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ICAM-5 Modulates Cytokine/Chemokine Production in the CNS during the Course of Herpes Simplex Virus Type 1 Infection

Margaret C.L. Tse¹, Crystal Lane¹, Kevin Mott², Nattawat Onlamoon¹, Hui-Mien Hsiao¹, and Guey Chuen Perng^{1, #}

¹ Department of Pathology and Laboratory Medicine, Emory Vaccine Center, Emory University School of Medicine, Atlanta, GA.

² Center for Neurobiology & Vaccine Development, Ophthalmology Research, Department of Surgery, Cedars-Sinai Medical Center, Los Angeles, CA.

Abstract

Chemokines are important in HSE development in the CNS but underlying regulatory events are unknown. Two-hybrid binding assays identified that intercellular adhesion molecule 5 (ICAM-5), an immune modulator in the CNS, interacted with neurovirulence factor, UOL, of HSV-1. Viral load and interleukin levels were similar in UOL deletion virus (Δ UOL), and wild type virus infected mouse brains. However, higher numbers of lymphocytes, but unaltered soluble ICAM-5 and chemokine levels were detected in Δ UOL infected mouse brains. In contrast, lower lymphocyte numbers, reduced soluble ICAM-5, and higher chemokine levels were detected in wild type virus infected brains. Our results suggest that ICAM-5 plays a critical role in modulating chemokine production in the CNS.

Keywords

ICAM-5; chemokines; HSV-1; neurological disorder; HSE

1. Introduction

Herpes simplex encephalitis (HSE) is induced by herpes simplex virus type 1 (HSV-1) infection, a neurotropic double-stranded DNA virus that belongs to the herpes viridae family. HSE is the commonest fatal sporadic encephalitis in humans (Whitley, 1990). While the exact incidence of HSE is not known, it has been estimated that at about 2000 cases occur annually in the United States (Johnston, 1998). Serological studies estimate that over five billion people are presently infected with HSV-1 worldwide (Whitley, 2001), thus the actual incidence is probably an underestimate. HSE is a devastating disease and more than 70% of untreated patients die with only 2.5% of surviving patients returning to normal neurological function (Whitley and Roizman, 2001). In adults, HSV-1 accounts for 95% of all fatal cases of sporadic HSE and usually results from reactivation of the latent virus.

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#Corresponding author, Guey Chuen Perng, Dental School Building, Room 429, Emory Vaccine Center, 1462 Clifton Road, Atlanta, GA 30322. Phone: 404-727-5490 gperng@emory.edu.

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HSV-1 is one of the most prevalent viruses in humans, 90% of normal people show past exposure to the virus (Whitley and Roizman, 2001). More than 33% of HSV infections of the world population are evident as recurrent (Whitley et al., 1998). Upon primary infection with viral replication, HSV-1 viral particles may travel through axons (Whitley and Roizman, 2001) by anterograde flow to sensory neuron in trigeminal ganglion, where viral replication occurs briefly and become latent in the sensory neurons for life. Latent HSV-1 is periodically reactivated, either spontaneously or following various triggering events such as trauma, sunlight, and stress, to cause blisters in the mouths and lips, and occasionally HSE in the brain (Whitley and Roizman, 2001).

HSV-1 infects via mucosal surfaces or damaged skin. Thus, ocular infection with HSV-1 can induce HSE in infected animals (Perng et al., 1994; Perng et al., 2001). The high mortality rates and the potential burden of neurological disorder in recovered individuals in HSE, has lead to extensive studies of the neuropathogenesis of HSE, and numerous factors associated with HSE have been suggested, such as viral load or strains of the virus, imbalances of the immune response, the individual's genetic background, nutritional status, or cytokine and chemokine storms (Aurelius et al., 1994; Baringer, 2008; Rosler et al., 1998; Wuest and Carr, 2008). All in all, the information collected indicates that the main feature of HSE is the propensity of the disease for the frontotemporal region (Johnston, 1998) and that infiltration of immune cells into the brain during HSV-1 infection cause as much damage as the virus itself (Chan et al., 1989). Cytokines, including chemokines, produced from resident immune functional cells or from infiltrating immune cells have been implicated in playing an important role in the pathogenesis of HSE (Aurelius et al., 1994; Rosler et al., 1998). However, differentiation of viral tropism from immune tropism has not been well delineated and the exact mechanism(s) by which modulation of these cytokines lead to the development of HSE is largely unknown.

