in PVG rats. Notably, the tissue localisation of CalcR staining differed between the infected DA and PVG rats. In PVG rats, CalcR was visualized more in the periphery of the epineurium (i.e. the outer connective tissue layer surrounding several nerve fascicles/bundles), whereas in DA rats the staining was mainly present in perineurial cells and in the endoneurium (i.e. the connective tissue layer surrounding a nerve fascicle/bundle and a nerve fiber, respectively). The tissue distribution pattern of CalcR staining succeeds that of HSV-1 in both strains at 5 dpi.
Calcitonin receptor (Calcr) is a seven-transmembrane G protein-coupled receptor. It binds the calcitonin hormone which is secreted from the thyroid gland by the parafollicular cells. Calcitonin is responsible for the calcium homeostasis, thus involved in bone formation and metabolism (Naot and Cornish 2008). The CalcR is expressed in a various number of tissues (Mori, Ishii et al. 2006). However, up to now little is known about the role of the CalcR in other tissues and it has not been previously described with regard to infectious diseases. However, the calcitonin precursor, procalcitonin can be up to 100,000 fold up-regulated in bacterial infections and can be used as a diagnostic biomarker, even though its role has not been fully understood (Müller, White et al. 2001). In addition, procalcitonin has also been reported to be elevated in the cerebrospinal fluid of patients suffering from Alzheimer's disease, vascular dementia, dementia with Lewy bodies, frontotemporal dementia and acute neuroinflammation (encephalitis, meningitis) compared to non-demented controls (Ernst, Morgenthaler et al. 2007).

HSV-1 latency is known to decrease the level of the neuropeptide calcitonin gene-related peptide (CGRP) in trigeminal sensory neurons (Hamza, Higgins et al. 2007). However, CGRP signals through calcitonin receptor like receptor (CLR) (Naot and Cornish 2008).

The Amylin hormone is similar in structure to calcitonin hormone and signals through Calcr. It is secreted by pancreatic β-cells parallel to insulin and is associated with type II diabetes development (Marzban, Park et al. 2003). The in vivo use of CalcR agonist rat Amylin to modulate CalcR enhanced the survival of DA rats significantly after HSV-1 infection compared to controls. Notably, the modulation using the in vitro system using CalcR transfection of cell lines suggested that the presence of CalcR does not directly influence the infectivity of cells. However, Amylin signaling through CalcR could decrease the viral infection and/or replication inside cells through a more complex mode of action.

In conclusion, the use of this hypothesis free system of genetic mapping, in paper I we identified Calcr as a candidate for regulating susceptibility to peripheral neuronal infection and HSE development. Additionally, Calcr could provide an alternative route of virus transport through perineural cells, or signaling through the receptor and/or the intracellular calcium concentrations could influence cells infectivity and viral replication.

Innate immune responses after HSV-1 infection

In paper II, the main focus was to explore the role of immune responses after infection with HSV-1 in DA and PVG rats. Thus the detailed kinetics of immune activation after infection was investigated by IHC and qRT-PCR.

The HSV-1 spread in the whiskers tissue within the first 24hrs was similar in both strains, mostly found around the epineurium and in some parts around the
At the same time point the activated macrophages (MΦ), Iba1+/ED1+ cells were visualized as well surrounding the epineurium.

After 2dpi, the most conspicuous finding in DA rats was the presence of HSV-1 surrounding perineural cell layers forming ring-like shapes, with an increase in the staining intensity in the following days; detectable morphological changes in Schwann cells and more virus spreading to the trigeminal ganglia and into the brainstem. In contrast, the HSV-1 staining was reduced in PVG rats after 2dpi.

Recruitment of different immune cells to the whiskers area after infection was seen from 12 hpi. NK and CD8+ T Cells were more visualized in DA rats from 12 dpi compared to PVG rats, even though more NK cells were present. In parallel, activated phagocytic MΦs from 2dpi were surrounding the perineurium in a ring-like pattern in DA rats similar to the distribution of HSV-1. However, this was not seen in PVG rats. Nevertheless, more MΦs were counted in the resistant PVG, indicating more efficient and rapid phagocytic activity.

