Herpes Simplex Virus Infection, Pathological Pain and Recurrent Lymphocytic Meningitis

Katariina Kallio-Laine

ACADEMIC DISSERTATION
To be publicly discussed with the permission of the Medical Faculty of the University of Helsinki, in Lecture Hall 2, Biomedicum Helsinki on December 4, 2009, at 12 noon.

Helsinki 2009
To my family
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which are referred to in the text by their roman numbers.


IV Kallio-Laine K, Seppänen M, Aittoniemi J, Seppälä I, Valtonen V, Färkkilä M, Kalso E, Lokki ML. HLA-DRB1*01 allele and low plasma IgG concentration may predispose to herpes-associated recurrent lymphocytic meningitis. Accepted to be published in Human Immunology on Oct 22, 2009.

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## ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACC</td>
<td>anterior cingulate cortex</td>
</tr>
<tr>
<td>ADCC</td>
<td>antibody-dependent cellular cytotoxicity</td>
</tr>
<tr>
<td>AMPA</td>
<td>alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid</td>
</tr>
<tr>
<td>AP</td>
<td>alternative pathway of complement</td>
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<tr>
<td>ATP</td>
<td>adenosinetriphosphate</td>
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<tr>
<td>BOLD</td>
<td>blood oxygenation level dependent</td>
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<tr>
<td>Ca&lt;sup&gt;2+&lt;/sup&gt;</td>
<td>calcium-ion</td>
</tr>
<tr>
<td>CGRP</td>
<td>calcitonine gene related peptide</td>
</tr>
<tr>
<td>C</td>
<td>complement factor (e.g. C3 and C4)</td>
</tr>
<tr>
<td>CP</td>
<td>classical pathway of complement</td>
</tr>
<tr>
<td>CRPS</td>
<td>complex regional pain syndrome</td>
</tr>
<tr>
<td>CSF</td>
<td>cerebrospinal fluid</td>
</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
<td>EAA</td>
<td>excitatory amino acids</td>
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<tr>
<td>EEG</td>
<td>electroencephalography</td>
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<tr>
<td>Elisa</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>GABA</td>
<td>γ (gamma)-aminobutyric acid</td>
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<tr>
<td>IASP</td>
<td>International Association for the Study of Pain</td>
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<tr>
<td>IC</td>
<td>insular cortex</td>
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<td>IENF</td>
<td>intraepidermal nerve fiber</td>
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<td>Ig</td>
<td>immunoglobulin</td>
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<td>IGH</td>
<td>immunoglobulin heavy chain gene</td>
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<tr>
<td>fMRI</td>
<td>functional magnetic resonance imaging</td>
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<tr>
<td>HLA</td>
<td>human leukocyte antigen</td>
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<td>HSV</td>
<td>herpes simplex virus</td>
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<tr>
<td>IL</td>
<td>interleukin</td>
</tr>
<tr>
<td>LEP</td>
<td>laser evoked potential</td>
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<tr>
<td>LP</td>
<td>lectin pathway of complement</td>
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<tr>
<td>MBL</td>
<td>mannose binding lectin</td>
</tr>
<tr>
<td>MBL2</td>
<td>mannose binding lectin gene</td>
</tr>
<tr>
<td>MEG</td>
<td>magnetoencephalography</td>
</tr>
<tr>
<td>mGluR</td>
<td>metabotropic glutamate receptor</td>
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<tr>
<td>MHC</td>
<td>major histocompatibility complex</td>
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<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartic acid</td>
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<tr>
<td>NO</td>
<td>nitric oxide</td>
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<tr>
<td>PAG</td>
<td>periaqueductal gray</td>
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<tr>
<td>PCR</td>
<td>polymerase chain reaction</td>
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<tr>
<td>PET</td>
<td>positron emission tomography</td>
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<tr>
<td>PFC</td>
<td>prefrontal cortex</td>
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<tr>
<td>PGP</td>
<td>protein gene product</td>
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<td>QST</td>
<td>quantitative sensory testing</td>
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<td>RLM</td>
<td>recurrent lymphocytic meningitis</td>
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<tr>
<td>Acronym</td>
<td>Definition</td>
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<tr>
<td>RVM</td>
<td>rostral ventromedial medulla</td>
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<tr>
<td>SMT</td>
<td>spinomesencephalic tract</td>
</tr>
<tr>
<td>SRT</td>
<td>spinoreticular tract</td>
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<tr>
<td>STT</td>
<td>spinothalamic tract</td>
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<tr>
<td>TNF</td>
<td>tumor necrosis factor</td>
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<tr>
<td>VLF</td>
<td>ventrolateral funiculus</td>
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<td>WDR</td>
<td>wide-dynamic range</td>
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ABSTRACT

**Background:** Aims of the study were: (i) to characterise the clinical picture, immunological features and changes in brain morphology and function in patients with widespread unilateral pain and HSV-infections (I, II), and (ii) to analyse the prevalence, clinical symptoms and immunological predisposing factors of HSV-2 induced recurrent lymphocytic meningitis (RLM) in Southern Finland (III, VI).

**Methods:** Patients for the studies were recruited from the Pain Clinic, Department of Anaesthesiology and Intensive Care Medicine, from the Division of Infectious Diseases, Department of Medicine, and from the Department of Neurology, Helsinki University Central Hospital. Controls for laboratory analyses were recruited from the Vita Laboratories, Helsinki, Finland. Plasma concentrations of IgM, IgA, IgG, and IgG1-4, serum concentrations of C3, C4 and serum classical pathway hemolytic activity (CH50) were measured (I and IV). Serological anti-HSV-1 and -2 antibody status was tested (I and III). C4 phenotyping, and the analyses of C4 gene copy numbers by isotype-specific genomic real-time PCR amplification, HLA-A, HLA-B and HLA-DRB1 typing (I and IV), MBL2 genotyping (IV), and IgG1 and IgG3 allotyping (Gm) (I and IV) were performed. Clinical neurological examination (I-IV), quantitative sensory testing (I and II), skin biopsy (I), and functional magnetic resonance imaging (II) were also performed.

**Results:** We found support to our hypothesis that HSV, or inflammatory reaction triggered by the virus, has a role in the generation of a pathological pain state. We characterized the clinical picture of the patients with widespread unilateral pain and present found subtle immunological abnormalities, including low serum IgG1 and IgG3 levels, which made the patients vulnerable for recurring HSV infections. We also demonstrated both functional and structural changes in the brain pain-processing areas in these patients: they had less pain-related activity in the insular cortices (IC) bilaterally, in the anterior cingular cortex (ACC), and in the thalamus, and the gray matter density was lower in the ACC, and in the frontal and prefrontal cortices.

In the meningitis studies we presented a period prevalence of RLM, showed that RLM is more common and less benign than previously reported, and that neuropathic pain is frequently present both during and after meningitis episodes. We also showed that *HLA-DRB1*01, *HLA-B*27, and low IgG1 levels are predisposing factors for RLM.

**Conclusions:** Patients are vulnerable to recurrent HSV infections because of subtle immunological abnormalities. HSV causes diverse clinical manifestations. First, the herpes simplex virus, or the inflammatory process
triggered by it, may cause pathological widespread pain probably by activating glial cells in the CNS. In these patients, signs of alterations in the brain pain-processing areas can be demonstrated by functional brain imaging methods. Secondly, HSV-2 induced RLM is a rare complication of HSV-2 virus. The predisposing factors include low IgG1 subclass levels, HLA-DRB1*01 and HLA-B*27 genotypes. Neuropathic pain is frequently associated with RLM.
1. Introduction

Pain in an unpleasant experience associated with actual or potential tissue damage by noxious stimuli. It includes not only the conscious perception of the sensory event, but also the cognitive analysis and emotional response associated with the experience. Acute pain has an adaptive function. It makes us move away quickly from the pain-inducing stimulus, and teaches us to avoid pain inducing dangers in the future. While becoming chronic, the pain looses its adaptive function, and becomes harmful.\textsuperscript{154}

Pain is characterized as chronic when it is present for longer than six months. Chronic pain may be classified as nociceptive, neuropathic or idiopathic. Nociceptive pain arises, when the nociceptors in the skin, muscles, joints and viscera are stimulated by pressure, heat, irritant chemicals etc., whereas neuropathic pain arises as a consequence to a lesion or a disease of the somatosensory system. In idiopathic pain, the aetiology of chronic pain remains unsolved.\textsuperscript{138}

The term “pathological pain” is occasionally used, when speaking about debilitating, chronic, widespread – and often neuropathic – pain. It is characterized by an amplified response to normally innocuous stimuli, and an amplified response to acute pain. Pathological pain is not adaptive, and it may not be limited to the area of damage. Body regions beyond the site of damage may also exhibit exaggerated pain responses although they are not innervated by the damaged nerves, i.e. the pain is extra-territorial. In addition, a small proportion of patients may also report pain as arising from the corresponding body part on the healthy contralateral side of the body. That is, the exaggerated pain is “mirrored” on the other side, and called as “mirror-image” pain.\textsuperscript{90, 125, 154}

Long-lasting neuropathic pain is often pathological. The treatment of neuropathic, as well as pathological pain is challenging. Neuropathic pain responds poorly to nonsteroidal anti-inflammatory drugs, and only moderately to opioids. Patients may get relief from drugs that are targeted to neurons, but the relief is usually only partial – a 30% reduction in pain is considered as a moderate to good result.\textsuperscript{36, 63} One reason for failures in drug therapy may be that drugs are targeted to neurons, whereas immunologically active glial cells, which are considered to be important in both induction and maintenance of pathological pain states, are ignored.\textsuperscript{143, 154}

Glia is activated by different pathogens (including bacteria and viruses), infections, and inflammation. The activation of glial cells is followed by release of inflammatory mediators from the peripheral nerve endings, which may further activate other glial cells. This is supposed to cause the spread of pain in extraterritorial, and mirror-image pain.\textsuperscript{144, 147}

Herpes simplex viruses 1 and 2 are among the most common viruses causing infections in man. They are neuroinvasive, they have an ability to
maintain latency in the nervous system after primary infection, and they are able to periodically reactivate from their latency. Because of their viral properties, herpes simplex viruses are good candidates for activating glia. Microglia has been shown to detect HSV, most likely via Toll-like receptors, and induce the release of cytokines, including tumor necrosis factor-α, as an attempt to defend against the virus.

Viral reactivations become clinically evident only in a minority of patients. The severity of disease manifestations depends on a variety of virus and host factors. Immune competence seems to be crucial to the maintenance of latency. Some individuals may be more vulnerable to severe manifestations and frequent reactivations of HSV due to defects in cell-mediated immunity, as e.g. transplant recipients and patients with AIDS.

Clinical manifestations of HSV-1 and HSV-2 are diverse. In addition of probably being able to activate glia and to induce pathological pain, one rare manifestation of HSV is recurrent lymphocytic meningitis (RLM). It is a seldom seen disease, mostly caused by HSV-2. There is significant patient-to-patient variability regarding the time to recurrence and the total number of episodes. Patients are most often young or middle-aged, and women are more often affected. RLM is considered a benign disease with no neurological sequelae. Neuropathic pain in association with RLM has only rarely been reported. Predisposing factors for RLM are not known. There is one case report of RLM associated with Ig-sublass deficiency, and in another study, an association between MBL deficiency and RLM has been implied. It has also been suggested that complications of HSV infections are more common when the infection is primary, instead of initial. In developed countries, seroprevalence to HSV-1 has decreased, and so a larger proportion of HSV-2 infections in the future will be primary. Thus, RLM may be more common in the years to come.
2. Review Of The Literature

2.1. The neuroanatomy of pain

The first documented attempt to understand pain, was done by Rene Descartes, a philosopher and scientist, who in 1664 introduced a theory of pain transmitted through a single channel from the skin to the brain (Figure 1). This simplified theory directed both the theory and the treatment of pain for more than 300 years. Today we know that pain transmission is much more complicated.

Figure 1. Rene Descartes: Tractatus De Homine 1664.

Peripheraффerents

The sensory experience begins in the periphery, where the peripheral terminals of primary afferent fibers respond to stimuli. There are three main types of these fibers. Aβ-fibers are large in diameter and highly myelinated fibers with low activation threshold. They respond to light touch and quickly conduct tactile information to spinal cord. Aδ-fibers are smaller in diameter and only thinly myelinated. They are slower than Aβ-fibers, and have a higher activation threshold. They respond to both thermal and mechanical stimuli. C-fibers are the smallest type of afferent fibers, they are unmyelinated, and thus also the slowest fiber type. They have the
highest activation threshold, and respond only to nociceptive stimuli.\textsuperscript{31} The A\textgreek{d}- and C-fibers are called nociceptors. Tissue damage or inflammation can excite the peripheral terminals of these nociceptors, causing the release of endogenous chemical mediators, such as prostaglandins, bradykinin, and substance P. These substances may then sensitize the nociceptors so that the afferent activity to a given stimulus increases. This phenomenon is called primary hyperalgesia.\textsuperscript{24, 31}

**Dorsal horn**

A\textgreek{d}- and C-fibers synapse to the spinal cord dorsal horn, which is organised into different laminae. Most nociceptive fibers terminate to superficial laminae I-II. In the spinal cord dorsal horn there are various neuronal cell types which respond to different sensory stimuli. Nociceptive specific cells are mostly found superficially, and synapse with A\textgreek{d}- and C-fibers only. Wide dynamic range (WDR) neurons receive information from all three types of sensory fibers (A\textgreek{b}, A\textgreek{d}, C) and therefore respond to all kind of stimuli from light touch to noxious pinch, heat and chemicals. In addition, there are excitatory (glutamatergic), and inhibitory (GABAergic) interneurons in the spinal cord, which are able to increase or decrease the response of the nociceptive and WDR neurons. In addition to these, evidence has accumulated during the last decade suggesting that also non-neuronal cells of the dorsal horn (astrocytes and microglia) influence pain transmission through the dorsal horn, particularly under pathological conditions.\textsuperscript{31}

**Spinal cord**

Neuronal activity in the spinal cord dorsal horn is under dynamic modulation; messages received by the spinal cord may be suppressed, relayed unaltered, or amplified. The relationship between the stimulus and response to pain is not always linear, spinal neurons which respond to nociceptive information are under constant control by peripheral inputs, interneurons, and descending controls. The response may also be greatly altered by various neurotransmitter systems, particularly during pathological conditions.\textsuperscript{154}

**Ascending and descending pathways in the spinal cord**

Signals from the dorsal horn to supraspinal structures are carried by spinal projection neurons along the ascending pathways. The most prominent ascending pathway is the spinothalamic tract (STT), which transmits sensations of pain, temperature, and touch. It crosses the midline at the spinal nerve level, and ascends in the ventrolateral funiculus (VLF) to the
thalamus. The second pathway is the spinomesencephalic tract (SMT). It also crosses the midline, and ascends in the VLF to midbrain, especially periaqueductal grey (PAG) and nucleus cuneiformis. The third tract is the spinoreticular tract (SRT), which terminates in the reticular formation of the medulla. In addition to the primary target structures, the ascending pathways also project to the brainstem area, such as the rostral ventromedial medulla (RVM), which has descending pathways back to dorsal horn. 