Several genes or gene products from HSV-1 have been associated with neurovirulence in infected animals (Stevens, 1987) even though the majority of the effects are likely due to the inability of replication and/or invasion of these mutant viruses in infected animals. Thus, several of these genes may indirectly contribute to HSE development rather than directly interact or interfere with host factors. We reported recently that a gene product, UOL, is involved in neurovirulence since mice infected with virus in which UOL has been deleted (Δ UOL) in the HSV-1 genome had significantly higher survival rates compared to wild type virus (Chan et al., 2006; Naito et al., 2005). The uniqueness of the Δ UOL virus is that the Δ UOL virus replicates as efficiently as the wild type virus in infected mouse brains. Thus, Δ UOL virus provides an excellent tool to investigate host factors directly modulating HSV-1 infection.

We report here that mice infected with Δ UOL virus had higher survival rates and higher ocular logID₅₀ than wild type virus, even though the peak viral load was similar in infected mouse brains. The number of lymphocytes infiltrating mouse brains infected with Δ UOL was higher than with wild type virus. To our surprise, levels of cytokines/chemokines were higher in mouse brains infected with wild type virus than with Δ UOL virus. Using mouse brain cDNA library as the prey target and the UOL as bait in the BacterioMatch 2-hybrid system, we found that UOL bound ICAM-5, a molecule that has been shown to play an important immune modulation role in CNS (Tian et al., 2000). In mouse brains infected with wild type virus, expression of ICAM-5 was reduced at early time points of infection and returned to normal when infectious HSV-1 virus was undetectable, while the levels of ICAM-5 remained the same during the course of Δ UOL virus infection. Thus, we hypothesize that UOL–ICAM-5 interactions modulate cytokine/chemokine production in HSV-1 infected mouse brains.

2. Materials and methods

2.1 Virus and cell culture

Δ UOL virus, HSV-1 McKrae strain with a deletion of 437 nucleotides at the 5' end of the UOL gene, has been described (Chan et al., 2006). Both Δ UOL virus and parental wild type McKrae virus strains were triple plaque purified. Rabbit skin (RS-1) cells were grown in Eagle's minimal essential medium supplemented with 5% fetal calf serum (FCS).

2.2 Animals and inoculations

Female Swiss-Webster mice were bilaterally infected ocularly without corneal scarification or anesthesia as previously described (Perng et al., 2001). Virulence death owing to encephalitis was determined by survival rates at 21 days post infection as previously described (Perng et al., 2001).

2.3 Virus replication in RS-1 cells and mouse brains

Monolayer of RS-1 cells seeded in 60mm tissue culture plates were infected with Δ UOL or McKrae wild type at a multiplicity of infection (MOI) of 0.1 or 0.01. Cells were harvested at different time points with three cycles of freeze and thaw. After removal of cells with low speed centrifugation, virus titer was determined by standard plaque assays described previously (Perng et al., 1994). To determine virus replication in mouse brain, infected mice were euthanized at various times after ocular infection. Brains were removed and individually homogenized. Cell debris was removed by low speed centrifugation, and virus titer in the supernatant was determined by standard plaque assays.