In recent years, the role of PRRs in recognizing invading pathogens has been extensively investigated. A special focus had been addressed to the role of TLRs in viral recognition, activation of immune cells and proinflammatory cytokines release. It has been shown that TLR2 and TLR9 are involved in DNA viruses’ detection in particular HSV (Dasgupta, Chentoufi et al. 2011; Takeda, Miyazaki et al. 2011) and protection against CNS infections (Sorensen, Reinert et al. 2008; Lima, Zolini et al. 2010). Interestingly, we found that the mRNA expression of Tlr2 and Tlr9 in the whiskers area was significantly increased in PVG resistant rats after 1 and 2 dpi, the exact time point with most number of observed phagocytic cells. Also, since Tlr2 is found on the surface of MΦ and DCs, it is likely that the increase in expression reflects a more rapid and vigorous recruitment of phagocyting cells in the resistant PVG strain.

Thus our findings in paper II, displayed the defective immune responses in the susceptible DA strain. The reduced activation of Tlr2 and Tlr9 which lead to the delayed infiltration of MΦ, as well as the early recruitment of NK and T cells to the infection site all in turn influenced HSV-1 entry, replication and propagation into the CNS. Additionally, the discernible differences in host strain dependent spread and replication of HSV-1 in the perineurial cell layer might support the hypothesis that the genetic properties of the perineurial cells could possibly serve as an alternative route of viral entry to the CNS and progression to HSE. It also remains possible that inflammation around perineural cells could affect axons indirectly, such as through the effects of decreased axoplasmic flow or increased intrafascicular pressure (Bove, Weissner et al. 2009). Notably, these differences in HSV-1 and phagocytic cells tissue distribution in the periphery and around the PNS go hand-in-hand with more Calcr staining around the perineural cell layers in susceptible DA rats.
Genetic and immune regulation of HSE in RILs

We believe that several genes are regulating the susceptibility vs. resistance to HSE development. This was evident to us when we infected a panel of different inbred rat strains to run the haplotype map in paper I. The susceptibility to HSE clinical symptoms development varied across the different inbred strains tested, mainly we found that SHR rats were susceptible to HSE, while BN rats were totally resistant. Strikingly, by IHC we detected HSV-1 presence in the CNS of both SHR and BN, which was not the case in resistant PVG rats. However, this has been described previously in resistant mice (Halford, Balliet et al. 2004). Activated immune cells, mostly NK cells were also observed by IHC and found to be more abundant and wide spread in the brain stem of SHR rats, suggesting that the encephalitis was due to the severe immune reaction in this strain.

To identify gene(s) predisposing to susceptibility in SHR or resistance in BN we used an unbiased approach using the BXH/HXB RILs between these two strains. We infected 29 RIL, determined their phenotypes and performed linkage analysis. Most RILs were susceptible to HSE symptoms while only 5 RILs were completely resistant. The linkage analysis in the panel of RILs revealed a significant QTL, named Hse6 at the end of rat chromosome 4 between (160.9 – 174 Mb) regulating HSE incidence. Several genes in the Hse6 region are NK cell receptor genes, such as the killer cell lectin-like receptor genes (Klr) and Ly-49 receptor genes. Previously Pereira and colleagues, identified a NK complex-linked locus in mice, Rhs1 (resistance to herpes simplex virus 1), on chromosome 6 that controls resistance to acute and latent HSV-1 infections resulting in HSE (Pereira, Scalzo et al. 2001). Another region identified in mouse chromosome 6 was Hrl (Herpes resistance locus) influencing the survival to HSV-1 infection (Lundberg, Welander et al. 2003). Interestingly, both these regions correspond to Hse6 on rat chromosome 4.

