



Karolinska Institutet

Institutionen för Medicin Solna
Enheten för Infektionssjukdomar
CNS-gruppen

Studies on Host-Related Pathogenesis of *Herpes simplex* Type-1 Encephalitis in Rat

AKADEMISK AVHANDLING

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ABSTRACT

In order to explore the molecular mechanisms of *Herpes simplex* encephalitis (HSE), a severe infection of the central nervous system (CNS) caused by *Herpes simplex* type 1 virus (HSV-1), a rat model resembling the human condition was characterized in the DA (Dark Agouti) strain. After injection into the whiskers' area HSV-1 entered the CNS at the level of the brain stem via the trigeminal ganglion, subsequently spreading to the thalamus, cortex and olfactory bulb, leading to death at five days post infection (dpi). In contrast, the Piebald Virol Glaxo (PVG) strain was found to be completely resistant to disease and without signs of immunological reactions within the CNS, since HSV-1 virus did not penetrate beyond the site of inoculation. The kinetics of HSV-1 infection in the two strains was thoroughly characterized by magnetic resonance imaging, quantitative polymerase chain reaction, virus isolation in green monkey kidney cells, histology and immunohistochemistry (IHC).

Kinetics of virus propagation and primary immune reactions following HSV-1 infection were compared between the susceptible DA and the resistant PVG strain at 12 hours post-infection (hpi), 1, 2, 3 and 4 dpi. A low expression of Toll-like receptors 2 and 9 and slower recruitment of macrophages was associated with viral replication in the perineurial cell layer and consecutive propagation to the CNS in the DA rats, while virus spread was confined to the epineurium of the peripheral nerve in the resistant PVG strain.

The underlying genetic mechanisms for the difference in susceptibility between the two strains were dissected in a F2 (DAXPVG) intercross, with genome-wide microsatellite-based genotyping. Linkage analysis revealed a very strong quantitative trait locus (QTL) on chromosome 4 regulating susceptibility to HSE. Fine mapping of the QTL by infection of additional rats with recombinations in the region, haplotype mapping of disease susceptibility in a panel of inbred rat strains, infection of congenic strains, sequencing and mRNA expression studies of the genes in the interval indicated the calcitonin receptor (*Calcr*) as the candidate gene. Functional experiments with treatment using calcitonin receptor agonists *in vivo* provided further support of the candidate gene status of *Calcr*.

Additional genetic determinants of susceptibility to HSE were studied in two other rat strains: Spontaneously Hypertensive Rat (SHR) and Brown Norway (BN), which are susceptible and resistant, respectively, to HSE, as well as in 29 BNxSHR recombinant inbred lines (RIL). The use of an already existing database of single nucleotide polymorphisms (SNPs) differing between SHR and BN revealed another significant QTL on chromosome 4 regulating susceptibility to HSE. Further analysis of the QTL using immunohistopathology indicated the Von Willebrand Factor homologue (*Vwf*) gene, which has a role in blood-brain-barrier homeostasis, as a possible candidate for regulating differences in susceptibility between the BN and SHR strains.

In summary, the present study has demonstrated a strong genetic influence on the susceptibility to HSE in a rat model that displays many similarities to the corresponding human condition. Further genetic and functional studies are needed to confirm the candidate gene status of *Calcr* and *Vwf* regulating HSE and these may ultimately lead to more effective treatments of this severe CNS infection.

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