2.4 Isolation of leukocytes for flow cytometry analysis

Four brains at each time point from each infected group were used for FACS analysis. Mouse brains were removed, washed in ice cold phosphate buffered saline (PBS), dissociated by passage through a 70 μ m cell mesh. Cell suspension was pelleted, resuspended in RPMI supplemented with 10% (v/v) FCS, and was separated on a 90%/37%/30% Percoll (Amersham Biosciences, Piscataway, NJ) gradient. After centrifugation with 500 g for 30 min, leukocyte fraction was isolated from 37%/30% interface. Mononuclear cells were washed in PBS/2% heat inactivated FCS (HIFCS) and the cells were incubated with antibodies as follows: T lymphocytes: peridinin chlorophyll protein-conjugated anti-CD14 (clone Sa2-8; eBioscience, San Diego, CA), fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (BD Pharmingen, San Diego, CA), phycoerythrin (PE) Texas Red-conjugated anti-CD4 (clone RM4-5, Invitrogen) and Allophycocyanin (APC)-Alexa Fluor[®] 750-conjugated CD8 (Invitrogen); monocytes: peridinin chlorophyll protein-conjugated anti-CD14 (clone Sa2-8; eBioscience, San Diego, CA), Pacific Blue-conjugated anti-CD11b (clone M1/70.15; Invitrogen), phycoerythrin-Cy7 (PECy7)-conjugated anti-CD11c (clone HL3, BD Pharmingen, San Diego, CA). Cells were incubated at room temperature in dark for 15 min, and followed by washing with PBS/2% HIFCS. Cells were then fixed in 0.3 ml 4% (w/v) neutral buffered paraformaldehyde for FACS analysis. Data were collected on LSR II flow cytometer (BD Biosciences, San Jose, CA) and analyzed using FlowJo Software (Treestar Inc., Ashland, OR). Lymphocytes were rare in the CNS of uninfected mouse brains. Thus, only results from infected mouse brains were presented.

2.5 Brain cytokine arrays

Mouse cytokine antibody arrays were purchased commercially (R&D Systems Inc., Minneapolis, MN). Experiments were carried out according to the suggested instructions. Briefly, Δ UOL or wild type infected brains were removed and homogenized in medium with protease inhibitor. Samples were frozen at -80°C , thawed and cellular debris removed by

centrifugation. Total protein concentration of the supernatant was measured by BioRad Protein assay (Bio-Rad, Hercules, CA). Two hundreds microgram of brain tissue lysates were mixed with a cocktail of biotinylated detection antibodies. The mixture was incubated with the Mouse Cytokine Array, a nitrocellulose membrane spotted with capture antibodies to multi-analyte profiling of 40 cytokines and chemokines, for overnight at 4°C. The array was then incubated with streptavidin-horseradish peroxidase followed by chemiluminescent detection. Intensity of the signals was quantified using NIH ImageJ software.

2.6 Reverse transcription-PCR (RT-PCR)

For selected cytokines, total RNA (3 µg) was extracted from infected brains at different days using RNeasy mini kit (Qiagen, Germany) according to the recommendations of the manufacturer. First-strand cDNA was synthesized by SuperScript II Reverse transcriptase (Invitrogen) with oligo (dT)₁₂₋₁₈ primer. The sequences of specific primers used were as follows: (1) TNF-α (307 bp) forward primer 5'-GGCAGGTCTACTTTGGAGTCATTGC-3', reverse primer 5'-ACATTCGAGGCTCCAGTGAATTCGG-3'; (2) β-actin (348 bp) forward primer 5'-TGGGAATCCTGTGGCATCCATGAAAC-3', reverse primer 5'-TAAACGCAGCTCAGTAACAGTCCG-3'. The PCR reaction was performed with an initial denaturation at 94°C for 2 min, followed by cycles of 30 s at 94°C, 30 s at 55°C, and 45 s at 72°C using SuperMix (Invitrogen). The PCR products were analyzed on a 1.5% agarose gel and stained with ethidium bromide.

2.7 BacterioMatch 2-hybrid

The BacterioMatch 2-hybrid system (Stratagene, La Jolla, CA) was carried out according to the manufacturer's protocol. Briefly, UOL open reading frame (ORF) was fused in frame into the pBT bait plasmid. After verification with sequencing, pBT/UOL was screened with prey target, mouse brain cDNA library. Repeated screenings were carried out in positive identified clones by ampicillin selection and β-galactosidase expression as reporter. Colonies with blue color appearance were indicative of interactions of UOL and the target protein. The positive clones were picked and subjected to sequencing to verify the identity of the gene. Sequencing results suggested that all positive clones (5/5) belonged to the ICAM-5 gene.