Sequencing variants and expression QTL (cis-eQTLs) analysis of all the genes in the Hse6 region identified the von Willebrand factor Vwf gene as the candidate for HSE regulation. The Vwf of SHR origin was the only gene that showed variants in the putative essential splice site, harbored 3 SNP variants upstream of the gene, 2 non-synonymous coding variants and 18 synonymous variants. Interestingly, Vwf was cis-regulated in 6 tissues including the brain.

The vWF is a glycoprotein synthesized by the Weibel-Palade bodies in endothelial cells. It functions in the blood coagulation system as a mediator between the platelet and the vessel wall and it is an anti-hemophilic factor carrier having a role in hemostasis (Sadler 1998).

In the brain, we visualized the vWF staining in both strains by IHC. Conspicuously, in the brain stem of SHR rats interrupted or irregular vWF staining was seen in the larger blood vessels but not in the capillaries and less visible around small blood vessels. This could suggest a disruption of the BBB in SHR rats, resulting in increased cellular infiltration after infection leading to encephalitis development. In an earlier EAE
study, vWF has been proposed to influence the BBB permeability leading to more infiltration of inflammatory cells into the CNS (Noubade, del Rio et al. 2008).

We then went on and established our model in mice and found C57BL/6 mice resistant and BALB/C mice susceptible to HSE after infection with $2 \times 10^6$ PFU of HSV-1. We infected C57BL/6 \textit{Vwf}\textsuperscript{−/−} (Denis, Methia et al. 1998) to investigate the influence of \textit{Vwf} on infection compared to C57BL/6 control. Surprisingly, neither the \textit{Vwf}-deficient, nor the wild type mice got diseased or presented any symptoms of clinical disease. This could be explained by the fact that in this mouse strain other genes are more essential for resistance or that perhaps their immune responses are more efficient in eradicating the infection or causing less damage to the CNS. Also, knock-out systems resemble mendelian traits, thus in this artificial system the interactive influence of other genes might not be involved or to compensate for the deficiency created other genes might replace the function. Thus making it difficult to compare to normal mice and amend the real complex disease process.

In human HSE patients, we measured elevated expression levels of vWF protein by enzyme-linked immunosorbent assay (ELISA) in the CSF compared to controls. This suggests a role of vWF in human disease. A number of studies on cerebral malaria indicated increase in plasma vWF levels after \textit{Plasmodium falciparum} infection (Hollestelle, Donkor et al. 2006; Bridges, Bunn et al. 2010). Interestingly, this suggests a role of endothelial cells activation after different infections leading to BBB permeability. In addition, elevated cytokine levels in CSF of HSE cases were reported in a number of studies (Sköldenberg, Aurelius et al. 2006; Ichiyama, Shoji et al. 2008; Kamei, Taira et al. 2009; Khademi, Kockum et al. 2010). The value of our novel finding of vWF in CSF of HSE cases is that it can be used as a diagnostic biomarker.

Further studies will be needed to identify the role of \textit{Vwf} at the molecular level in susceptibility to HSE and to determine the association of vWF to human HSE. This is of particular importance to all CNS inflammatory conditions, as trafficking across the BBB is an essential target for effective therapeutics development.
GENETIC REGULATION OF INNATE IMMUNE RESPONSES AFTER CNS INJURIES IN RAT

In recent years it became apparent that the immune privileged state of the CNS is no longer the case. The CNS exhibits a tightly regulated immune activation system, in which the microglia cells are the major players. However, the involvement of other neuronal cells such as astrocytes, oligodendrocytes and neurons in immune responses are still under extensive investigation.

Microglia cells are the macrophages of the CNS. They remain inactive under normal conditions, however after exposure to external or internal insults, such as after infections or injuries they become activated and more MHC class II expression is detected. Earlier studies from our laboratory identified polymorphisms in the class II transactivator gene, $Mhc2ta$, regulating MHC II after VRA in rat (Lidman, Swanberg et al. 2003; Harnesk, Swanberg et al. 2008). Also, MHC2TA in humans was associated with the risk for developing autoimmune diseases such as multiple sclerosis (MS), rheumatoid arthritis (RA) and myocardial infarction (Swanberg, Lidman et al. 2005).