Activation of the descending pathways from the brainstem modulates the pain experience, and is influenced also by limbic areas, hence adding the emotional, affective component to the pain experience. The descending pathways may be both facilitatory and inhibitory. Descending facilitatory pathways from the brainstem RVM have been shown to be involved in the maintenance, but not in the initiation, of nerve injury induced pain. This facilitatory descending tract modulates neuronal responses to tactile stimuli in the dorsal horn, and may be behind mechanical allodynia. 

Descending inhibitory actions involve the release of noradrenaline, which inhibits transmitter release from primary afferents, and the release of substance P in the dorsal horn suppressing the projection neurons. This is accomplished by interneurons, and indirectly by the release of endogenous opioids, and is an endogenous system for pain control. 

Both descending facilitatory and inhibitory noradrenergic projections undergo plastic changes in chronic pain states, like an increase in descending noradrenergic inhibition after peripheral inflammation (Figure 2).

Figure 2. Dynamic modulation of pain. CNS contains distinct circuits that either inhibit or facilitate pain. Inhibitory systems are activated by opiate drugs, environmental factors, and learned danger signals. Facilitatory systems are activated by infection, inflammation, and learned sickness and safety signals. Pain inhibitory systems suppress pain by inhibiting neurons in the spinal cord dorsal horn, and pain facilitatory systems exaggerate pain by increasing excitability in these neurons. There is also dynamic modulation of the release of neurotransmitters from pain fibers arriving in the spinal cord. The Figure is modified from Watkins LR and Maier SF. The pain of being sick. Annual Review of Psychology 2000;51:29-57.
The thalamus

The thalamus is the main relay site for nociceptive information to cortical and subcortical structures. Within the thalamus, nociceptive information regarding the type, intensity, and location of pain is encoded prior to sending the information onward to limbic structures and cortex.\(^{106}\)

The lateral pain system consists of STT neurons projecting through the ventral posterolateral (VPL) nucleus of the thalamus to the primary and secondary somatosensory cortex (SI and SII), to parietal operculum, and to the insula. It is mainly associated with the sensory-discriminative aspects of pain processing, and provides information on stimulus location and intensity. The medial pain system includes the STT neurons projecting through the posterior ventromedial (VMpo) nucleus of the thalamus, and further to the ACC, amygdala, hippocampus, hypothalamus, parabrachial nuclei and to periaqueductal gray (PAG). It plays a crucial role in the motivational-affective and cognitive-evaluative aspects of pain processing.\(^{15,73}\) Functional and anatomic divisions of the thalamus have been made on the basis of their connections to specific spinal cord laminae. The relevance of anatomically defined thalamic nuclei has also been confirmed in humans with high-resolution imaging studies: direct stimulation of the principal somatosensory nucleus (Vc) in human thalamus produces two types of pain responses: an on-off type signalling of the presence of painful stimulus, and a firing rate signalling of the intensity of painful stimuli.\(^{19}\)

Thalamus is implicated in chronic pain, and thalamic hypoperfusion reflects the pain state. In cancer pain patients, decreased blood flow contralateral to pain has been registered.\(^{28}\) In neuropathic pain it has been argued that hypoperfusion of the thalamus could also reflect deafferentation. On the other hand, in one study of deafferentation-related pain, thalamic hypoperfusion was restored following pain relief.\(^{44}\)

The pain pathways from periphery to the brain are seen in Figure 3.
Figure 3. Pain pathways from periphery to brain. Primary afferent fibers transmit impulses from the periphery, through the dorsal root ganglia (DRG) to the dorsal horn in the spinal cord. Nociceptive specific (NS) cells are mainly found in the superficial dorsal horn (laminae I-II), whereas most of the wide dynamic range cells (WDRs) are located deeper (lamina V). Projection neurons from lamina I innervate areas such as the parabrachial area (PB) and periaqueductal grey (PAG), and are affected by limbic areas. Descending pathways from brainstem modulate spinal processing. Lamina V neurons mostly project to the thalamus, and from there to the various cortical regions, including SI, SII, IC, ACC, and PFC. The Figure is reprinted from D’Mello R and Dickenson AH. Spinal cord mechanisms of pain. British Journal of Anaesthesia 2008;10:8-16 with permission of Oxford University Press and the authors.

Central processing of pain

Pain is a complex, multi-factorial subjective experience, and its measurement and imaging are difficult. A large brain network is subsequently active during nociceptive processing. Melzack (1999) first described the term pain
“neuromatrix”, now more commonly called “pain matrix”. It means all brain regions, which have more or less active roles in sensory-discriminatory or affective-cognitive-evaluative components of pain.85, 86, 133

There are several meta-analyses available of human data from positron emission tomography (PET), functional magnetic resonance imaging (fMRI), electroencephalography (EEG), and magnetoencephalography (MEG) providing evidence of the most common regions active in an acute experimental pain (Figure 4).133 Five major cortical areas have been identified consistently responding to acute pain. These areas include the primary and secondary somatosensory cortices (SI and SII), the insular cortex (IC), the anterior cingulate cortex (ACC), and prefrontal cortices (PFC).

The ACC is reported as the area most often activated by different noxious or painful stimuli; also the IC is generally activated. Neurons in the ACC and IC are likely to encode multiple forms of pain. Results from human brain imaging studies suggest that not all pain is processed by similar brain regions, and each particular form of pain is coded by several brain regions.133, 157 In addition, there are other regions which may be active especially in chronic pain; such as basal ganglia, cerebellum, amygdala, and the areas within the parietal and temporal cortices.133

Pain-related somatotopy has been observed in the contralateral insula, where face activation was anterior to both hands and feet, and in the SI cortex, where the organization follows the same somatotopy as observed for tactile input.8, 15

Less is known about clinical/chronic pain than of experimental acute pain. In studies of acute pain in healthy subjects, the stimulus is easily controlled and healthy subjects are easy to find. On the contrary, the chronic pain patients are heterogeneous, and many factors affect the evaluation of pain they report. Despite this, some information of chronic pain in patients is available.

Sustained high level of spontaneous pain results in increased activity within the medial PFC and the ACC.127 Activation in rostral anterior insula and PFC is consistently seen in clinical pain conditions, irrespective of underlying pathology. It has been suggested that clinical pain is located more rostrally in the anterior insula than nociceptive pain in healthy volunteers.113 It seems that especially the middle short gyrus in the anterior part of insular cortex is implicated in processing chronic pain.2 The anterior insula is active not only during pain, but is also associated with anxiety, depression, fatigue, fibromyalgia, somatization and fear.

Both activation and deactivation of different brain areas seem to be crucial. Deactivation of cortical areas may reflect a reduction in neuronal activity. It might mean that excitation and inhibition occurs not only at the neuronal level, but also at the cortical network level.157 It is not known if deactivation occurs only at the inhibitory neurons, thus reflecting decreased pain inhibition as a cause of chronic pain. Gabapentin, a drug commonly used to treat neuropathic pain, has been shown to reduce chronic pain-
related deactivation, suggesting that deactivation might, indeed, contribute to chronic pain.59

A variety of brain regions are involved in the descending modulation of pain. These areas include the frontal lobe, ACC, insula, amygdala, hypothalamus, PAG, nucleus cuneiformis, and RVM. This is one way for the brain to alter pain processing, and perhaps the route by which sleep, anxiety, coping, and catastrophizing may impact the pain experienced.31 Attention, mood and emotional state have a significant impact on the resultant pain perception and the ability to cope. Cerebral neuronal plasticity may underlie all these conditions. A common clinical and experimental observation is that anticipating and being anxious about pain can exacerbate the pain experienced. Anticipating pain is adaptive in acute pain, but in chronic pain it becomes maladaptive, and can lead to fear of movement, avoidance, and anxiety. We are only beginning to unravel the specific roles of different brain areas in pain processing and pain-related phenomena like depression and anxiety.133

Chronic pain may cause structural changes in the brain. In chronic back pain patients, decreased prefrontal and thalamic gray matter density has been demonstrated.9 In fibromyalgia patients, there is a loss of gray matter in the ACC, IC, PFC and parahippocampal gyri.72 It is unclear whether the loss of neurons is limited to local inhibitory neurons. Furthermore, it is unclear whether these structural changes are a consequence of chronic pain or of secondary changes due to pain-related mental disorders, such as depression.157
2.2. Cellular and synaptic mechanisms of chronic pain

Transmitters

Suppression of pain is essential in life-threatening situations, when in spite of tissue damage the animal has to be able to escape. During that kind of intense stress, pain-suppressing neurotransmitters (endorphins) are released. Endorphins can act within the spinal cord dorsal horn, and prevent nociceptive signals from reaching the brain.

On the other hand, nociceptive information may be amplified, often as a result of increased nerve excitability, resulting in exaggerated pain responses. Increased sensory nerve excitability increases the release of glutamate and substance P, which are the classic pain neurotransmitters in the spinal cord. Glutamate is an excitatory amino acid expressed throughout the nervous system. This neurotransmitter is essential for pain signalling at all anatomical levels. The majority of primary afferents synapsing in the dorsal horn utilize this transmitter. The main inhibitory transmitter in turn is gamma-aminobutyric acid (GABA).31, 157
Receptors

Glutamate exerts its excitatory effect via different receptor subclasses: AMPA receptors, NMDA receptors, mGluR receptors, and Kainate receptors. Most is known about the function of AMPA and NMDA receptors. During acute or persistent noxious stimulus glutamate is released from the peripheral terminals. AMPA receptors are activated to the stimulus first, and set the baseline response in the dorsal horn. With repetitive and high-frequency stimulation of the C-fibers, the NMDA receptors are activated. Activation of the NMDA receptors is not possible with only acute or low-frequency noxious stimuli. It is likely that the co-release of peptidergic transmitters, such as substance P and CGRP (calcitonine gene related peptide) are needed to activate NMDA. Hence, NMDA receptor activation has been shown to be in a key role in hyperalgesia, or enhancement of pain seen in chronic pain states such as neuropathic pain. Activation of NMDA receptors leads to an increase in postsynaptic calcium ions (Ca\(^{2+}\)) and to influx of Ca\(^{2+}\) to the cell. Once inside, Ca\(^{2+}\) serves as an important intracellular signal for triggering, of e.g. neuronal nitric oxide (NO) syntheses, which in turn can promote mechanisms of plasticity, such as long-term potentiation. Overall, it is likely that aberrant activity is amplified and enhanced by NMDA receptor-mediated mechanisms in tissue damage and neuropathic pain, and that this receptor is critical for both the induction and maintenance of pain.\(^{31, 157}\)

2.3. Genetics and pain

Several genetic variants that either prevent, decrease or increase pain have been identified in humans. Examples of variants which increase pain include the δ-opioid receptor genetic variant \(OPRD1\), which is known to increase pain sensitivity, and variants in cytochrome \(P450\) \((CYP)\) \(2D6\), which are associated with decreased codeine effects. Genetic variants, which reduce pain, can either prevent it completely, almost completely, or they may increase analgesic drug effects, thus reducing pain.

Complete prevention of perceiving of pain has so far been reported in six different rare hereditary syndromes, namely the “channelopathy-associated insensitivity to pain”, and in five distinct hereditary sensory and autonomic neuropathies (HSAN I-V). Channelopathy patients suffer from a loss of affective-motivational component of pain perception. They recognize the painful stimuli but do not show any withdrawal responses. In these patients, the mutation is in \(SCN9A\) gene, which codes the voltage-gated sodium channel (Nav1.7). HSAN patients have peripheral neuropathy, they have a sensory-discriminative deficit and do not perceive pain. Five types of HSAN are caused by mutations in five distinct genes \((SPTLC1, HSN2, IKBKAP, NTRK1\) and \(NGFB)\).\(^{29, 30, 95}\)

Genetic variants that decrease pain have been identified in healthy
individuals, without any hereditary syndrome. These variants only moderately modulate the expression or function of the biological structures. A variant in μ-opioid receptor gene (OPRM1) is associated with a decrease in pressure pain intensity. A variant in COMT gene is associated with a reduction in the activity of an enzyme, which metabolizes catecholamines (e.g. dopamine, norepinephrine and epinephrine), thus having an effect in pain transmission. Genes may also influence nociceptive processing in the brain: in a PET study, individuals homozygous for the met158 allele of the COMT polymorphism showed diminished regional μ-opioid system responses to pain, and higher sensory and affective ratings of pain compared with heterozygotes. In addition, there are gene variants, which decrease pain by increasing analgesic drug effects, e.g. the cytochrome P450 2D6 gene, which increases the metabolism of prodrugs into active analgesics.

Associations between genotype and phenotype in pain have been difficult to reproduce (e.g. COMT), probably because of high frequency of pain-relevant genetic variants in healthy population. In epidemiological studies, genetic factors are thought to account for half of all low back pain heredibility. Because of the strong linkage disequilibrium in the MHC region, associations between HLA haplotypes and pain are difficult to identify, and large population-based studies are needed. In Japanese patients, HLA haplotype HLA-A*3303-B*4403-DRB1*1302 has been shown to be associated with an increased risk to develop neuropathic pain after herpes zoster infection, but not with the onset of herpes zoster. TNF gene in the MHC region codes cytokine TNF-α, which induces hyperalgesia. It has been suggested that polymorphisms in genes in the MHC region may be associated not only with autoimmune diseases, but also with the susceptibility to develop neuropathic pain.

2.4. Neuropathic pain

According to a new definition, neuropathic pain is “pain arising as a direct consequence of a lesion or disease affecting the somatosensory system”. Because of lack of a specific diagnostic tool for neuropathic pain, a grading system for definite, probable, and possible neuropathic pain has been proposed (Figure 5). The former IASP (International Association for the Study of Pain) definition date back to year 1994: neuropathic pain is “initiated or caused by a primary lesion or dysfunction in the nervous system”. The problem with this definition was that it lacked defined boundaries. Indeed, the sensitivity of nociceptive system is modulated by its adequate activation (e.g. central sensitization), so it is difficult to distinguish neuropathic dysfunction from physiologic neuroplasticity.
Neuropathic pain is characterized as pain in the absence of a stimulus. Nociceptive thresholds are reduced, so that normally innocuous stimuli produce pain. Stimuli may also evoke abnormal or unpleasant perceptions of pain (paraesthesia, dysesthesia). Neuropathic pain arises from trauma, inflammation or infection of peripheral nerves. Traditionally neuropathic and inflammatory pain were considered distinct entities. Today, however, infection or inflammation is estimated to be responsible for up to half of all cases of neuropathic pain, and the immune system is considered to actively participate in creating and maintaining neuropathic pain conditions of diverse aetiologies (Figure 6).112, 145, 146

Neuropathic pain should be divided into peripheral or central neuropathic pain based on the anatomic location of the lesion or the disease. Most (90%) neuropathic pain states have been considered to arise from the peripheral rather than the central nervous system.33 Examples of peripheral neuropathic pain states include polyneuropathies of various aetiologies, postherpetic neuralgia, and iatrogenic or traumatic injuries. Central pain syndromes represent a form of neuropathic pain that is associated with lesions of the brain or the spinal cord. The most common aetiologies are stroke, traumatic spinal cord injury or multiple sclerosis.93

In neuropathic pain the activity, properties, and transmitter contents of the nerves change.31, 121 Animal models of inflammatory and traumatic neuropathy have revealed a remarkable degree of plasticity in sensory nerves, sensory nerve somas, and the spinal cord. Damaged nerves may develop spontaneous activity in peripheral nerve terminals, on axonal level and in neuronal cell bodies, and become increasingly actively responsive to pain-inducing substances. Ectopic activity is due to the accumulation and clustering of sodium (Na+) channels around the damaged axon. This aberrant activity may spread to the cell bodies in the dorsal root ganglia. Nerve
fibers may also start to cross-excite each other. In addition, sympathetic efferents may start to activate sensory afferents, which results in spreading of excitation between different cell types.\textsuperscript{143}

Figure 6. In up to half of the cases neuropathic pain is caused by infection or inflammation. In addition, inflammatory mechanisms are often activated in neuropathic pain.