2.8 Glutathione S-transferase (GST) pull down

The open reading frame of UOL was cloned in frame using the GST expression vector. Purification of GST-UOL fusion protein was as described (Naito et al., 2005). GST-UOL was expressed in *E.coli* and purified according to the manufacturer's instructions. Infected brains were homogenized in brain lysate extraction buffer (250 mM sucrose, 10 mM Tris, 1 mM EDTA, cocktail protease inhibitors). Homogenates were spun at 5000 rpm for 10 min at 4°C to remove cell debris. The supernatants were collected by further spinning at 20000 rpm for 1 h at 4°C. Pellet was resuspended in LSB buffer (75 mM NaCl, 10 mM Tris, 1mM EDTA, pH7.4, 2% CHAPS). Protein concentration was measured by BioRad Protein assay (Bio-Rad, Hercules, CA). Extracted brain lysates (150µg) were incubated with 100 µg of purified GST-UOL protein overnight at 4°C, and 50 µl of EZ View Glutathione Red affinity gel beads (Sigma, Saint Louis, MO) were added and incubated for 2 h at room temperature. Beads were washed with LSB buffer containing 0.5% Triton X-100 for several times. Beads were resuspended in Laemmli loading dye and subjected to SDS-PAGE separation and immunoblotting.

3. Results

3.1 Replication of ΔUOL virus is similar to the wild type both in RS-1 cells and in mouse brain

Replication of ΔUOL virus in RS cells and in infected mouse brains was similar to that of wild type McKrae virus (data not shown), compatible with our previous report (Chan et al., 2006).

Importantly, the peak of the virus titers at day 7 post infection (P.I.) was similar in Δ UOL and McKrae infected mouse brains, which is also in accordance with previous findings (Chan et al., 2006). The results suggest that deletion of the UOL gene does not affect replication of the virus either in vitro or in vivo, indicating that the UOL mutant virus can enter and invade the CNS as efficiently as wild type McKrae virus.

3.2 HSV-1 survival is enhanced in mice deficient in UOL expression

Next, we determined the survival rate of mice infected with the Δ UOL strain. Mortality was assessed following ocular administration of 10^6 , 10^5 , 10^4 , 10^3 and 10^2 PFU of Δ UOL virus or wild type virus (five mice per dose per group). All mice survived following infection with 10^4 , 10^3 and 10^2 PFU of both virus strains (data not shown). Infection of mice with high dose HSV-1 resulted in a severe encephalitis as revealed by prominent hind limb paralysis (Perng et al., 2001). Mortality of mice was dose dependent for both viral strains. Mice inoculated with 10^6 PFU of wild type virus and Δ UOL virus had survival rates of 40% and 60%, respectively in two independent experiments (Fig. 1). Wild type virus infected mice began to die earlier than those infected with Δ UOL virus (Fig. 1B). Mice infected with 10 fold less (10^5) of wild type virus increased survival rate to 80%, whereas all mice survived following Δ UOL virus infection at this dosage. Overall, the trend for the survival rate was significantly different (Long-rank test, $P=0.04$) between Δ UOL and wild type virus infected mice. These results are consistent with previous reports in mice infected with Δ UOL virus (Chan et al., 2006). Accordingly, ocular log ID₅₀ was calculated using the Reed and Muench method (Thakur and Fezio, 1981). Δ UOL virus was significantly less virulent than the wild type virus as the ocular log ID₅₀ of wild type and Δ UOL strain were 5.75 and 6.25, respectively.

3.3 Less weight gain in Δ UOL than wild type infected mice

Body weight gain was compared in mice infected with different virus strains to the controls throughout the experimentation (Fig. 2). Weight loss was observed as early as day 4 p.i. with the most dramatic body weight lost on day 7 p.i., which then slowly recovered but never reached the same levels as control mice. In addition, to our surprise, a significantly higher percent of body weight loss on days 7 and 11 ($P<0.01$) was observed in mice infected with Δ UOL virus than with wild type virus.