In paper IV, we investigated the microglial activation in two inbred rat strains after VRA, the mechanical nerve injury model. BN rats showed higher MHC II at an earlier time point after VRA when compared to MHC haplotype LEW 1N rats that carry the same $Mhc2ta$. This suggested that other gene regions outside Vra4 containing the $Mhc2ta$ gene are responsible for MHC II expression in BN rats.

To investigate this possibility we crossed BN rats with LEW 1N rats and performed a whole genome linkage scan in $F_2$ (BNxLEW 1N). Two QTLs were identified to regulate MHC II expression (CD74 invariant chain; MHC II marker) after 5 days VRA, Neuinflam4 and Neuinflam5 on chromosomes 1 and 7, respectively. BN alleles increased MHC II expression in Neulinflam4, while LEW 1N in Neuinflam5 increased the expression. Epistatic interactions (iQTL) were present between Neuinflam1 (D1Rat159) and Neuinflam5 to regulate MHC II and other inflammatory molecules.

In parallel we studied the regulation of a number of inflammatory molecules including beta-2 microglobulin ($\beta 2m$, MHC class I marker), complement components 1 ($C1q$) and 3 ($C3$), $Cd11b$ (C3 receptor) and $Il1b$ to identify the genomic regulation of the activation of these inflammatory markers. $C3$ was found to be suggestively linked by Neuinflam4 and Neuinflam5. On chromosome 10, Neuinflam9 was identified to regulate the expression of $C1q$, $Il1b$ and $Cd11b$, whereas; Neuinflam10 on chromosome 11 regulated the expression of $C3$, $Cd11b$ and $\beta 2m$. The expression of these molecules did not differ between the parental strains. Nonetheless, the fact that several of them were linked to the same QTLs in the genome, suggests a common regulatory effect. Notably, several of these QTLs overlap with QTLs regulating different autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) the model of MS and RA models; especially Neuinflam9 which overlaps with Eae18a and Eae18b on chromosome 10 (Jagodic, Becanovic et al. 2004).
The neuroinflammatory regulation after VRA seems to be complex and under polygenic control.

**Preliminary Results**

**Influence of Neuinflam9 on neuroinflammation**

Different neurological diseases activate CNS resident cells, mostly microglia through exhibiting early innate immune responses by activating toll-like receptors signaling pathways. To identify the genes predisposing CNS disorders through innate responses regulation, we performed a genome-wide linkage scan in the same F_2 (BNxLEW^{1N}) cross used in paper IV, to investigate the regulatory regions for the expression of innate immune molecules after 5 days VRA.

We identified several expression quantitative trait loci (eQTLs) responsible for the regulation of different toll-like receptors (TLRs) and interferon regulatory factors (IRFs). Among these were the previously identified *Neuinflam9* QTL on rat chromosome 10, regulating *C1q, Il1β* and *Cd11b* expression. This QTL also regulates the expression of *Tlr2* and was suggestive for the regulation of *Irf7* as well as *Tlr4, 6* and *7*. A second significant QTL was identified on chromosome 5, regulating *Irf3* expression. Two suggestive QTLs on chromosome 1 and 12 regulated *Irf7* expression.

*Neuinflam9* overlaps with *Toxo1* region which regulates resistance to *Toxoplasma gondii* infection (Cavailles, Sergent et al. 2006). The influence of *Neuinflam9*, on the expression of these innate immune molecules after nerve injury using VRA and TBI models was verified in two different BN.LEWc10 congenic lines; lineages BN.LEWc10-B (D10Rat100 – D10Mco4) and BN.LEWc10-E(D10Got12 – D10Rat238). *Irf7* expression was significantly lower in BN.LEWc10-E congenic after VRA and TBI, compared to BN.LEWc10-B.