2.5. The immune connection in neuropathic pain and the role of glia

Previously pain was thought to be mediated solely by the neurons. During the last decade it has became evident that spinal cord glia (astrocytes and microglia) plays an important role in exaggerated, pathological pain (Figure 7). Glia was first suggested a contributing factor to pathological pain by Garrison et al. in 1994. They found that (i) manipulation that creates pathological pain also activates astrocytes, and (ii) a drug that blocks pathological pain also blocks astrocyte activation. Since then, every animal model of pathological pain has revealed a positive correlation between exaggerated pain and activation of spinal cord glia.\textsuperscript{46, 154}

Activation of glia is necessary for both induction and maintenance of pathological pain. Microglia and astrocytes may have a distinct role in pathological pain. It has been proposed that early activation of microglia leads to activation of astrocytes, which serves to maintain pathological pain.\textsuperscript{105} Exaggerated pain may be blocked by blocking the activation of spinal cord glia.\textsuperscript{90} Similarly, development of exaggerated pain may be blocked by administration of a microglia specific inhibitor, minocycline.\textsuperscript{132}

Glia is activated
by several substances, including viral pathogens, as well as by excitatory amino acids and prostaglandins, which activate also neurons. Selective activation of only glia is possible by using foreign invaders like viruses, which bind to specific receptors in glia. It has been shown that glial activation, without neuronal activation, is sufficient to induce exaggerated pain states. Microglia has been shown to be able to detect HSV, most likely via Toll-like receptors. In response, it releases proinflammatory cytokine TNF-α and upregulates GLT-1 (glutamate transporter), leading to increased microglial glutamate uptake.

Glia is not involved in acute pain. Normally glia is quiescent and releases nothing that enhances pain. Once activated, it releases substances that essentially contribute to the amplification of pain.

Neurons are able to release classical pain neurotransmitters (EAA, substance P, prostaglandins, ATP, and NO, among others), thereby activating glia in vivo. When activated, glia activates other glial cells by releasing excitatory substances like interleukin-1β and NO. As immunocompetent cells, activated glia releases classical immune proteins including proinflammatory cytokines (IL-1β, IL-6, TNF-α). In the spinal cord these proinflammatory cytokines are signal molecules that activate neurons as well as glia, and thus glia can signal neurons. Blocking of proinflammatory cytokine activation in the spinal cord blocks diverse exaggerated pain states.

Theoretically, the release of pain enhancing substances by newly activated astrocytes distant from the initial site of trauma could explain the spread of pain as “mirror image pain” and “extraterritorial pain”. Astrocytes are able to communicate with each other via gap junctions. In animal models, spread of pain has been blocked by spinal administration of substrates that disrupt gap junctions.

Glia can also compromise the ability of opioids to suppress pain. Evidence exists that chronic morphine activates both astrocytes and microglia, and that it also activates microglial p38 MAP kinase and stimulates the production of spinal proinflammatory cytokines. Morphine tolerance is attenuated or reversed by either inhibition of spinal proinflammatory cytokines or by knockout of IL-6 signalling. This activation of glia by opioids may be an explanation to “paradoxical” opioid-induced pain enhancement, where prolonged opioid treatment leads to loss of antinociceptive efficacy, and also to decrease of nociceptive thresholds. In addition, glia may be an important factor in morphine dependence and withdrawal. In animal models, AV411 (ibudilast), a blood-brain-barrier permeable glial activation inhibitor has prevented brain mediated withdrawal signs over time. Glial activation takes place in multiple sites along the pain pathway, including peripheral nerves, dorsal root ganglia and spinal cord. Study of the involvement of the brain glia in pain regulation is still in its infancy. It is already clear that glia in the brain regulates responses to opioids, such as morphine. There is evidence that glia in the medullary trigeminal ganglia regulates pain, and it seems likely that glia in the brain play an important regulatory role in pain enhancement, too.
Figure 7. Classical and nonclassical views of pain transmission and pain modulation. (a) Classical pain transmission pathway. Peripheral Aδ- and C-fibers respond to noxious stimulus. The axons relay action potential to dorsal horn, where the neurotransmitters of the sensory nerve are released, leading to activation of postsynaptic receptors on pain transmission neurons (PTNs). Axons of the PTNs then transmit information of the noxious event to the brain. (b) Normal pain. Glia is quiescent, and does not release neuroexitory substances. Information of the noxious event is carried by Aδ- and C-fibers. Substance P and excitatory amino acids (EAAs) are released in amounts appropriate to stimulus intensity and duration. NK-1 and AMPA receptors are activated, and the generated action potentials are relayed to brain. (c) Pathological pain, classical view. In response to intense or prolonged pain, the PTNs become sensitized. The magnesium ions exit to NMDA-linked channel. This results in influx of calcium ions, and nitric oxide (NO) synthesis. Presynaptically NO causes exaggerated release of substance P and EAAs, and postsynaptically PTN hyperexcitability. (d) Pathological pain, new view. Glial activation is considered as a driving force for creating and maintenance of pathological pain. Glia is activated (i) by bacteria and viruses which bind to specific receptors on microglia and astrocytes; (ii) by substance P, EAAs, fractalkine, and ATP, or by brain-to-spinal cord pain enhancement pathways; or (iii) by NO, prostaglandins (PGs) and fractalkine released by PTNs. Following activation, microglia and astrocytes cause PTN hyperexcitability and the exaggerated release of substance P and EAAs from presynaptic terminals. These changes are created by the glial release of NO, EAAs, reactive oxygen species (ROS), PGs, proinflammatory cytokines (IL-1, IL-6, and TNF), and nerve growth factor. The Figure is reprinted from Watkins LR and Maier SF. Immune regulation of central nervous system infections: from sickness response to pathological pain. Journal of Internal Medicine 2005;257:139-155 with permission from the publisher.
Inflammatory mediators (cytokines and chemokines) and pain

Cytokines are small regulatory proteins that are produced by white blood cells and a variety of other cells, including those in the nervous system. Cytokines act on hormonal concentrations through high-affinity receptors. They are produced upon demand and travel only short distances. Due to this local action, the serum levels do not necessarily reflect local activation. Cytokines have numerous effects on cells of the immune system and they modulate inflammatory mechanisms. Some cytokines are labelled pro-inflammatory, and the others anti-inflammatory. Three main pro-inflammatory cytokines are interleukin-1β, interleukin-6 and tumor necrosis factor-α.123, 124

Inflammation is a pathophysiological state associated with pain. The free nerve endings of peripheral nerve fibers in systemic tissues respond directly to inflammatory factors, such as lowered pH, bradykinin, histamine or prostaglandins, by generating electrical activity that is normally interpreted as painful. In models of inflammatory pain, pain is generally enhanced by pro-inflammatory cytokines and reduced by cytokine blockades. Pro-inflammatory cytokines stimulate the production of the traditional chemicals of inflammation, such as prostaglandins.92

Evidence has emerged that cytokines link the immune and the nervous system and that they may be involved in the generation of pain and hyperalgesia. The organisms’ response to infection is called the “illness response”, which is mediated via cytokines. The illness response is associated with fever, fatigue, loss of appetite, and hyperalgesia. During acute infections, hyperalgesia is one of the adaptive changes, which forces animals to decrease their use of energy.123, 146

Cytokines are produced by peripheral immune cells, and by CNS immunocompetent cells. In the CNS, glia is the predominant source of cytokines.145 In the peripheral nerve, axonal injury upregulates cytokine production.92, 123 Cytokine expression increases in human neuropathies such as chronic inflammatory polyneuropathy and Guillain-Barre syndrome.25 Treatment of a neuropathic condition of erythema nodosum leprosum with thalidomide, reduced TNF-α secretion in peripheral blood mononuclear cells, and greatly reduced pain.13 Intravenous immunoglobulin reduced IL-1α and IL-1β levels in the plasma of patients with previously elevated levels, and reduced pain in a subgroup of patients. Models of painful nerve injury reveal changes in cytokine expression in the injured nerve itself, in the DRG, in the spinal cord dorsal horn, and in the CNS.54, 123, 131

Chemokines are a family of over 50 low molecular weight proteins, which are central to innate immune responses following tissue damage, injury and some diseases. They orchestrate the migration of leucocytes to inflamed tissues. Chemokines are divided to α-chemokines, β-chemokines, lymptactins and fractalkine. Synthesis of chemokines or receptor expression within the nervous system is linked to glial activation states and neuronal
hyperexitability, and has recently been implicated in the development and persistence of many pathological pain states. As an example is fractalkine, a factor released by neurons of the pain pathway. Fractalkine induces leucocyte migration and facilitates inflammation. Under normal conditions it is expressed on the extracellular surface of neurons, and fractalkine receptors are seen mostly on microglial cells. During pathological pain, fractalkine receptor expression is increased in microglia, in pain-related areas. Application of exogenous fractalkine produces exaggerated pain responses by activating microglia and, on the other hand, intrathecal injection of fractalkine antagonist attenuates both thermal hyperalgesia and mechanical allodynia in experimental models of neuropathic pain.89, 151

2.6. Examples of different neuropathic pain syndromes – different mechanisms

Peripheral neuropathic pain: Small-fiber neuropathy

Small-fiber neuropathy is a subtype of sensory neuropathy, which exclusively or predominately affects small A-δ and C-fibers and their functions. Small somatic or autonomic fibers, or both, may also be involved. The most common aetiologies of small-fiber neuropathy are diabetes, followed by amyloidosis and Sjogren’s syndrome. The majority of small-fibre neuropathies remain idiopathic despite extensive evaluation.

Most patients present symptoms between the age 45 and 70 years. Eighty percent of patients report burning pain in the feet. Pain, numbness, and paraesthesias occur in half of the patients. Reduced sensitivity to pin-prick and temperature is almost always seen. Patients may also have loss of vibratory sensation at the toes or absent ankle reflexes. Nerve conducting studies are generally normal.

Symptoms are usually distal, and “length-dependent”, starting from the feet. The hands often become affected over time. Symptoms restricted to upper limbs or cranial nerves are exceptional. A small number of patients develop more acute, diffuse or multifocal symptoms. These patients may slowly recover after a plateau, but symptom fluctuations may persist. In these cases, antecedent infectious illnesses have been reported.

Abnormal results in quantitative sensory testing (QST) have been reported in 60-85% of patients. However, QST has relatively low sensitivity and specificity. A more objective method is quantitation of intra-epidermal nerve fiber density. Reduced intra-epidermal nerve fiber density is observed in 50-88% of patients.6, 75, 94
Lesion at the level of dorsal or cranial sensory nerve ganglia: Postherpetic neuralgia (PHN)

Herpes zoster is a neurocutaneous disease caused by reactivation of Varicella-Zoster virus (VZV) from latency in dorsal or cranial sensory nerve ganglia. Herpes zoster predominately affects elderly people, and occurs as a result of ageing-related waning of cell-mediated immunity to VZV. In addition to the elderly, also immunocompromised patients are at risk to develop VZV reactivation. The most common complication of herpes zoster is PHN, persistence of pain for more than three months after the rash has healed. Like the zoster disease itself, the risk of postherpetic neuralgia increases with advancing age. The pain in PHN may be spontaneous, or stimulus-evoked, and its intensity varies. Dynamic allodynia (pain in response to normally non-painful stimuli) is a typical clinical feature. The diagnosis of PHN is based on clinical history of herpes zoster, and typical sensory findings in clinical testings.\textsuperscript{34, 111, 149}

Central neuropathic pain: Central post stroke pain (CPSP)

Central pain is caused by a lesion in the brain or the spinal cord. In brain-related central pain, approximately 90\% of cases have vascular aetiology. CPSP is not a rare syndrome: 8\% of stroke patients suffer from it, and in 5\% of them, pain is moderate to severe. CPSP is characterized by constant or intermittent pain occurring after stroke in those parts of the body which correspond to the cerebrovascular lesion. Hypersensitivity and sensory loss are typical features in clinical sensory examination.\textsuperscript{64} Most often CPSP is caused by a thalamic lesion, particularly within the ventroposterior inferior nucleus. Treatment of CPSP has remained as a challenge; the pain responds poorly to anti-analgesic drugs.\textsuperscript{7, 93}

Interaction of central and peripheral mechanisms and sympathetic nervous system: Complex regional pain syndrome (CRPS)

CRPS is a chronic neurological disorder characterized by disabling pain, swelling, vasomotor instability, sudomotor abnormality, and impairment of motor function. CRPS typically develops to an extremity after surgery or trauma. It may be divided into two subtypes depending on whether there is a nerve lesion present or not. Allodynia and hyperesthesia are often described as clinical features. The symptoms are not confined to the innervation zone of an individual nerve. It is probable that both peripheral and central mechanisms interact in the development of CRPS. Signs of inflammation with oedema and vasodilatation and increased cutaneous
temperature are often seen, therefore an inflammatory process in the periphery has been discussed. Changes within the pain-processing areas of the brain have frequently been reported in functional imaging studies.\textsuperscript{4, 61, 62, 66}

2.7. Herpes simplex viruses -1 and -2

Herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) are large double-stranded DNA viruses of the \textit{Herpesviridae} family. They share a 83\% sequence homology of their protein-coding regions and the structure of their genomes is similar, but can be distinguished serologically.\textsuperscript{52} HSV-1 and -2 are among the most common human viral infections. They have been recognised since ancient Greeks, and because of their worldwide occurrence and ability to achieve a state of latency, they belong to the most harmful human pathogens. HSV-1, HSV-2, and VZV are neurotrophic, and classified as “alpha” herpes viruses.\textsuperscript{152}

Transmission takes place usually through intimate contact, as HSV-2 is transmitted predominantly sexually and HSV-1 mainly horizontally in childhood. HSV has no seasonal variation and it naturally infects only human beings. Most human beings have been infected and harbour latent virus that can reactivate, hence there is a vast HSV reservoir for transmission.\textsuperscript{100, 109, 152}

HSV enters the body through skin or mucosal surfaces, and it initiates cytolytic replication in epithelial cells at the entry site. Then it penetrates through the dermis and enters the ends of peripheral sensory nerves innervating the infected cells. The nucleocapside, containing the HSV DNA, is transported in a retrograde manner to the neuronal soma in the sensory root ganglia, where it converts to a latent state, and persists for the whole life of the infected person. Immune competence seems to be crucial for maintenance of latency, but the exact mechanisms of reactivation are unknown. Recurrence takes place, when HSV reactivates in sensory root ganglia, and is transported in the peripheral nerves back to the mucosal or skin surface. Recurrence may be triggered by physical or emotional stress, fever, ultraviolet light and tissue damage. Recurrences tend to be confined to the dermatome of primary infection but do not necessarily occur at precisely the same anatomic location. Reactivation of HSV becomes clinically evident only in a minority of patients.\textsuperscript{52, 119}