3.4 Increased infiltration of T-lymphocytes in Δ UOL virus infected mouse brains

Brain leukocyte kinetics during the acute infection period was analyzed as described in Materials and Methods. The CD140⁺ population was first gated using forward and side scatter, followed by CD3⁺ to examine the lymphocyte population. On day 5, the frequency of CD3⁺ T cell in brains infected with wild type virus was significantly ($P<0.01$) higher than Δ UOL virus (Fig. 3A). In contrast, on day 10, significantly ($P<0.01$) higher frequency of CD3⁺ T cells in brains infected with Δ UOL virus was observed (Fig. 3A). Subpopulations of CD3 lymphocytes, CD4⁺ (Fig. 3B) and CD8⁺ (Fig. 3C), were significantly ($P<0.01$) higher in Δ UOL infected mouse brains on day 10 p.i., but were significantly higher in McKrae virus infected mouse brains on day 5 p.i.. A higher frequency of CD4-CD8- T cells was observed in Δ UOL virus infected mouse brains on days 7 and day 10 (Fig. 3D). Myeloid lineage populations were also analyzed and first gated using forward and side scatter, followed by CD14⁺ vs. side scatter. The CD14⁺ population was further divided into CD11b⁺CD11c⁺, CD11b⁺CD11c⁻ and CD11b⁻CD11c⁺ populations. As a whole, there were no salient differences in monocytes populations infiltrating the brains of mice infected with Δ UOL virus and wild type virus throughout infection, even though higher frequencies of these myeloid lineage cells were apparent compared to the control (Table 1).

3.5 Cytokine/chemokine protein expression profile

To investigate whether lymphocyte infiltration could reflect cytokine/chemokine expression patterns, the cytokine/chemokine profile in infected brains was analyzed on day 3 (Fig. 4, A and B) and day 10 (Fig. 4, C and D) after infection. The fold increase was calculated by comparison of day 10 to day 3 (Fig. 4E), and the cytokine/chemokine expression profile is summarized in Table 2. The most significant differences in expression levels were IL-1 α , CXCL10 and CCL2 in wild type virus infected mouse brains at 10 days after infection. Additionally, while most interleukins expression levels were not obviously altered in brains infected with wild type virus and Δ UOL virus; chemokines or soluble factors protein expression levels including CXCL11, CXCL9, CCL3, RANTES, TIMP-1, sICAM-1 and IFN γ were moderately up-regulated in wild type infected mouse brains with at least 1.5 fold on day 10 p.i. compared to day 3 p.i (Table 2).

On the other hand, a subtle difference in chemokine expression in Δ UOL virus infected brains was detected in the cytokine/chemokine array. Cytokine expression levels of CCL11 and IL-1 α were moderately up-regulated on day 10 p.i. compared to day 3 p.i. With much more sensitive RT-PCR, TNF- α mRNA expression was the highest on day 3 in brains infected with wild type virus and gradually reduced to the basal level by day 14. In contrast, TNF- α mRNA peaked at day 7 after infection in brains infected with Δ UOL virus (Fig. 5).

3.6 UOL interacts with soluble ICAM-5

The BacterioMatch 2-hybrid system was used to screen for the potential UOL interaction partner. Mouse brain cDNA library was used as the target for screening using UOL as the bait. Among about 1000 colonies displayed from mouse brain cDNA library, 5 clones which showed highly positive interactions with UOL were identified as ICAM-5 after sequencing. All 5 clones were 100% identical to ICAM-5 sequence. ICAM-5 has been suggested to play a crucial role in CNS immune modulation (Tian et al., 2000), and has also been used as a marker for acute HSV encephalitis (Lindsberg et al., 2002). To confirm the binding of UOL to ICAM-5, GST pull down assays were performed, in which GST-UOL fusion protein was incubated with brain lysates infected with wild type virus and Δ UOL virus, respectively (Fig. 6). Neither ICAM-5 protein (Fig. 6A, lane 1) nor in TG (Fig. 6A, lane 2) could be pulled down in the uninfected brain by glutathione beads. ICAM-5 protein after incubation with GST-UOL fusion protein was pulled down by glutathione beads (Fig. 6A, lane 3). Beginning from day 2 p.i., reduced binding of soluble ICAM-5 were detected by GST-UOL pull down in brain lysates prepared from wild type infected mice (Fig. 6A, lane 4-7). By day 7, the level of soluble ICAM-5 in wild type infected brain (Fig. 6A, lane 8) were similar to that of the control (Fig. 6, lane 3). In contrast, the levels of ICAM-5 protein were unaltered throughout the infection period in Δ UOL infected mouse brains (Fig. 6B).