Additionally, the congenic strain BN.LEWc10–E was found to be protected from developing EAE phenotypes after MOG immunization. This strongly suggests that the same gene(s) that regulated expression of innate immune molecules also regulate predisposition to autoimmune disease in the CNS, proposing the involvement of a common immune regulatory pathway essential for neuroinflammation.

Interestingly, the identification of the genes underlying these QTL regions regulating innate immune activation molecules in the CNS would be of great value to unravel common pathways regulating innate responses of relevance to neuroinflammatory and neurodegenerative conditions and identify new targets for therapeutic interventions.
CONCLUDING REMARKS

Through the studies included in this thesis, we were able to investigate and describe different aspects of neuroinflammatory conditions after infections and injuries.

Of great interest are our novel findings in HSE regulation using experimental rat strains. We were able to identify two candidate genes regulating different aspects of HSE disease development. \textit{Calcr} is identified as a candidate for peripheral neuronal infection and further spread to the CNS, whereas \textit{Vwf} as a candidate for disease progression in the CNS and increasing BBB permeability.

Remarkably, the peripheral distribution of virus, immune cells and CalC protein around the perineurium has not been reported previously. In addition, the differences described with regard to immune activation and responses in the PNS and CNS are of great value to understand the mechanisms of virus - host interaction. Thus, our work sheds light on new insights with regard to HSE pathogenesis which could be of relevance to human disease.

On the other hand, studying the non-infectious or sterile immune responses within the CNS, in particular after neurotraumas is of great significance to identify common regulatory pathways. The genetic regulation of neuroinflammation is under complex polygenic control that can be explored through studying the different aspects of regulation using experimental animal intercrosses.

Two QTLs were identified regulating MHC class II activation after VRA, \textit{Neuinflam4} and \textit{Neuinflam5} on chromosome 1 and 7. Notably, \textit{Neuinflam9} on chromosome 10 regulates several immune related molecules including \textit{C1q}, \textit{Il1β}, \textit{Tlr2} and \textit{Irf7}. This region on chromosome 10 also coincides with \textit{Toxo1} region which regulates resistance to \textit{Toxoplasma gondii} infection; in addition a part of this region conferred resistance to EAE development. Thus we were able to describe new regulatory regions that could be essential for CNS protection.

Interestingly, it will be of great significance to further explore what initiates the cascade of events in the CNS. Thus whether it is the neurodegenerative processes causing more inflammation which in turn will lead to more destruction of neuronal tissue or is it the neuroinflammation that generates irreversible degeneration.
FUTURE PERSPECTIVES

A key goal behind the results generated from the work in this thesis is to inquire the relevance to human disease, in which our findings are of great implication to be further followed.

It will be of importance to explore the association of Calcr and Vwf to human HSE disease. As well as, to perform functional studies to further increase the knowledge and understanding on virus-host interactions and the functions of these candidate genes at the molecular level in HSE pathogenesis.

The QTL regions identified regulating different aspects of neuroinflammation after injuries need to be studied in more detail. Sequencing of these regions to detect common genetic variants, as well as global expression profiling would be of interest to select possible candidate genes. In addition, the use of smaller congenic strains across these regions would facilitate narrowing down of the confidence intervals of these QTLs and allow in-vitro functional investigations to understand the precise pathways regulated influencing neuroinflammatory conditions.
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“My Lord, bestow me guidance so that I thank you for the favour which You bestowed upon me and my parents, and so that I may perform the good deeds which please You, and by Your mercy include me among Your bondmen who are worthy of Your proximity.” Holy Qur’an; Al-Naml, Ch.27; verse 19.
REFERENCES


Echchannaoui, H., K. Frei, et al. (2002). "Toll-like receptor 2-deficient mice are highly susceptible to Streptococcus pneumoniae meningitis because of reduced..."


Rasmussen, S. B., S. B. Jensen, et al. (2009). "Herpes simplex virus infection is sensed by both Toll-like receptors and retinoic acid-inducible gene-like receptors, which synergize to induce type I interferon production." The Journal of general virology 90(Pt 1): 74-78.