Diagnosis of HSV can be confirmed by virus isolation in cell culture or PCR detection of HSV DNA.\textsuperscript{58} The development of type specific enzyme immunoassays (ELISA) that reliably distinguish between antibodies to HSV-1 and HSV-2, enables serological studies which can measure both symptomatic and asymptomatic infections.\textsuperscript{100, 152}

Clinical, epidemiological and biological properties in HSV-1 and HSV-2 are different. Seroprevalence to HSV-1 varies from 52\% (in Finland) to 84\%
Antibodies to HSV-1 reduce the disease severity of HSV-2. Changes in seroepidemiology of HSV-1 and HSV-2 have been reported; the seroprevalence of HSV-1 has declined, while that of HSV-2 has increased in developed countries. Consequently, a greater proportion of HSV-2 infections will be primary (the patient is seronegative to HSV-1 when getting infected with the first HSV-2 infection), instead of initial (the patient is already HSV-1 seropositive during the first HSV-2 episode). In primary HSV-2 infection, complications after the disease may be more frequent than in initial herpes infections.22

Historically, aciclovir has been used for treatment and suppression of various HSV-related conditions. Aciclovir is available in oral and intravenous formulation. Other effective antiviral agents for treatment and suppression of HSV include valaciclovir and famciclovir. They have a substantially higher bioavailability than aciclovir. Valaciclovir and famciclovir are available as oral preparations only.52, 152

Clinical manifestations of HSV

HSV causes a range of diseases from mild uncomplicated mucocutaneous infections to life threatening ones. The severity of disease manifestations is dependent on a variety of viral and host factors. Patients with defects in cell-mediated immunity suffer from more frequent and more severe recurrences of HSV.109

Gingivostomatitis is a symptomatic primary HSV-1 infection, usually occurring in children. After primary infection the virus remains latent usually in the trigeminal ganglia, and when reactivated, it causes facial herpes or cold sores. Symptomatic outbreaks of cold sores are estimated to affect 20-40% of adults.119 More rare manifestations of HSV-1 in cranial region are ocular HSV-infections or herpetic facial paralysis. A serious neurological complication of HSV-1 infection is encephalitis which, if untreated, may cause death and in survivors permanent neurological defects, e.g. seizures, mental status changes, aphasia or motor deficit.49

HSV-2 causes predominantly genital herpes. Latency is established in the sacral root ganglia. One third of patients are estimated to have more than six recurrences per year, one third will have two recurrences per year and the remaining third only rare recurrences. Over time the recurrences will become less frequent. Transmission usually occurs from symptomless virus shedding.152 Neurological complications of HSV-2 include (recurrent) aseptic meningitis, myelitis and brainstem encephalitis. Radiculopathy caused by HSV-2 has been reported occasionally.53 HSV-2 encephalitis occurs mainly in newborn babies, and more rarely, in immunocompromised adults.49

Disseminated HSV-infections, with a progressive disease involving
respiratory tract, oesophagus, or gastrointestinal tract, are only seen in patients with an immunodeficient state, such as bone marrow transplantation, malignancy, malnutrition, alcoholism or pregnancy.\textsuperscript{109, 152}

**Neuropathic pain and HSV**

There is limited information about the association of neuropathic pain and HSV. Recurrent mucocutaneous HSV lesions are often preceded by pain, a burning or tingling sensation at the lesion site – the type of pain which resembles neuropathic pain. In acute phase of recurring HSV-2 genital herpes, transient neuralgic symptoms may occur, but there are also case reports of chronic neuropathic pain in sacral area associated with the disease.\textsuperscript{53} HSV-2 induced radiculomyelitis may present clinically with neuralgic pain of the buttocks, perineum or legs, in addition to numbness, paraesthesias and urinary retention (Elsberg syndrome).\textsuperscript{109} In a one-year follow-up of patients with HSV-2 induced meningitis/meningomyelitis, chronic radicular pain, weakness, and paraesthesia of the lower limbs was reported.\textsuperscript{12} In addition, a painful syndrome of brachial neuritis (Parsonage-Turner Syndrome) has repeatedly been associated with viral infections, including HSV-1.\textsuperscript{71, 84}

**Recurrent lymphocytic meningitis (RLM)**

RLM was first described in 1944 by a French neurologist, Pierre Mollaret. Hence, the term “Mollaret meningitis” is frequently associated with this syndrome.\textsuperscript{116}

RLM is a rare illness that manifests as recurrent episodes of aseptic meningitis. Symptoms during the meningitis episodes include fever and meningismus lasting for a few days, followed by spontaneous recovery. Symptomatic periods of the disease usually occur during 3 to 5 years.\textsuperscript{91} There is a significant patient-to-patient variability regarding the time to recurrence, which may vary from weeks to years, and the total number of episodes, which may reach 30.\textsuperscript{18, 116} Women are affected more often than men, and the mean age of the patients is around 35-40 years.\textsuperscript{74, 130} Over time, recurrences become less common, although data from prospective studies that support this is limited.\textsuperscript{116}

In 1991, Yamamoto et al. first reported a case of RLM attributed to herpes simplex virus; the viral DNA in CSF was detected by PCR. Since then, increasing evidence has accumulated that HSV-2 is the most common cause in RLM.\textsuperscript{104, 130} Almost 10% of patients may develop meningitis after the primary HSV-2 infection.\textsuperscript{20, 109} In patients with only a single episode of lymphocytic meningitis, enteroviruses are the most common etiologic agents.\textsuperscript{74, 76} RLM may also be induced by autoimmune disorders, or medication, e.g.
nonsteroidal anti-inflammatory drugs, trimethoprim-sulfamethoxazole and intravenous immunoglobulin. The clinical disease resolves spontaneously. Approximately one-half of the patients have transient neurological manifestations, including seizures, hallucinations, diplopia, cranial nerve palsies, or altered level of consciousness. Anecdotal evidence suggests that acyclovir, valacyclovir, and famciclovir, which have been administered to some patients for both episodic therapy and suppression of recurrences, might be beneficial, although there is no controlled trial data to support their efficacy and safety.

The current diagnostic criteria state that RLM is a benign illness, and the patients should experience spontaneous recovery within a week. However, in addition to HSV recurrences, diffuse neurological complaints during the year following the acute phase have been reported in approximately one-fourth of the patients. The characteristic cerebrospinal fluid (CSF) abnormality in RLM is lymphocytic pleocytosis. Protein levels in CSF are mildly elevated, and the glucose levels are usually normal. Analysis of CSF by PCR for HSV DNA is considered to be the gold standard for diagnosis.

In RLM probably all episodes are caused by the same etiologic agent. However, HSV-2 DNA is not always found in the CSF of the patients during a recurrence, despite an earlier HSV-2 DNA positive episode. Viral load and leucocyte counts reach significantly higher levels during the first episode compared with recurrent cases. False negatives may be caused by lower viral load in recurrences, which may also be due to earlier timing of the CSF sample.

2.8. Immunology

The cells and molecules responsible for immunity constitute the immune system, and their collective and coordinated response to the introduction of foreign substances is called the immune response. The physiologic function of the immune system is defence against infectious microbes. However, even non-infectious substances, and mechanisms that normally protect individuals from infections and eliminate foreign substances, are capable of causing immune responses.

Defence against microbes is mediated by early reactions of innate immunity and later responses of adaptive immunity. The innate immune response to microbes stimulates adaptive immune responses, and influences the nature of the adaptive responses. Adaptive immunity in turn, uses many of the effector mechanisms of innate immunity to eliminate microbes, and it also enhances the antimicrobial activities of the defence mechanisms of innate immunity.
**Innate immunity**

Innate immunity is the immediate ability of the host to prevent and limit infections. It consists of cellular and biochemical defence mechanisms that are in place even before infection, and it provides rapid but incomplete host defence until the slower, more definitive, adaptive immune system is activated. Previous contact with an antigen is not needed. The innate immune system is phylogenetically older than the adaptive immune system. Innate immunity reacts only to microbes, and the response is essentially the same during repeated infections. Major participants of innate immunity are (i) physical and chemical barriers such as epithelia; (ii) phagocytic cells (neutrophils, macrophages) and NK cells; (iii) blood proteins including the complement system and other mediators of inflammation; and (iv) proteins called cytokines. Of these, the complement system is a key component, and plays a central role in host defence against pathogens.

**Adaptive immunity**

Adaptive immunity develops as a response to exposure to infectious agents. The response is increased in magnitude, and in its defensive capability with each exposure to a particular microbe. Characteristic for adaptive immunity are specificity for distinct molecules and an ability to “remember” and respond more vigorously to repeated exposures to the same molecule. Adaptive immunity is able to recognize and react to a large number of microbial and nonmicrobial substances. It has a capacity to distinguish even closely related microbes and molecules, and for this reason it is also called “specific immunity”. The components of adaptive immunity are lymphocytes and their products. There are two types of adaptive immune responses, called humoral immunity and cell-mediated immunity.

Humoral immunity is mediated by molecules in the blood and mucosal secretions, called antibodies (immunoglobulins). They are glycoprotein molecules, produced by B lymphocytes that bind to antigens with high specificity and affinity. Antibodies recognise microbial antigens, neutralise the infectivity of the microbes, and target microbes for elimination by various effector mechanisms. The basic structural unit is an antibody composed of two identical heavy chains and two identical light chains. Every individual has millions of different antibodies, with unique binding sites. B lymphocyte is the only cell type capable of producing antibodies and therefore the central cellular component of humoral immunity. B cells develop in the bone marrow and mature cells are mainly seen in lymphoid follicles, in the bone marrow, and in low numbers in the circulation.

Cell-mediated immunity is mediated by T lymphocytes. T cells mature in the thymus, circulate in the blood, populate secondary lymphoidal tissues, and are recruited to peripheral sites of antigen exposure. Intracellular
microbes, such as viruses, survive and proliferate inside phagocytes and other host cells, where they are inaccessible to circulating antibodies. Cell-mediated immunity is crucial in defence against such infections.1

**Immunoglobulin (Ig)**

There are five isotypes of antibodies, determined by which of the five different forms of heavy chains are present. These antibody isotypes include IgM, IgD, IgG, IgA, and IgE. Each isotype has different functions. IgA and IgG have additional structural variations.1

**Immunoglobulin G (IgG)**

Human immunoglobulin G (IgG) can be subdivided into four subclasses: IgG1, IgG2, IgG3, and IgG4, each having unique biological and functional properties. IgG1 makes up most of the total IgG (66%), followed by IgG2, IgG3 (7%), and IgG4.8 Immunoglobulin G (IgG) subclasses IgG1 and IgG3 mediate antibody-dependent cellular cytotoxicity (ADCC) and are efficient activators of the classical complement pathway.87 Deficiency of IgG1 results in low levels in total IgG and is often, although not invariably, associated with susceptibility to bacterial infections. Deficiency of IgG3 is rather common. A clear clinical “phenotype” of IgG3 deficiency has not been defined, although susceptibility to viral infections, or prolonged clinical course of viral infections has been suggested.9 It is possible that isolated IgG3 deficiency is clinically relevant only in combination with other deficits (e.g. MBL deficiency). IgG1 and IgG3 deficiencies often occur together, and are thought to result from aberrant regulation of the expression of immunoglobulin heavy chain genes (IGH) encoding specific IgG allotypes.87

**IgG allotypes**

Variations in IgG subclass levels are associated with Ig allotypes. Allotypes are genetic variants of Ig subclasses and light chains. Thirteen different IgG allotypes have been described; high and normal levels associated with (b) and (g) allotypes and low levels with the (g) allotype.99, 114

**The Complement System**

The complement system is a part of the innate immune system and also one of the major effector mechanisms in antibody-mediated immunity. It consists of more than 30 proteins in plasma and cell surfaces, and has three...
physiologic activities: (i) defending against pyogenic bacterial infections, (ii) bridging innate and adaptive immunity, and (iii) disposing of immune complexes and the products of inflammatory injury. Recognition of the microbes by complement occurs through three distinct pathways: the alternative pathway (AP), the lectin pathway (LP) and the classical pathway (CP). All these pathways are activated in sequential manner, with the action of one component leading to the activation of the next one.

Classical pathway

The classical pathway (CP) uses a plasma protein called C1 to detect IgM and IgG antibodies bound to microbe surfaces. Among the IgG subclasses, the IgG1 and IgG3 are the most efficient activators of the complement. Activation of the classical pathway is initiated by binding of the antigen-antibody-complex to the C1 molecule. This induces a proteolytic cascade involving multiple complement proteins. In addition to the antigen-antibody immunocomplex, various pathogens and molecules can bind to C1 and initiate the cascade. The classical pathway is an effector arm of the humoral immune system. It generates inflammatory mediators, opsonins for phagocytosis of antigens, and lytic complexes that destroy cells. 

Complement protein C4

Complement protein C4 (C4) is essential for the propagation of the classical and lectin pathways. It is the most polymorphic protein of the complement system. C4 has two isotypes, C4A and C4B, and is encoded by the closely linked C4A and C4B genes in the major histocompatibility complex (MHC) class III on chromosome 6. The plasma protein levels of C4A and C4B are mainly determined by the corresponding gene dosage. Deficiency or excessive amounts of C4 may affect an individual’s immune system. C4 deficiency may lead to defective processing of immune complexes, impairment of B cell memory, and persistence of bacterial and viral infections. Excessive amounts of C4 could possibly cause overactivation of the complement pathways and exacerbate the inflammatory response at local tissues.

Lectin pathway

The lectin pathway (LP) is the most ancient pathway, triggered by a plasma protein called mannose-binding lectin (MBL). MBL bound to microbes activates one of the proteins of the classical pathway. The rest of this pathway is similar to the classical pathway.
Mannose-binding lectin

Mannose-binding lectin (MBL) is a liver-derived plasma protein that activates the lectin pathway. It is a major pattern-recognition molecule of the innate immune system. It primarily recognizes sugar groups on the surfaces of microorganisms. It has been shown to bind to a wide range of pathogenic organisms including HSV-2. It is capable of promoting clearance of apoptotic cell debris and immune complexes from the circulation.

The serum levels of MBL are determined by polymorphisms in the MBL2 gene. Low serum concentrations of MBL are caused by point mutations in codons 52, 54, and 57, in exon 1 of the MBL2 gene. The concentration is also dependent upon several promoter region polymorphisms of the gene. Approximately 5% of the Finnish population have MBL deficiency, defined as an undetectable level of MBL in the serum. A large proportion of these individuals are asymptomatic.

The clinical relevance of MBL deficiency is controversial. In some studies MBL deficient patients seem to be prone to severe diseases caused by encapsulated bacteria, and to illnesses caused by a broad range of viruses, fungi, and parasites. However, many of these associations are weak, or contradictory. In most cases, other components of the immune system can compensate for the lack of MBL. It is possible that MBL deficiency becomes clinically manifest only when it occurs in association with some other form of immunodeficiency.