4. Discussion

Over the years, HSE has been an interesting subject of investigation since the clinical presentation of the disease is associated with a high mortality rate, and there are suitable animal models available. Numerous viral or host factors that contribute, associate, or correlate, either directly or indirectly, to the pathogenesis of the HSE have been documented (Sancho-Shimizu et al., 2007; Whitley, 2001) with viral gene knockout mutants in infected animals. While the viral gene knockout mutants are a great tool to investigate viral factors contributing to the pathogenesis of diseases, the majority of these gene knockout mutants are unable to replicate efficiently in infected animals (Chou et al., 1990; Moyal et al., 1992; Orvedahl et al., 2007). These genes, therefore, may only indirectly contribute to the observed phenotypes.

We recently identified that the UOL gene product plays a virulence role in infected mice (Chan et al., 2006), since mice infected with the Δ UOL virus had a significantly higher survival rate compared to wild type virus. The importance of the UOL gene products in virulence is further supported by finding that the UOL transcript is absent in the avirulent strains, KOS and F, the HSV-1 strains with a genome sequence of UOL that is 98% identical to the virulent strains, McKrae and 17syn+ (Zhu et al., 1999). In addition to higher survival rates in the Δ UOL virus infected mice, the virus replicates as efficiently as wild type virus in infected mice (Chan et al., 2006), suggesting that UOL is not involved in neuroinvasion and has no effect on virus entering into CNS. However, the increased survival rate and higher ocular ID₅₀ in Δ UOL virus infected mice obviously indicate that UOL disruption results in a decrease in neurovirulence. The observed phenotype of Δ UOL virus in infected mice, therefore, provides a great tool to dissect the host factors, in particular the immune factors, directly involved in HSE during the course of acute HSV-1 infection.

It has been documented that upon HSV-1 infection, transient body weight loss is expected as stress and immune responses to the infection (Cao et al., 2004). To our surprise, a significantly higher degree of body weight loss was observed in mice infected with Δ UOL virus than with wild type virus. In mice infected with Δ UOL virus, although higher levels of T lymphocytes infiltrating the mouse brains on day 10 compared with wild type virus, lower amounts of cytokine/chemokine production was seen. In contrast, in mice infected with wild type virus, T lymphocyte infiltration peaked on day 10 p.i., and robust production of cytokines/chemokines on the same day was observed. The observed phenomena are consistent with an LCMV study, in which it was suggested that immunopathologic disease (Cole et al., 1972) and weight loss (Hildeman and Muller, 2000) were not caused by the virus itself but was mediated by the T cell response and cytokine production. We therefore presume that cytokines/chemokines produced by the infiltrating immune cells acting within CNS might be involved in CNS pathology in the course of HSV-1 infection.

Dynamic production of cytokines, chemokines, and soluble cellular factors were observed in mouse brains in the course of HSV-1 infection. These factors including anti-inflammatory, pro-inflammatory, and anti-viral factors integrate into a complicated balance networks in the CNS. The up and down expression levels of the cytokines/chemokines in mouse brains infected with wild type virus infection were consistent with previous reports (Archin and Atherton, 2002; Marques et al., 2008; Wuest and Carr, 2008; Hartlage-Rubsamen and Schliebs, 2001; Irani and Griffin, 1996). The interesting observations in this study are those cytokines and chemokines detected in mouse brains infected with the Δ UOL virus. Thus, the expression levels of these factors such as unaltered TNF- α mRNA and protein levels, higher IFN- γ and tissue-inhibitor of metalloproteinase 1 (TIMP-1) levels, no changes or down-regulated Interferon-gamma inducible protein-10 (IP-10, CXCL10), macrophage chemoattractant protein-1 (MCP-1, CCL2), monokine induced by interferon-gamma (MIG, CXCL9), macrophage inflammatory protein-1 α (MIP-1 α , CCL3), RANTES (CCL5), in Δ UOL virus infected mouse brains may provide a critical coordinated network of how these cytokines and chemokines being regulated in the course of HSV-1 infection.