On the other hand, high frequencies of variant alleles in certain populations suggests that MBL deficiency may also be advantageous. Some intracellular pathogens use C3 opsonization to enter their host. Any reduction in complement-activating function may reduce the probability of parasitization. Hence, MBL deficiency may protect against parasitic infections (e.g. malaria).

MBL deficiency may have an impact on protective immunity at the population level, as susceptibility to many common infectious diseases is under complex genetic control.

Major Histocompatibility Complex (MHC)

MHC was first discovered as a genetic locus containing highly polymorphic genes that determined the outcome of transplants exchanged between individuals. Now it is known that the MHC region takes part in innate and adaptive immunity. The physiological function of MHC molecules is the presentation of microbial peptides to the appropriate subset of T cells (adaptive immunity). Human MHC molecules are called human leucocyte antigens (HLA).

MHC genes are the most polymorphic genes present in the genome. For some HLA loci, more than 250 alleles have been identified by serological
Review Of The Literature

assays, and molecular sequencing has showed even greater polymorphism than predicted by serologic studies. The set of MHC alleles present in one chromosome is called the MHC haplotype. An individual's response to infections and inflammatory insults is affected by inherited haplotypes. Both disease-predisposing and protective HLA haplotypes, and alleles associated with various rheumatic and infectious diseases have been reported.16

There are two main types of MHC gene products, called class I and class II MHC molecules. The classical class I loci, HLA-A, -B, and -C, encode molecules that bind antigens usually derived from the intracellular pathogens and present them to CD8+ T cells, thereby initiating a cytotoxic T cell response. The classical class II loci, HLA-DR, -DQ, and -DP, specify molecules that primary bind peptides of extracellular origin and present them to CD4+ T cells, resulting in cytokine production and T cell help in antibody production.1

The MHC region encodes at least 240 genes, of which about 130 are considered to be functional (Figure 8). Of these, at least 40% are involved in immune response.16 A high diversity is crucial to defence against microorganisms. MHC genes have been associated with more than 100 diseases, including common diseases, such as diabetes, rheumatoid arthritis, psoriasis, asthma and various autoimmune disorders. Identification of these associations is complicated by the strong linkage disequilibrium that exists between the HLA genes and by environmental factors such as infectious agents and allergens.118 Conclusive association studies regarding the influence of HLA on infectious diseases require large samples, proper ethnic-background stratification, accurate clinical information, and use of models that consider other known genetic effects on the disease. Fulfilling these criteria is difficult.16

Despite this, a number of convincing HLA class I and class II associations with infectious diseases have been identified. Previously, HLA-DRB1*01 has been associated with periodontal pathogens in Finnish coronary artery disease patients, as and HLA-B*27 and HLA-B*35 with infectious or post-infectious complications in chronic herpes simplex virus infections, HIV, reactive arthritis and Chlamydia pneumoniae infections.16, 77, 96, 97, 120

The haplotype HLA-A*3303-B*4403-DRB1*1302 has been shown to predispose to neuralgia after herpes zoster in Japanese patients.110
2.9. Methods of evaluating the pain pathway

Peripheral level

Pain is a complex physiological and clinical phenomenon characterized by
subjective sensory qualities, such as its time of onset or specific location on the body. Strong affective reactions associated to pain heighten its complexity. Despite these challenges, the patients are able to provide reliable information of their pain experience on structured physiological testing. The evaluation of the functional status of the nervous system that conveys pain information is based on the subjects’ ability to report and quantify their pain experience. The perception of pain depends on activation of specific peripheral nerve fibers and their central connections.\textsuperscript{68} C- and A\textsubscript{\(\delta\)}-fibers mediate pain, and damage to them or their central connections underlie all neuropathic pain.\textsuperscript{94}

Diagnosis of neuropathic pain is often possible in clinical bed-side examination. Location and quality of pain may be determined, pain intensity may be assessed by using visual analogue scale (VAS), and sensory symptoms may be evaluated by simple equipment: a piece of cotton wool, wooden cocktail stick, warm and cold objects and a tuning fork. Nerve conduction studies and somatosensory-evoked potential, which do not assess small fiber function, may demonstrate and localize a peripheral or central nerve lesion. Quantitative assessment of A\textsubscript{\(\delta\)}- and C-fibers is possible by using quantitative sensory testing, laser-evoked potentials (LEPs), and skin biopsy. The LEPs are an easy and reliable method of assessing function of nociceptive pathway, but in clinical practice they are available in few centres only.\textsuperscript{21}

Quantitative Sensory Testing (QST)

In the peripheral nervous system, pain is mediated by myelinated A\textsubscript{\(\delta\)}- and non-myelinated C-fibers. Cold perception and the first (sharp and localized) pain are mediated by the relatively fast (5-30 m/s) conducting A\textsubscript{\(\delta\)}-fibers and warm perception and the second (dull) pain by the slow conducting (1-2.5m/s) C-fibers.\textsuperscript{107}

Determination of the thermal perception thresholds is an established method for the study of small nerve fiber function. Commonly used measurements are the reaction-time-inclusive method of limits and the reaction-time-exclusive method of levels; of these the method of limits is most commonly used because it is easy and fast to use. In the method of limits, the stimulus temperature increases or decreases with constant rate, starting from the temperature of +32 °C. When the patient gets the sensation of warm or cold, or heat or cold pain, he pushes a button, after which the temperature returns to 32°C. In this method, the subjects’ state of alertness and motivation influence the results.\textsuperscript{107}

Patients with neuropathic pain may have negative sensory phenomena, for example thermal sensory deficits, where thermal innocuous and pain thresholds are elevated. They may also have positive sensory phenomena (hyperalgesia and hyperesthesia), where the thermal pain thresholds are lowered. Both these abnormal states can be measured with QST.
One limitation of the method has been the lack of comprehensive normative data. The perception thresholds may vary according to the specific body area, and in some studies according to age and gender. Age-related differences appear to be most prominent with patients above the age 60 years. Thermal sensation and heat pain thresholds may also slightly vary when same subjects are measured repeatedly. Instead, there are no significant differences in QST-parameters observed between the right and left sides of the body.

There is greater variability of noxious thresholds than innocuous thresholds. Pain is strongly influenced by individual modulatory mechanisms, at the spinal cord, brainstem, thalamic and cerebral levels of the pain-pathway, which cannot be controlled by measurement protocols. Cold pain is the most susceptible modality in this regard.

**Skin biopsy**

Intraepidermal nerve fiber density (IENF) quantitation is established as a diagnostic and research tool for conditions affecting unmyelinated C- and Aδ-fibers, such as idiopathic painful small-fiber neuropathy, diabetic neuropathy, HIV neuropathy, and Fabry’s disease. This method is quantitative, it can be used for serial testing, the skin biopsies are easy to perform, and the method has high sensitivity and specificity. In different studies slightly variable techniques are used. Usually 3 mm punch biopsies are taken under local anaesthesia at the ankle, 10 cm above lateral malleolus. The biopsy specimens are fixed, frozen or embedded in paraffin, cut into 10-100 μm thick sections, and immunostained for the panaxonal marker, PGP 9.5. The nerve fibers are counted by laser scanning confocal microscopy or light microscopy. Some of the methods are methodically demanding, which is why the acceptance of the method for wider use has been relatively slow. Counting the nerve density per epidermal area by light microscopy after immunohistochemical staining may prove to be easier.

Controversial results exist concerning the influence of age, gender, and anthropometric variables on IENF density in normal individuals. Most studies have reported a reduction of IENF density with increasing age. The influence of gender, height, and weight are controversial. There are reports suggesting that women have greater epidermal nerve fiber density than men. Most studies have shown a length-dependent pattern of IENF density in normal individuals: IENF density at the distal thigh is about 60% higher than at the distal leg segment. No correlation with the duration of diabetes or level of glycemic control with IENF density was observed. There is limited longitudinal information on patients undergoing serial biopsies. In a small study of patients with sensory complaints of the feet, the swelling of large nerve fibers in the initial biopsy, correlated to a decline in IENF density in repeated biopsies. In patients with CRPS, IENF density was
diminished at the site affected by CRPS, but not in the ipsilateral unaffected control site, or contralateral matching control site.94

Functional brain imaging in pain

Current functional neuroimaging methods for human brain mapping in pain are based on two different principles: measuring of either hemodynamic or electrophysiological changes (Figure 9). Available methods based on hemodynamic principles are functional magnetic resonance imaging (fMRI), positron emission tomography (PET), single-photon emission computed tomography (SPECT) and near-infrared spectroscopy (NIRS). Electrophysiological techniques include electroencephalography (EEG), magnetoencephalography (MEG), and transcranial magnetic stimulation (TMS). Hemodynamic methods are superior in spatial resolution, compared to electrophysiological methods, which have a theoretical advantage of detecting cortical activities within a millisecond's resolution (Table 1).67, 117

The first human brain imaging studies using modern technologies were published in early 1990s. Those studies indicated that multiple cortical and subcortical regions are activated during painful cutaneous heat stimuli in healthy subjects.

![Brain imaging techniques used in the study of pain. MEG and EEG provide the most direct measures of neuronal activity, whereas PET and fMRI measure an indirect hemodynamic response. The Figure is reprinted from Kupers R and Kehlet H. Brain imaging of clinical pain states: a critical review and strategies for future studies. The Lancet Neurology 2006;5:1033-1044 with permission from the publisher.](image)

Figure 9. Brain imaging techniques used in the study of pain. MEG and EEG provide the most direct measures of neuronal activity, whereas PET and fMRI measure an indirect hemodynamic response. The Figure is reprinted from Kupers R and Kehlet H. Brain imaging of clinical pain states: a critical review and strategies for future studies. The Lancet Neurology 2006;5:1033-1044 with permission from the publisher.)
Table 1. Characteristics of different brain imaging techniques used in the study of pain. The Table is reprinted from Kupers R and Kehlet H. Brain imaging of clinical pain states: a critical review and strategies for future studies. The Lancet Neurology 2006;5:1033-1044 with permission from the publisher.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Temporal resolution</th>
<th>Spatial resolution</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tr>
<td>fMRI</td>
<td>100 ms-3 s</td>
<td>&gt;2 mm</td>
<td>Measures activity in cortical and subcortical structures. Excellent spatial resolution.</td>
<td>Poor patient comfort. Requires non-magnetic equipment. Stimulus-dependent technique.</td>
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**Functional Magnetic Resonance Imaging (fMRI)**

fMRI imaging is a powerful tool for imaging functionally active brain regions in health and diseases. The most common method is blood oxygenation level-dependent (BOLD) imaging. fMRI BOLD imaging is based on the magnetization difference between oxyhemoglobin and deoxyhemoglobin, which constitutes a measure of haemodynamic response. The BOLD contrast is thought to reflect the population synaptic activity of given brain regions. In the area of brain where a neuronal group is activated, the concentration of deoxyhemoglobin is relatively decreased, and that area can be visualized as high signal intensity on T2-weighted image.117

Both primary somatosensory cortex (SI) and secondary somatosensory (SII) cortex are commonly activated in heat pain studies. Evidence suggests that nociceptive input to these regions at least partially underlies the perception of sensory features of pain. Anterior cingulate (ACC) and insular cortices (IC), both components of the limbic system, are activated during the majority of PET and fMRI studies of heat pain. These regions have been implicated in the affective processing of pain. Prefrontal cortical areas, as well as parietal association areas, are also sometimes activated by heat pain and may be related to cognitive variables, such as memory or stimulus evaluation. Subcortical activation is also seen, most commonly in thalamus, basal ganglia, and cerebellum.8

fMRI of pain has produced results that are in agreement with prior reports using PET. It has revealed novel regions of the brain involved in pain...
processing. High-resolution fMRI has allowed the imaging of the brainstem in human subjects exposed to noxious stimuli, and measurement of neuronal activity in the spinal cord in both healthy and injured subjects. fMRI also demonstrates that the magnitude of the fMRI signal reflects the perceived intensity of pain. Experimental studies suggest that hemodynamic response is significantly correlated to neuronal activity, especially synaptic activity. However, the hemodynamic response tends to be more widespread in space and lasts longer in time as compared with neuronal activity.

Different types of pain have been associated with different patterns of brain activation, for example thermal and mechanical hyperalgesia produce substantially different brain activation patterns. There may be sub-regional differences in the processing of different types of pain. In several studies, ACC has been divided into different components, proposing different responses to different stimuli. Further, it has been suggested that affective reactions to pain would be localized to rostral ACC, while cognitive processes to the mid-cingulate region.

The processing of painful stimuli in the brain always involves psychological modulation of pain. Studies examining the effects of distraction show pain-evoked activity in SI, ACC, IC, and thalamus. Other regions, including PAG, parts of ACC, and the orbitofrontal cortex are activated when subjects are distracted from pain, suggesting that these regions may be involved in the modulatory circuitry related to attention. Emotional state can influence pain perception, and negative emotional states enhance pain evoked activity in limbic regions, such as ACC and IC. The anticipation of pain can activate pain-related areas such as SI, ACC, PAG, IC, PFC and cerebellum, in the absence of physical pain stimulus.

fMRI (and other hemodynamic methods) in clinical pain

It is probable that different clinical pain states generate different brain activation patterns. In clinical pain studies reported, the patient populations studied vary greatly in terms of the aetiology of pain, duration of pain, localisation of pain, type of pain, distribution of pain and psychological factors. All these differences make interpretation of the results challenging.

It is difficult to find patients with pain in the same anatomical region. Differences in pain afflicted areas may affect to activation patterns in regions with known pain-related somatotopic organization. Varying duration of pain may affect the results: in one study, hyperperfusion of the contralateral thalamus was observed in CRPS patients, if the duration of pain was only 3 to 7 months, whereas in long-term pain, hypoperfusion of the same area was observed. Supraspinal activation patterns for acute pain in healthy subjects differ from those seen in clinical pain conditions. When attempting to study clinical pain states with hemodynamic methods, the earliest approach was
application of similar painful stimuli to healthy subjects and patients with different pain states. In those studies, when compared to healthy controls, patient brain activity to thermal stimuli was reported to be decreased in rheumatoid arthritis, in patients with atypical facial pain, and in patients with post-tooth extraction pain. Those early studies had several methodological weaknesses. Later, low back pain patients compared to matched normal controls showed no significant changes to responses to thermal stimuli applied to the hand.

In neuropathic pain, a number of studies have investigated cerebral responses to stimulation of allodynic areas in a nerve-injured territory, and compared it to the healthy, contralateral side. Peyron et al. found that responses to allodynic stimuli were enhanced in the hemisphere ipsilateral to allodynia, whereas in the contralateral hemisphere, there was a complex pattern of response attenuation (SII and insula) and enhancement (ACC, sensorimotor and posterior parietal cortices). Hypoesthetic skin areas have rarely been studied, and it is likely that responses to stimulation of allodynic and hypoesthetic skin areas produce different brain activation patterns.

Increases in cerebral blood flow in the ACC have been reported in psychiatric patients with obsessive-compulsive disorders, anxiety, post-traumatic stress or mood disorders like depression. Anxiety and depression are likely to be present also in chronic pain, thus affecting the pain experience and making the interpretation of observed hemodynamic changes difficult.