To understand further the possible cause of our observations, BacterioMatch 2-hybrid system was used to screen mouse brain cDNA library with UOL gene as the bait. ICAM-5 or telencephalin, an immune modulator in the CNS (Kilgannon et al., 1998; Tian et al., 2000), was found to partner with UOL protein. Interestingly, soluble ICAM-5 has been detected in experimental hypoxic-ischemic injury of the brain (Guo et al., 2000) and herpes simplex encephalitis (Lindsberg et al., 2002). Furthermore, a recent report shows that neuronal ICAM-5 suppresses T cell activation (Tian et al., 2008). Thus, with the pull-down assays, levels of ICAM-5 were not altered in lysates prepared from brains infected with Δ UOL virus, suggesting that ICAM-5 may be critical in modulating the releasing of cytokine/chemokines in activated

immune cells infiltrating the brain. Further studies on molecular details on how HSV-1 evades microglia and CNS immune systems related to UOL-ICAM-5 interaction remains to be elucidated and the role of UOL-ICAM-5 interaction is thus highly warranted.

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Fig. 1. Higher survival rate in mice infected with Δ UOL virus than wild type virus
Mice were infected intraocularly with indicated dosages of viruses. Survival rates were recorded for 21 days p.i.. (A) and (B) were two independent experiments with $n=5$ mice/group/experiment. * $P<0.05$, Long-rank test.

Fig. 2. Dramatic body weight change ΔUOL virus infected mice

Mice were infected intraocularly with ΔUOL virus or wild type virus at 1×10^5 PFU per eye except for the mock-treated control. Body weight (A) was monitored for 28 days p.i. and % body weight change (B) was assessed compared to the individual body weight change at Day 0 of the corresponding group. Data are presented as daily average (starting with $n=20$ /group).

*ANOVA analysis revealed that significant body weight loss ($P < 0.01$) in ΔUOL virus infected mice compared to the wild type and the control on days 7 and 11 p.i..

Fig. 3. Profiling T cell phenotype in mouse brains infected with Δ UOL virus and wild type virus
Mice were infected intraocularly with Δ UOL virus, or wild type virus at 1×10^5 PFU per eye. Leukocytes were isolated from infected brains as described in the Materials and Methods, stained with leukocyte cell surface markers, CD3, CD4, and CD8, and subjected to FACS analysis. (A) Total CD3⁺ leukocytes, (B) subpopulation of CD4⁺, (C) subpopulation of CD8⁺, and (D) subpopulation of CD4⁺CD8⁺ (DN) subsets. $n=4$ per group per time point ; * $P<0.05$, ** $P<0.01$, Student's t test.

Fig 4. Profiling of cytokines/chemokines expression in brain lysates from mice infected with ΔUOL virus and wild type virus

Brain lysates were prepared as described in Materials and Methods and subjected to analysis with R&D mouse cytokine antibody array. (A) and (C) brain lysates from mice infected with wild type virus, day 3 and day 10 p.i., respectively. (B) and (D) brain lysates from mice infected with ΔUOL virus, day 3 and day 10 p.i., respectively. Six spots on the top-left, the top-right, and the bottom-left of each array were the positive control, whereas the bottom-right 2 spots were the negative control. (E) The intensity of the signal on each spot was calculated with NIH ImageJ software to generate a protein histogram. The error bars were derived from the two spots on the array. The intensity of each array was normalized with the positive controls and

the background was subtracted before quantitation as suggested by the manufacturer. Proteins were categorized into cytokine families: interleukin, chemokine and others.

Fig 5. Kinetics of mRNA of cytokines/chemokines mouse brains infected with Δ UOL virus or wild type virus

In selected cases, total TNF- α mRNAs expression in mouse brains infected with wild type virus or Δ UOL virus were verified by RT-PCR. Actin mRNA was used as a control.