In some clinical pain states it is possible to provoke the pain and measure the underlying brain activity. For example, in patients with migraine with aura, there is a decrease observed in blood flow and activity in occipital area, during a migraine attack. In a few reports, ongoing neuropathic pain has been alleviated by anaesthetic blocks or central pain has been treated with motor cortex stimulation. However, in most pain conditions, including neuropathic pain, manipulation of ongoing pain is not possible. In those conditions application of equated stimuli (equalization of perceived intensity of pain in VAS-scale) between the patients and normal controls has been used in several studies.

Responses to painful stimuli may be activation or deactivation of pain-related areas. In healthy subjects the response to painful stimuli is often increased activity in those areas. In clinical pain, however, deactivation and attenuated responses have been reported. Attenuation of cortical responses has also been considered as a sign of possible lesion in spinothalamic tract.

Contralateral thalamus is activated in response to pain in healthy subjects. Thalamic activation may also be bilateral, which suggests that thalamic activation in pain may reflect arousal reaction to pain. In contrast to experimental pain in normal controls, chronic pain is often associated with decreased activity in the contralateral thalamus. This deactivation may be reverted by analgesic procedures. In normal subjects the stimulus is transmitted via the spinothalamic pathway through the thalamus to SI,
SII, IC and ACC. It has been suggested that in chronic pain, the activity of this pathway decreases, and the PFC activity seems to increase, indicating that pathways outside the spinothalamic tract, such as spinoparabrachial, spinohypothalamic, and spinoreticular projections, may become more important in chronic pain.\(^8\) It has also been thought that the hypofunctional thalamus does not allow for adequate descending inhibition during chronic pain states.\(^5\)

In a meta-analysis of several studies on clinical pain states it was concluded that chronic clinical pain conditions more frequently involve PFC, while experimental pain involves SI, SII, thalamus and ACC. The stronger activation of PFC suggests that chronic pain has stronger emotional and cognitive components than experimental pain.\(^8\)

**Voxel-based morphometry (VBM)**

Voxel-based morphometry involves a voxel-wise comparison of the local concentration of gray matter between two groups of subjects. There is a number of morphometric features that may be difficult to quantify by inspection, meaning that many structural differences may be overlooked. VBM is not biased to one particular structure and it gives a comprehensive assessment of anatomical differences throughout the brain. VBM deals with differences in the local composition of brain tissue after macroscopic differences in shape have been excluded. The procedure involves spatially normalizing images from all subjects in the study into the same stereotactic space. This is followed by segmenting and smoothing of the gray-matter segments. Statistical methods are used to compare the two groups.\(^11\)

**Voxel-based morphometry in clinical pain**

Voxel-based morphometry has demonstrated a significant reduction in gray matter volume of the dorsolateral prefrontal cortex and the right thalamus in patients with chronic back pain. The magnitude of the decrease was equivalent to the loss of gray matter volume during 10 to 20 years of normal ageing. There was also a correlation between the loss of brain volume and duration of pain.\(^9\) The gray matter volume was reduced in the brains of fibromyalgia patients, especially in the posterior cingulate gyrus, left IC, and the medial frontal cortex.\(^72\)

The observed atrophy was distinct from that described in chronic depression, anxiety, or neurodegenerative conditions such as Alzheimer’s disease. Common pathological hallmarks of neurodegeneration and pain include inflammation, glial activation, and apoptosis. This shared pathology may explain the atrophy in seen pain patients, although it is not clear how universal this finding is in different pain states.\(^9\)
Magnetoencephalography (MEG)

Magnetoencephalography is a non-invasive method, where magnetic fields are measured outside the skull. The magnetic fields are generated by simultaneously firing neurons. The skull, scalp, and brain do not distort magnetic fields, so the cortical activity can be easily measured.\textsuperscript{55} The following four areas are constantly activated in experimental pain in healthy subjects in MEG: (i) primary somatosensory cortex (SI) contralateral to the stimulated side, (ii) secondary somatosensory cortex (SII) in the bilateral hemispheres, (iii) insula in the bilateral hemispheres, and (iv) cingulated cortex in the bilateral hemispheres.\textsuperscript{67} Very few EEG or MEG studies demonstrate activity in IC and neither method shows activation of the thalamus or PFC. IC and the thalamus may be missed because of their position deep inside the brain, since location accuracy of both these techniques decreases with increasing distance from the scalp.\textsuperscript{8} Compared with hemodynamic imaging studies, magnetic recordings are highly sensitive to describe activity in the SII region. MEG is more sensitive than EEG in recording the sources in SI and SII, which are oriented tangentially to the scalp. Radially oriented current flow in ACC activity is more frequently detected in EEG.\textsuperscript{8} The temporal resolution of MEG is superior to hemodynamic methods. The sensation of pain elicited by a brief painful stimulus is reflected in two sequential brain activations in MEG recordings from SI, SII, and ACC. This is due to different conduction velocities in nociceptive A\textdelta- and C-fibers.\textsuperscript{8} Different sensory stimuli can be used during the measurements. Tactile stimuli can be delivered to fingertips with diaphragms driven by compressed air. A painful stimulus which selectively stimulates nociceptive A\textdelta-fibers may be delivered by laser beam. A selective stimulation of C-fibers is possible by decreasing the total energy of a laser beam and by restricting the size of the stimulated skin area. The activity generated by these methods is termed as laser evoked potential (LEP).\textsuperscript{67}

MEG in clinical pain

Bodily representations of the SI cortex vary according to sensory input. Excessive training may lead to an increase in cortical representation areas of the fingers.\textsuperscript{35} On the contrary, loss of sensory input, e.g. after amputation of the arm, leads to changes in cortical representations, and the amount of cortical reorganizations correlates with the magnitude of phantom-limb pain.\textsuperscript{29} Hence, in amputation patients, the changes may be due to both deafferentation, and the pain. However, cortical reorganization also occurs, when there is no loss of peripheral input to brain, indicating that pain itself may be associated with reorganization of the primary somatosensory cortex. In patients with CRPS type I (when there is no evident lesion of afferent nerves), the distance between the thumb (D1) and little finger (D5)
representation areas decreased in distance, and the representation areas shifted to more lateral and inferior position toward the lip.\textsuperscript{66, 80} Changes also correlated with pain intensity and the presence of mechanical allodynia. During healing process, a reduction of CRPS pain correlated with recovery from cortical reorganization. Reorganization of SI cortex has also been reported in low back pain patients. In MEG measurements, the amplitude of the response depends on the number and synchrony of the activated neurons. In CRPS, the stronger SI activation to tactile stimulation of the painful than to the non-painful hand has been suggested to reflect central sensitization, as these patients have allodynia and hyperesthesia in the affected limb.\textsuperscript{38, 66, 80, 81} In addition to CRPS and phantom-limb pain, there are only few other clinical pain conditions examined by MEG, including small studies of low-back pain and carpal tunnel syndrome.\textsuperscript{38, 129}
3. Aims Of The Study

The aims of the study were to characterize two subgroups of patients with a HSV-associated clinical condition. The specific objectives of this Thesis were the following:

1. To characterise the clinical picture, immunological features and changes in brain morphology and function in patients with widespread unilateral pain and HSV-infections (I, II).

2. To analyse the prevalence, clinical symptoms and immunological predisposing factors of HSV-2 induced RLM in the southern part of Finland (III, IV).
4. Subjects And Methods

Widespread unilateral pain

Patients: Twenty-two patients with widespread unilateral pain covering a large area of the body during the time period of 12/2002 and 1/2004, were evaluated for the study. The patients were recruited from the Pain Clinic and from the Division of Infectious Diseases of the Helsinki University Central Hospital. In addition to infectious problems, the patients had severe pain which had spread outside the original territory but remained on one side of the body (inclusion criteria: the painful area covered face or trunk or both, and one or two extremities). As the majority (n=17) of patients had recurring HSV-1 and HSV-2 infections, we decided to study the role of HSV-1 and -2 infections in the pain condition. A subgroup of patients (n=8), with the most widespread clinical pain and hypoesthesia as a clinical sensory finding were also recruited to a fMRI study.

Controls: For laboratory analyses (except for the frequencies of the HLA alleles), 51 age- and sex-matched controls (regardless of seropositivity or negativity to HSV-1 or HSV-2) were selected from a population of 150 consecutive voluntary subjects who visited the Vita Laboratories Ltd (Helsinki, Finland) for health survey before accepting a new job. An additional statistical analysis was made by using 34 age- and sex-matched, HSV-1 or HSV-2 seropositive controls also selected from the original 150 subjects. By two controls groups we tried to assess whether the immunological profiles potentially predisposed to complicated HSV infections or to neuropathic pain. For the frequencies of the HLA alleles, 90 Finns were used as historical controls (population matched historical control material available in: http://www.ncbi.nlm.nih.gov/gv/mhc/main.fcgi?cmd=init IHWG projects-Anthropology/ Allele frequencies -Select population -Finn90). A control group for VBM comprised 28 healthy adults (18 females, 10 males, mean age 32). Of these, eleven subjects (5 females, 6 males, 10 right-handed, mean age 30) served as controls also for the fMRI. These controls were not age- nor sex –matched.

Clinical examination: A questionnaire designed for this study (including questions about general symptoms, infectious diseases, musculoskeletal and neuropathic pain, and treatments) was used to interview the patients. Neurological examination (evaluation of the brainstem function, balance, coordination, muscle strength, tendon reflexes, cognition, fine motor skills, sensory evaluation) was performed. Evaluation of sensory function included responses to pressure, pin-prick, cotton-wool, cold and warm objects in
Quantitative sensory testing (QST) (Medoc TSA II; Medoc, Ramat Yishai, Israel) was performed at normal room temperature on four sites; two on the painful area of the body, and for reference, two on the corresponding contralateral side of the body. The method of limits was used to determine thresholds for warm and cold detection, and for heat and cold pain. The time between different stimuli was 60 seconds, or - in case of hyperpathy - until the sensation had passed. When cold (skin temperature under +31°C), the hands were warmed under warm water. In addition, in patients recruited to a fMRI study, the current pain intensity was evaluated with VAS-scale, and tactile sensitivity and two-point discrimination on the hands was tested with monofilaments (Touch-Test Sensory Evaluator, North Coast Medical, Morgan Hill, CA).

Laboratory tests: Plasma concentrations of IgG, IgA, IgM (Dade Behring BN Prospec, Deerfield, IL) and IgG subclasses (Ig1-4) (Peli-Class BN, Sanguin Reagents, Amsterdam, The Netherlands), and serum concentrations of C3 and C4 (Behringswerke AG, Marburg/Lahn, Germany) were measured by nephelometry. Serum classical pathway haemolytic activity was determined by an enzyme-linked immunosorbent assay technique (CH50; Quidel Corporation, San Diego, CA). The Manufacturers’ reference values for levels below two standard deviations from the mean were used to define low levels (= values below two standard deviations from the mean). The cut-off values were 6.8 g/l for IgG, 0.88 g/l (males) and 0.52 g/l (females) for IgA, 0.36 g/l (males) and 0.47 g/l (females) for IgM, 4.9 g/l for IgG1, 1.5 g/l for IgG2, 0.2 g/l for IgG3, 0.08 g/l for IgG4, 0.71 g/l for C3, and 0.12 g/l for C4. Type-specific HSV-1 and HSV-2 antibodies were measured by commercial EIAs according to the Manufacturers’ instructions (HerpeSelect 1 & 2 IgG; Focus Diagnostics, Cypress, CA; ElAgen HSV IgM, Adaltis, Bologna, Italy). Complement C4 allotypes were determined electrophoretically. The absence of an isotype was confirmed by isotype-specific PCR amplification of the C4A and C4B genes. DNA samples were genotyped for HLA-A, HLA-B, and HLA-DRB1 by means of commercial kits from Biotest (ABDR SSP), Dreieich, Germany, or One Labda (ABDR SSP), Canoga Park, CA; with low to intermediate allele resolutions. In a subgroup of 12 patients, also IgG1 and IgG3 allotyping (Gm) was performed: allotypic marks of IgG1 were detected with double diffusion precipitation in gel (IsoGel Agarose, FMC BioProducts, Rockland, ME), and allotyping for IgG3 was obtained as a by-product of their quantitation with inhibition ELISA.

Skin biopsies: As a pilot study, skin biopsies were taken from six patients. The biopsies were taken 10 cm above the lateral malleolus from both sides. In all patients, one biopsy was taken from the painful area, and could be compared with the one taken from the corresponding contralateral non-painful and non-painful skin areas.
painful area. The samples were fixed in 10% formalin, after which they were embedded in paraffin. Thin 10 µm sections were cut and stained with polyclonal gene product (PGP) 9.5 (Ultraclone, Isle of Wright, UK). Using light microscopy, immunopositive intraepidermal nerve fibers were counted per epidermal area.

Functional magnetic resonance imaging: During fMRI measurement, thermal stimuli to the right and left hand dorsa were delivered via 16 mm x 16 mm Peltier thermodes of the TSA II devices, and tactile stimuli to the index, middle and ring fingers via diaphragms driven by compressed air. fMRI was performed with a 3.0 T Signa Excite scanner (General Electric, Milwaukee, WI) using a gradient-echo (GRE) echo planar imaging (EPI) sequence. Imaging the whole brain required 31 oblique slices, each 4 mm thick. The tactile stimulation series was conducted first and the pain stimulation series immediately afterwards. The subjects were instructed to lie still and keep their eyes open during the imaging. High resolution T1-weighted anatomical images were acquired during the same MRI session. The fMRI data was preprocessed with BrainVoyager QX (Brain Innovation, Maastricht, The Netherlands). Both functional and anatomical images were normalized to Talairach space for subsequent analysis. The data of the patients with pain in the left side of the body (n=4) were mirrored across the midsagittal plane. In group comparison half of the controls were mirrored in the same manner, to minimize the possible effects of unsymmetrical activations to left- and right-hand stimuli. In VBM, T1-weighted anatomical images were obtained, and further preprocessing of the data was conducted according to the optimized VBM protocol with software package SPM2 (Wellcome Department of Imaging Neuroscience, London, UK).

**Recurrent lymphocytic meningitis**

Patients: All patients in Helsinki and Uusimaa Hospital District area (a total population of 1.4 million) with a recurrent HSV-2 lymphocytic meningitis (defined as two or more clinical episodes of lymphocytic meningitis, and HSV-2 DNA in at least one CSF sample) between January 1994 and December 2003, and willing to take part in the study, were recruited to the patient study (n=22). Of these, 21 patients went further to laboratory analysis (one patient had to be excluded due to a clinical condition, which probably affects on immunological analyses). For prevalence study, all patients diagnosed with RLM (n=37) between January 1996 and December 2006 were included.

Controls: For laboratory analyses (except for the frequencies of the HLA alleles), 62 age- and sex-matched healthy subjects were selected from a population of 150 matched consecutive voluntary subjects who visited the
Vita Laboratories Ltd (Helsinki, Finland) for health survey before accepting a job, to serve as controls. For the frequencies of the HLA alleles, 90 Finns were used as historical controls.