Fig. 6. Levels of ICAM-5 were not altered in Δ UOL virus infected mouse brains

The GST-UOL pulled down assay was performed as described in Materials and Methods. (A) GST-pull down of brain lysates from McKrae infected mouse brains. Reduced levels of ICAM-5 were observed from day 2 to day 5 p.i. On day 7 p.i., the levels of ICAM-5 returned to that of day 0 uninfected brain. (B) GST-pull down of brain lysates from Δ UOL virus infected mouse brains. Levels of ICAM-5 were unaltered from day 0 to day 14 p.i.

Table 1
Profiling of myeloid cell lineage in mouse brains infected with ΔUOL virus and wild type virus.

Day p.i. ^a	3	5	7	10	14
CD14⁺CD11b⁺CD11c⁺					
Wild type (% of control)	N/A	1.17±0.86	0.52±0.76	4.79±4.86	0.19±0.08
ΔUOL (% of control)	N/A	1.17±1.17	0.75±1.21	13.57±19.49	0.21±0.15
CD14⁺CD11b⁺CD11c⁻					
Wild type (% of control)	4.00±3.65	0.50±0.64	0.50±0.59	7.70±6.65	0.06±0.10
ΔUOL (% of control)	1.50±1.91	1.33±1.89	0.41±0.49	18.30±23.05	0.11±0.10
CD14⁺CD11b⁻CD11c⁺					
Wild type (% of control)	0.29±0.22	0.98±0.92	0.53±0.39	0.92±0.41	0.30±0.08
ΔUOL (% of control)	0.18±0.06	1.11±0.70	1.02±1.18	1.70±0.67	0.55±0.68

^aOcular infection with 1×10⁵ PFU per eye, isolation of leukocytes, and FACS analysis were described in the Materials and Methods. Results were from 4 mice per group per time point.

Table 2

Expression levels of cytokines/chemokines in HSV-1 infected mouse brains^a

Analyte (interleukin)	IL-3	IL-4	IL-5	IL-6	IL-7	IL-10	IL-13	IL-12	IL-1 α	IL-16	IL-1 β	IL-17	IL-1ra	IL-23	IL-2	IL-27
Fold change compared to Day 3p.i. of wild type	0.77	0.64	0.65	1.20	1.31	0.48	0.65	0.86	1.33	0.89	1.55	1.13	5.53	0.72	0.39	0.56
Fold change compared to Day 3p.i. of Δ UOL	0.88	1.24	1.13	0.97	2.45	1.43	1.58	0.83	0.83	0.55	1.66	0.60	1.00	0.35	0.78	1.23
Analyte (chemokine)	CXCL13	CXCL10	CCL17	CXCL11	KC	CCL1	CCL2	CCL11	CCL12	CXCL9	CCL3	CCL4	MIP-2	RANTES	CXCL12	
Fold change compared to Day 3 p.i. of wild type	0.86	22.79*	0.89	1.91	0.86	0.61	20.30*	0.90	0.61	2.86*	1.61	1.72	0.83	3.53	1.02	
Fold change compared to Day 3p.i. of Δ UOL	0.58	0.79	0.78	0.70	0.57	0.93	1.46	2.13	0.45	1.41	0.59	0.60	0.33	1.54	1.26	
Analyte (others)	C5a	TIMP-1	G-CSF	TNF- α	GM-CSF	M-CSF	TREM-1	sICAM-1	IFN γ							
Fold change compared to Day 3p.i. of wild type	1.09	2.57	1.10	0.77	1.23	1.12	0.86	1.52	3.57*							
Fold change compared to Day 3p.i. of Δ UOL	2.01	0.36	1.51	0.94	0.94	1.50	1.06	2.98	0.49							

^aThe levels of expression of each cytokine/chemokine in brain lysates on day 10 p.i. were compared to that of day 3 p.i., respectively. Cytokines are categorized by family: interleukin, chemokines, and others.

^{*} P<0.0, Student's t test indicates statistically significant difference for expression levels of cytokines between day 3 and day 10 p.i.