Prevalence study: The Helsinki and Uusimaa Hospital District Database of ICD-10 diagnostic codes A87, B00.3+G02.0, B01.0+G02.0, B02.1, G02*, G03.0, G03.1, and G03.2 were used to identify the study cases. The 11-year time period between January 1996 and December 2006 was covered. Since the World Health Organisation International Statistical Classification of Diseases and Related Health Problems (ICD) coding system changed in 1996, we chose a different time period in the patient study, to avoid the potential bias caused by two different coding systems.

Clinical examination: A questionnaire designed for this study (including questions about general symptoms, musculoskeletal and neuropathic pain, infections, treatments, and symptoms during and after meningitis episodes) was used to interview the patients. All patients underwent a detailed clinical neurological examination.

Laboratory tests: Plasma concentrations of IgM, IgA, IgG, and IgG1-4, serum concentrations of C3, C4 and serum classical pathway hemolytic activity (CH50) were measured, serological anti-HSV-1 and -2 antibody status was tested, C4 phenotyping, and the analyses of C4 gene copy numbers by isotype-specific genomic real-time PCR amplification, HLA-A, HLA-B and HLA-DRB1 typing, and IgG1 and IgG3 allotyping (Gm) were performed as described on page 51. Serological anti-HSV-1 and -2 antibody status was tested on the study entry day, at least one month from the latest meningitis episode. In addition, MBL2 genotyping was performed. In MBL genotyping, exon 1 of the MBL was amplified by PCR. Genotyping of codons 52, 54, and 57 was performed by restriction fragment length polymorphism.

All laboratory methods used in these studies (except IgG allotyping) are available in clinical practice. QST is in clinical use, especially when additional evidence of a suspected nerve lesion is needed. Skin biopsies are occasionally used in diagnostics of small-fiber neuropathy. In pain diagnostics, fMRI is still used for scientific purposes only.

**Statistics**

Comparison between the groups was made by using the permutation test or Fisher’s exact test (I,III,IV). For continuous variables, Mann-Whitney U test or independent samples’ two-tailed test were used (I). For pairwise comparisons, the paired samples t-test or the Wilcoxon signed-rank test were used (I). Wilcoxon signed-rank test was used to compare the healthy
and painful side of the patients (QST and monofilament testing) in the fMRI study (II). fMRI data were analyzed using a general linear model (II). The results are expressed as mean or median, standard deviation (SD) or interquartile range (IQR), and 95% confidence intervals (95%CI) (III). To illustrate information on the cumulative proportion, product limit estimate was used, and 95% CIs were obtained by bias corrected bootstrapping (III). The incidence ratios were calculated by using the exact Poisson regression model (IV). In HLA-analysis, no adjustment was made for multiple testing (IV).

**Ethical aspects**

All studies were approved by the Ethics Committee of the Helsinki University Central Hospital, and in all studies, the subjects gave a written informed consent.
5. Results

Widespread unilateral pain

The patients manifested a uniform clinical syndrome. They had long-lasting pain with fluctuating intensity, and exacerbations with HSV-reactivations. The patients often described their pain as burning, radiating or tingling. In clinical sensory examination, all but one presented either hypo- or hyperesthesia of the painful areas, when compared to the contralateral healthy side. The area of sensory abnormality was not always equal to the painful area; in half of the patients the painful area was larger. In QST, all patients had a side-to-side difference of more than two degrees at least in one sensory modality, when the healthy and painful sides were compared. At group level there was a significant difference observed only in the cold detection thresholds of the hands, where the cold detection threshold was lower on the painful side (Table 2).

Based on the patients’ description, sensory status and QST-results, the pain resembled neuropathic, although according to the IASP definition, an exact diagnosis could not be made, since the painful area was not necessary neuroanatomically logical, and signs of a lesion or disease affecting the somatosensory system were uncertain.

Skin biopsies were taken from only six patients as a pilot-study. These preliminary results did not support the hypothesis of the role of small fiber neuropathy in the patients’ clinical condition and thus the collection of biopsy samples was not continued. There were low values both in the painful and healthy side, and normal values on the painful side, with no uniform pattern.

In laboratory analyses, when compared to both seropositive and seronegative controls, total plasma IgG3 concentration, and the frequency of low IgG1 or IgG3 or both, were more frequent in the patients. Anti-HSV-2-IgG titers were higher in the patients than in the controls. When compared to seropositive controls, anti-HSV-2 titers were still higher in the patients, but plasma IgG3 concentrations, or in the frequencies of low IgG1 or IgG3 or both did not differ statistically -suggesting that IgG subclass deficiencies probably predispose to recurrent HSV infections, but not to pathological pain (Table 3).

In the fMRI study, during the measurement the average spontaneous pain on VAS-scale was 4.6/10. In this subgroup of patients, detection thresholds for cold and warm were within normal range, although lower in the painful side in quantitative sensory testing (p=0.04 for both). In monofilament testing, the tactile sensitivity was within normal limits, although lower on the painful side (p=0.04). Both the patients and the controls rated the pain...
intensity and the unpleasantness of thermal stimuli similarly.

In the fMRI of the control subjects, the contrast (Pain left-Warm left + Pain right-Warm right) showed bilateral activations in the IC, SII cortex, posterior parietal cortex (PCC), ACC and thalamus, and in the right frontal cortex, right inferior parietal cortex, right dorsolateral prefrontal cortex, and in the left cerebellum. In the patients the same contrast showed no statistically significant activations with the same threshold.

In the control subjects, when the hands were stimulated separately, the bilateral ICs, contralateral striatum, and parietal cortex were activated, and bilateral occipital and parahippocampal cortices and left sensorimotor region were deactivated. In the patients, separate stimulation of the painful and healthy hands showed only few activation areas; the contrast (Pain healthy-Warm healthy) showed a single cluster in DLPFC and the contrast (Pain painful-Warm painful) showed activations in the IC contralateral and in the IPC ipsilateral to the side of the chronic pain.

In the controls, the contralateral IC responses were equally strong and similar in their time courses to the stimulation of the left and right hands. In the patients, the contralateral IC response was significantly weaker to the healthy than the painful hand stimulation.

In conclusion; when comparing the differences in the pain-related brain activations between the two groups, the patients had significantly less activity than the controls in the ICs of both hemispheres, ACC and thalamus. In addition, the activations in the same areas in the patients were weaker, when the healthy hand was stimulated (Figure 10).

When the activations elicited by warm stimulation were compared between the controls and the patients, no differences were seen in any brain regions. The time courses and strengths of the SI and SII responses were similar in both groups, independently which hand was stimulated, and the touch-related activation areas were similarly found in the SI and SII cortices and the cerebellum of both groups.

In VBM analysis, gray matter density was lower in the patients than in the control subjects in the ACC, and in frontal and prefrontal cortices. No area showed lower gray matter density in the control subjects than in the patients.

The hemodynamic responses to tactile stimulation in SI and SII were highly similar both in patients and controls, despite the age difference between the groups. Also gray matter loss was significant in the patients compared to the controls, even when age was included as a covariate of no interest in the structural VBM analysis.
Figure 10. The differences in the pain-related brain activations (Pain left-Warm left + Pain right-Warm right) between the patients and the controls. The patients had significantly less activity than the controls in the ICs of both hemispheres, ACC and thalamus. The time courses of the contralateral IC responses were equally strong and similar in the controls to stimulation of the left and right hand. In the patients, the contralateral IC response was significantly weaker to the healthy than the painful hand stimulation.
<table>
<thead>
<tr>
<th>Modality</th>
<th>Painful side Mean (SD)</th>
<th>Healthy side Mean (SD)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arm</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold sensation</td>
<td>29.9 (1.7)</td>
<td>30.9 (0.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>Warm sensation</td>
<td>34.8 (2.6)</td>
<td>33.6 (0.9)</td>
<td>0.11</td>
</tr>
<tr>
<td>Cold pain</td>
<td>13.9 (12.0)</td>
<td>16.2 (11.3)</td>
<td>0.34</td>
</tr>
<tr>
<td>Heat pain</td>
<td>44.1 (4.6)</td>
<td>43.0 (4.9)</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>Leg</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cold sensation</td>
<td>29.0 (2.5)</td>
<td>29.5 (2.0)</td>
<td>0.43</td>
</tr>
<tr>
<td>Warm sensation</td>
<td>39.4 (3.6)</td>
<td>38.0 (3.2)</td>
<td>0.08</td>
</tr>
<tr>
<td>Cold pain</td>
<td>10.0 (11.2)</td>
<td>10.7 (11.4)</td>
<td>0.80</td>
</tr>
<tr>
<td>Heat pain</td>
<td>46.3 (4.1)</td>
<td>45.2 (4.0)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

Table 2. Quantitative sensory testing (QST). Comparisons were made between the painful and the respective contralateral areas.
Recurrence lymphocytic meningitis

Between January 1996 and December 2006, a total of 665 patients were treated at the Helsinki University Central Hospital for lymphocytic meningitis. In 37 of them (5.6%), the meningitis was recurrent. In 28 of the patients with recurrent disease (28/37), there was HSV-2 DNA in the CSF. In addition, three patients had a history of recurring genital herpes but no HSV-2 DNA in the CSF. Thus it may be approximated that the minimum period prevalence of the HSV-2 induced recurring meningitis is 2.2/100 000 in the Helsinki and Uusimaa Hospital district.

HSV-1 seropositivity was less common in the patients than in the controls (p=0.043). Anti-HSV-2 IgG titers in the patients during the asymptomatic period were higher than in the controls (p=0.034).

Serum IgG1 concentrations were lower (p=0.009), and low IgG1 (p<0.001) levels were more common in the patients than in the controls (Figure 11). Patients with low IgG1 levels had 0.40 meningitis episodes per follow-up
Results

year, compared with 0.20 meningitis episodes per follow-up year in the patients with normal IgG1 levels (incidence ratio 2.05). Despite a trend, serum IgG3 concentrations (p=0.078), or the frequency of low IgG3 (p=0.11) did not significantly differ between the patients and the controls. HLA-DRB1*01 and HLA-B*27 alleles were more common in the patients than in the historical controls (p=0.009 and p=0.050, respectively) (Table 4).

Immunological deficiencies associated to vulnerability to, or persistence of bacterial or viral infections, including partial or total C4B deficiency, low IgG1 or low IgG3, were detected in 12, 9 and 7 patients, respectively. HLA-allotypes associated to infectious diseases including HLA-DRB1*01, HLA-B*35 and HLA-B*27 were detected in 11, 9 and 7 patients, respectively. Combinations of these factors in a single patient were common (Table 5).

The sensitivity of PCR to detect HSV-2 was at its best, when the sample was taken during the first 2 to 5 days from the acute symptoms. During that time, HSV-2 DNA was present in 82% of the samples. The median leucocyte count during the first HSV-2 PCR positive episode was 350 (range 44-1410). In PCR negative cases, the leucocyte counts were lower and the samples were taken outside the optimal time period.

The time between the first and second episodes varied from one to 216 months (median 47 months). Paraesthesias, neuropathic pain, arthralgias, and urinary dysfunction were commonly reported during and after the clinical episode of meningitis.

![Graph showing IgG1 and IgG3 concentrations in patients with RLM and controls.](image)

Figure 11. Plasma IgG1 and IgG3 concentrations in the patients with RLM and in the controls. The limit of normal value has been indicated with a dashed line.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Patients (N=21)</th>
<th>Controls (N=62)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Means (95%CI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG1 g/l 5.61 (3.60-9.97)</td>
<td></td>
<td>6.37 (2.88-13.3)</td>
<td>0.0088</td>
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<tr>
<td>IgG3 g/l 0.28 (0.17-0.68)</td>
<td></td>
<td>0.30 (0.13-0.84)</td>
<td>0.078</td>
</tr>
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<td>Per cents</td>
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<tr>
<td>Low IgG1 43%</td>
<td></td>
<td>8%</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Low IgG3 33%</td>
<td></td>
<td>15%</td>
<td>0.11</td>
</tr>
<tr>
<td>C4B genes (0 or 1) 62%</td>
<td></td>
<td>39%</td>
<td>0.30</td>
</tr>
<tr>
<td>MBL2 structural variant carriage 43%</td>
<td></td>
<td>34%</td>
<td>0.459</td>
</tr>
<tr>
<td>MBL2 promoter variant carriage 19%</td>
<td></td>
<td>40%</td>
<td>0.112</td>
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<tr>
<td>HLA-B*27 19%</td>
<td></td>
<td>8%</td>
<td>0.050</td>
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<tr>
<td>HLA-B*35 21%</td>
<td></td>
<td>13%</td>
<td>0.151</td>
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<tr>
<td>HLA-DRB*01 31%</td>
<td></td>
<td>13%</td>
<td>0.009</td>
</tr>
</tbody>
</table>

Table 4. Immunological characteristics of the patients with RLM and of the controls.
* = 90 historical controls (Finn90). The statistical significance between the groups was evaluated either by permutation test or by Fisher’s exact test.
<table>
<thead>
<tr>
<th>Pat. No</th>
<th>Meningitis Episodes (N)</th>
<th>C4B deficiency</th>
<th>HLA-DRB1*01</th>
<th>HLA-B*35</th>
<th>HLA-B*27</th>
<th>Low IgG1</th>
<th>Low IgG3</th>
<th>MBL2 insufficiency</th>
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<tbody>
<tr>
<td>14</td>
<td>2</td>
<td>d</td>
<td>+</td>
<td>+</td>
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<td>20</td>
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<td>d(t)</td>
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<td>9</td>
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Table 5. Summary of the immune deficiencies found in the patients with RLM. N = number of meningitis episodes. d = deficiency (1 C4B gene present), d(t) = total deficiency (0 C4B genes present). + = low IgG1/ low IgG3/ combined MBL2 genotype representing MBL insufficiency (XA/YO or YO/YO)/ one HLA-DRB1*01/ HLA-B*35/ HLA-B*27 allele present. ++ = two HLA-DRB1*01/ HLA-B*35/ HLA-B*27 alleles present.
6. Discussion

HSV and widespread unilateral pain

The original hypothesis was that HSV has an important role in development of pathological pain in patients with widespread unilateral pain. Our hypothesis was based on two reasons: first, in over half of the patients there was a positive correlation between the activity of HSV infection and the severity of pain, and second, during the follow-up, 80% of the patients reported at least moderate pain relief with antivirals.

Viral pathogens activate glia, which is able to detect HSV via specific receptors. Activation of glia takes place at peripheral nerves, dorsal root ganglia, spinal cord, and most likely, also in the brain. Because of its viral properties (neuroinvasiveness, ability to maintain latency, and ability to reactivate from the latency) HSV is a good candidate for playing an active role in the glial activation, and creation of pathological pain. In addition, HSV has a predilection for frontal and temporal lobes, which are important pain-processing areas in the brain.

When activated, glia releases proinflammatory cytokines like IL-1, IL-6, and TNF-α. Repeated HSV infections cause repeated bursts of cytokines. Pro-inflammatory cytokines enhance pain in models of inflammatory pain, and stimulate the production of other inflammatory mediators, including prostaglandins. The free nerve endings of peripheral nerve fibers respond to this by generating abnormal electrical activity that is interpreted as painful. The spread of pain may be explained by activation of glia: astrocytes activate distant astrocytes via gap junctions by releasing pain enhancing substances.51, 88, 90, 105, 125, 142, 155

HSV is among the most common viruses of human population, and a majority of individuals harbour a latent virus that can reactivate. Despite this, pathological pain induced by HSV is a rare syndrome. Certain immunological deficiencies, such as low IgG1 or low IgG3, or both, were more common in the patients, which probably made them more vulnerable to recurring HSV infections. Anti-HSV-2-IgG titres were higher in the patients, most probably reflecting an increased activity of HSV-2 recurrences compared to the controls.

Besides herpes, recurrent or invasive infectious problems were reported by 65% of patients, and also other infections than HSV were reported to enhance pain - this fits the theory that in many cases, inflammatory mechanisms are active in chronic pain.

Three C4A genes, or a partial or complete deficiency of either C4A or C4B genes, were more common in the patients than in the controls, although the differences were not significant, probably due to the small number of
patients. It has been suggested that excessive amounts of C4 could possibly cause hyperactivation of the complement pathways, and exacerbate the inflammatory response at the local tissues. C4 deficiencies, in turn, have been proposed to lead to susceptibility to bacterial or viral infections.14

The relation between the HSV virus and pain is still uncertain. It may also be that HSV functions only as a trigger, which induces glial activation. Frequent HSV recurrences trigger glia repeatedly, hence leading to amplified response and pathological pain.

The characteristics of the pain

In Complex Regional Pain Syndrome, as in many other clinical pain conditions, central mechanisms are doubtlessly active. However, according to the IASP criteria for neuropathic pain, CRPS pain is not classified as neuropathic. Our patients with widespread, unilateral HSV-associated pain share many features with CRPS patients: they describe pain in similar words, and during time their pain has expanded to cover larger areas, even on the contralateral side. At the moment, until more evidence has been gained, the term “possible neuropathic pain” is perhaps appropriate when discussing the type of widespread unilateral HSV-associated pain. However, as in CRPS pain, the same treatment strategies as in neuropathic pain may be used.

Treatment aspects

Treatment options for neuropathic pain are still insufficient. Many patients have to undergo multiple drug therapy attempts, and use combinations of several drugs with frequent side-effects, to find anything to alleviate the pain. It is considered a good result, if the pain decreases by 30% from its original level. The problem with drugs used for neuropathic pain may be that they are originally targeted against neurons, instead of glial cells, which seem to be responsible for creation of pathological pain states.153

A poor response to traditional drugs used in neuropathic pain (antidepressants, anticonvulsants and weak opioids), and poor tolerability to side effects was typical in the patient group. Instead, the majority of them responded to antiderpetic medication. These drugs decreased the frequency of HSV recurrences, hence possibly reducing glial activation by reducing inflammatory responses in the nervous system.

Four of our patients had undergone a treatment attempt by intravenous immunoglobulins, either because of pain or because of infectious problems. In two of them, the treatment reduced the pain. There are a few small studies concerning intravenous immunoglobulins and pain. Intravenous immunoglobulins reduce IL-1α and IL-1β levels in the plasma, and in a subgroup of patients, also reduced pain.123 Theoretically, it might be a
treatment option in severe cases. However, in lack of good evidence, poor availability and high price, it will never be a treatment option to larger patient groups.

We already have a few drugs with an ability to inhibit microglial activation. Some of them are even in clinical use, although not in pain patients. One of these drugs is minocycline, which is an old drug, a semisynthetic tetracycline derivative. Besides antimicrobial actions, it has neuroprotective properties. It prevents apoptosis, reduces microglial activations, and prevents the release of proinflammatory cytokines. It has proved effective in animal studies in several models of different neurological diseases. In humans, minocycline significantly reduced white matter lesions in MS patients. Minocycline is a safe and cheap drug with high tolerability, but at the moment it is not commercially available in Finland.

Another drug possibility is ibudilast (AV411), which has been used in Asia to treat asthma, and stroke-related dizziness. Ibudilast suppresses proinflammatory cytokines and increases anti-inflammatory IL-10 in CNS glia. Also ibudilast has been in clinical use for years, and has high safety and tolerability. There has been a small study enrolling about ibudilast in diabetic neuropathic pain, but no results are available as yet.

Changes in functional brain imaging

Both functional and structural brain abnormalities were observed in the patients with chronic unilateral widespread pain. When compared to controls, the pain-related responses were attenuated in several pain-processing areas. Also gray matter density was decreased in areas related to pain-processing.

Acute experimental and clinical pain elicit different responses in functional brain imaging studies. It has been suggested that pain-related responses attenuate more in clinical than in experimental pain. In clinical pain, emotions, fear and anticipation of pain are more profoundly involved than in experimental pain. It has also been suggested that in clinical pain, pathways outside the spinothalamic tract, such as spinoparabrachial tract, spinohypothalamic tract, and spinoreticular projections are becoming more important. In addition, it is probable that different clinical pain states generate different brain activation patterns.

There is a balance between facilitatory and inhibitory systems of pain, and in chronic pain this balance probably changes. The inhibitory and facilitatory tracts of the spinal cord have been investigated for years. It is probable that also cortical areas are involved in pain facilitation and inhibition. Both activation and deactivation of cortical areas may be crucial in cortical pain-processing. Deactivation of cortical areas might reflect a reduction in neuronal activity. Deactivation of inhibitory areas in the
brain may theoretically be one of the causes of chronic pain. It has been shown that gabapentin reduces pain-related deactivation in chronic pain, suggesting that deactivation may contribute to chronic pain.59, 113, 157

Decrease in gray matter density has previously been associated to both HSV virus and the pain itself. HSV is neuroinvasive and able to reactivate in the CNS after latency. It prefers the frontal and temporal lobes, and has been shown to cause gray matter loss during encephalitis. On the other hand, gray matter loss has been described in pain conditions like fibromyalgia, and chronic back pain. It is impossible to say definitely whether gray matter loss in our patients is associated to HSV or pain, or both. On the other hand, decrease in gray matter density and reduced neuronal activity might be one explanation to attenuated pain responses in pain processing areas.

Unfortunately, age- and sex matched controls for fMRI and VBM analysis were not available. This is a drawback, because ageing is associated with changes in the hemodynamic response strength and decreased gray matter density. However, the hemodynamic responses to tactile stimuli were similar between the patients and the controls, which makes significant age-related differences between the groups unlikely. In VBM analysis, age was included as a covariate of interest to render the effect of age-difference between the groups.

Previously cortical reorganization has been observed in association with both extensive training, and loss of sensory input.35 Cortical reorganization itself may cause chronic pain, and a reduction in CRPS pain has been shown to correlate with a recovery from cortical reorganization.81 Eight of the patients with widespread unilateral pain (the same patients as in the fMRI study) also underwent MEG recordings.141 In seven out of eight patients, the D1-D5 distance (cortical representation areas to fingers 1-5) was shorter to the stimulation of the painful than the non-painful hand. There were no differences in fine motor skills between the hands, and no signs of damage to the sensory system in electrophysiological studies were observed, hence supporting the hypothesis that cortical reorganization may occur also in the absence of deafferentation, and may be associated with pain itself.

The characterization of this clinical syndrome seemed to be especially important to the patients themselves. Until this, the patients had been rigorously examined by laboratory, electrophysiological and imaging methods. Those methods had revealed some abnormality (e.g. entrapment of the ulnar nerve in a patient with a pain in the whole left side of the body), but did not sufficiently explain the spread of pain. This had even led to suspicion of only psychological reasons behind the pain. Although we are still unable to give an exact reason for the pain, the patients perhaps feel that they have been better understood.
Limitations of the study

The nature of pain in our patients remains uncertain. During the time of the study, we did not have detection questionnaires concerning neuropathic pain in Finnish. We should have included the McGill Pain Questionnaire to our study, which in part would have helped us to classify the pain.

The duration of pain in the patients varies. In some patients, the pain was at its most intensive phase, while others though that the pain had alleviated when compared to the worst phase. This difference most certainly affects to the results in the clinical sensory examination, in the QST, and possibly in the fMRI also.

At individual level, the side-to-side differences in the QST were remarkable. However, at the group level these differences were not significant. This is likely due to the small number of patients, and on the other hand, to a large variation of the sensory detection thresholds even on the healthy size. The patients were supposed to have abnormality in the cortical procession of pain – this may cause changes also in the procession of painful stimuli delivered to the healthy side. For that reason, age- and stimulus-location matched controls should have been used.

In the fMRI study, it would have been ideal to study a larger patient group. However, we did not have that possibility; the original patient group from where the patients were selected from was small, and when deciding to study patients as similar pain distribution as possible and with a similar sensory phenomenon, we ended up to a small patient cohort. In an ideal situation, a cohort of 16-20 patients would have given more reliable results. Also, the controls we used in the fMRI study should have been collected more carefully. Controls in the fMRI and VBM analysis were significantly younger than the patients. Although the similar tactile responses between the patients and the controls exclude major differences in pain-related responses, caution in the interpretation of the results should be used. In the VBM analysis, age was included as a covariate of no interest to minimize the effect of age difference between the groups.

In conclusion; the possibility of inflammatory mechanisms in chronic pain should be considered in clinical practise. Infectious problems in patients with chronic pain should be evaluated, and treated by antibiotics or antiviral medication when needed. Clinical studies about the drugs with possible effect on glial activation would be beneficial.

Prevalence, clinical symptoms and predisposing factors in RLM

The exact prevalence of HSV-2 induced RLM is difficult to determine, since patients may have long asymptomatic periods between the episodes of
meningitis. The time between the first and second meningitis episode varied from a few weeks to more than ten years. For that reason, we used the term “period prevalence”. Only in one earlier study an approximation of the prevalence of RLM has been given, which was 1/100 000. In our study the prevalence was higher, 2.2/100 000.

The diagnostic accuracy of the disease has increased during the last two decades because of the PCR method, which at least partially explains the increase in the prevalence ratio. On the other hand, in developed countries, seroprevalence of HSV-1 has declined, while that of HSV-2 has increased. Hence, a greater proportion of HSV-2 infections are primary instead of initial. In primary HSV-2 infections, complications (meningitis) after the disease (genital herpes) may be more frequent than in initial infections. This may be another explanation for the increase in the prevalence of RLM.22

**Associated symptoms**

RLM is less benign than previously reported: neuropathic pain, paraesthesias, arthralgias, and urinary dysfunction were common during and after meningitis, and the symptoms often lasted for months and even years before full recovery. In meningitis, the infection is not limited to meninges around the brain, but also to meninges around the spinal cord and spinal nerves may be affected. This explains the associated symptoms like radicular pain, paresthesias, and urinary difficulties. When clinical signs of myelitis were involved, the time of recovery was longer. Traditionally, RLM has been described as a benign disease with no neurological sequelae – and if not, this diagnosis should be excluded.116 Our results challenge this view.

**Laboratory diagnostics**

The PCR method is considered a gold standard in diagnosis of CNS infections. It is known that almost 90% of cases of RLM are caused by HSV-2 virus. Still, HSV-2 DNA is not always found from the CSF despite earlier HSV-2 DNA positive samples. In our study, the lowest leucocyte count in HSV-2 positive samples was 44 leucocytes in mm³. Similar sensitivity of the PCR method has been reported in other studies, too. Although the PCR method today is more sensitive than a few years ago, false negatives may result if the timing of a sample is early, and there are only a few leucocytes in the CSF. In those cases, PCR analysis should be performed from a CSF sample taken during the optimal time period, 2 to 5 days from the first symptoms.
Treatment aspects

Due to well tolerated antiherpetic medication, either intravenous or peroral antiviral medication may be recommended during acute meningitis episodes, as it seemed to shorten the acute phase of the disease, and alleviate the acute symptoms. Prophylactic antiviral medication seemed to decrease the number of meningitis episodes, but was not universally effective. Prophylactic treatment should be considered at least when the patient has frequently recurring symptomatic HSV-2 infections – although benign in nature, recurrent HSV infections decrease the quality of life. The patients with low IgG1, and HLA-B*27 or HLA-DRB1*01 alleles might be considered to have an increased risk for a new RLM episode. Especially those high risk patients might benefit from the prophylactic antiviral therapy.

Predisposing factors

We found several potential predisposing factors for RLM. Low IgG1 was more common, and there was also a trend of low IgG3 being more common in the patients than in the controls. Low IgG1 and IgG3 may increase the patients’ vulnerability to HSV-infections and their complications by diminished ADCC activity. It is probable that low IgG1 or IgG3 are not independent risk factors for RLM, but rather risk factors for more frequent or severe symptomatic HSV-2 infections, or a more severe primary infection, which increases the risk of meningitis.

MHC genes have been associated with more than 100 diseases. The causative genes responsible for the disease associations are difficult to identify, and usually large population based studies are needed. Despite the small number of patients we showed that HLA-DRB1*01 and HLA-B*27 were more common in the patients than in the controls. The prevalence of HLA-DRB1*01 (53%) in affected individuals is among the highest reported thus far in any patient population suffering from HLA-associated diseases. Previously HLA-DRB1*01 has been associated with peridontal pathogens in Finnish coronary artery disease patients, and HLA-B*27 and HLA-B*35 with infectious or post infectious complications in chronic HSV infections, HIV, reactive arthritis, and Chlamydia pneuonie infections.

Combinations of the factors which potentially affect to immune responses (complement C4B deficiency, low IgG1 and IgG3, and HLA-DRB1*01, -B*35 and B*27) were common among the patients. Two patients had five of these factors, seven patients presented four, two patients three, four patients two, three patients only one, and three patients showed none of these factors. It may be that single deficiencies do not increase susceptibility to infections, but the combined influence of multiple subtle impairments may be clinically relevant.
In conclusion; in patients with a second episode of aseptic lymphocytic meningitis, the aetiologic agent in most often HSV-2. PCR may be insensitive, if the leucocyte count in CSF sample is too low. Patients do not always totally recover from meningitis within a few weeks. Antiherpetic medication should be used due to its good effect and tolerability.
7. CONCLUSIONS

1. Infections and inflammation may have a significant role in creating pathological pain states, by activating immunologically active glia in the CNS. Activated glia is responsible for “extra-territorial” pain, where the pain spreads from the original territory to larger areas. Glia is able to detect HSV most likely via Toll-like receptors.

2. Herpes simplex virus is neurotrophic, it is able to maintain latency in the CNS, and able to reactivate periodically. HSV is thus a good candidate to activate the glia.

3. Functional brain imaging reveals attenuated pain-related responses and decreased gray matter density in the pain-processing areas in patients with widespread unilateral HSV associated pain. Attenuation of pain-related responses has previously been described most often in chronic pain. Gray matter loss may be associated to chronic pain, HSV infections, or both, and may also be one explanation for the attenuation of pain-related responses.

4. Immunoglobulin G subclass deficiencies may predispose to frequent HSV recurrences and to HSV associated diseases, including RLM. Frequent HSV recurrences may predispose to pathological pain.

5. RLM is more common and less benign than previously reported, and the prevalence of RLM may increase in the future.

6. In addition of low IgG1 subclass, predisposing factors to RLM include HLA-DRB1*01 and HLA-B*27. The combined influence of multiple subtle impairments may be clinically relevant.
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