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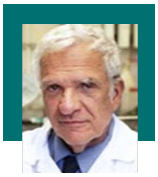

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Research Article

Implication of Uncoupling Protein 2 in Immunity to Herpes Simplex Virus Type I in Resistant and Susceptible Mouse Strains

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Abstract The present results demonstrate resistance to HSV-1 in C57Bl6 mice is associated with differential temporal body weight and GSH changes, compared to susceptible 129Sv mouse strain. Strong brain viral load TK and TLR-2 induction in the brain precedes TNF-α, iNOS, IκBα and UCP2 expression in 129Sv mice. Interestingly, we observe that UCP2 brain expression differs: UCP2 is found in higher quantities in the mid and hind brain in susceptible mice and in the forebrain in resistant mice. In contrast with previous data, UCP2 KO mice did not show differences in terms of survival, inflammatory gene expression and neurodegeneration compared to their WT controls. UCP2 brain expression is therefore a marker of resistance/susceptibility to infection but does not play a role in viral load or survival. In conclusion, UCP2s role in host survival to infection may be pathogen specific and largely subordinate to the direct effect of the toxicity of changes in antioxidants on the infectious agent itself.

Keywords mitochondrial transporters/exchangers; antioxidant; encephalitis; brain; microglial cells

1 Introduction

UCP2 is a member of the uncoupling protein (UCP) superfamily, itself derived from the broader mitochondrial carrier protein superfamily, which comprises metabolite transporters of the inner mitochondrial membrane such as the adenine nucleotide translocator (ANT), the phosphate carrier (PiC), the aspartate/glutamate carrier (AGC) [18, 21, 27]. Whereas the role of UCP1 has been only ascribed to brown adipose tissue thermogenesis, the precise physiological function of the ubiquitously expressed UCP2 largely remains obscure. In the past years, evidence has accumulated to associate UCP2 to immune functions mainly as a negative regulator of ROS production [2, 5, 17, 22, 23, 25]. This negative regulation has been particularly well exemplified by the resistance of UCP2 KO mice infected with *Toxoplasma gondii* [5] and with *Listeria monocytogenes* infection delayed in UCP2 KO mice [29]. UCP2 has also been implicated in the response to LPS administration [13, 30], during development of the atherosclerotic lesion [9], and in neurodegenerative processes [11, 37]. In some of these pathophysiological conditions, UCP2 was associated with a protective function possibly by preventing excessive ROS production [5, 22], regulating the mitochondrial pool of GSH [11] or modulating nitric oxide production [8, 17]. To our knowledge, the function of UCP2 in a viral model of disease remains to be identified.

Herpes simplex virus 1 (HSV-1) is a frequent neurotropic human pathogen commonly associated with orolabial and ocular infections [35] as well as with fatal encephalitis in some individuals with primary or reactivated infections [15, 38]. Animal models of human encephalitis have been described in mice using intranasal inoculation of HSV-1 suspension [10]. The immunological response and final outcome of the infections enabled classification of mouse, such as C57BL/6 and 129Sv, as respectively resistant and sensitive to HSV-1 infection. Interestingly, in both strains, a strong cerebral inflammatory response mediated by pro-inflammatory cytokines such as IL-1β and TNF-α has defined the innate immune system as a double-edged sword that is responsible for the sensitivity of 129Sv mice and simultaneously the main effectors of C57BL/6 strain resistance to HSV-1.

The purpose of the present study was (1) to explore the role of UCP2 in the response of resistant and sensitive mice strain to HSV-1 infection and (2) to investigate the significance of pro-inflammatory cytokines such as IL-1β and TNF-α in the control of UCP2 expression.

2 Materials and methods

2.1 Animals

129Sv and C57Bl/6 mice were obtained commercially from Charles River Canada (St-Constant, Quebec, Canada). WT and UCP2 KO mice were obtained from our own colonies and backcrossed on pure 129Sv or C57Bl/6 backgrounds for at least 8 generations ($> 99\%$) [5]. Double TNF-α and IL-1β KO mice were kindly provided by Dr. Serge Rivest (Université Laval, Québec City, Canada) [34]. Mice were acclimatized to standard laboratory conditions with a 12:12 h light dark cycle and had free access to a standard pelleted laboratory diet and water. The animals were handled in conformance with the Canadian Guide for the Care and Use of Laboratory Animals, and all the experimental protocols were approved by the Laval University's animal care committee.

2.2 HSV-1 infection

All animals used in this study were infected by intranasal inoculation at the age of 4 weeks with a viral suspension at a dose of 3.5×10^5 plaque-forming units (pfu) of the HSV-1 clinical strain H25 in 20 μL of MEM as described previously [10]. After infection, all mice were monitored for a maximum of 10 days or until the appearance of HSV-1 encephalitis-related symptoms, at which time they were sacrificed. The symptoms included the appearance of weight lost, ocular swelling and uncontrolled shaking movements [10]. At time of sacrifice, mice were deeply anaesthetized with an intraperitoneal injection of ketamine and xylazine and then intracardially perfused with cold saline followed by a 4% paraformaldehyde solution in 0.1 M borax buffer. For the time course protocols, 2 independent experiments were done: for in situ hybridization analysis (129Sv, $n = 8$ at day 4/day 5 when symptoms are maximum and C57Bl/6 mice, $n = 5$ at day 10) and for evaluation of the brain reduced glutathione levels (129Sv, $n = 8$–9 per group and C57Bl/6 mice, $n = 8$–9 per group). For IL-1β/TNFα double-KO study, in situ hybridization was done at day 6 when clinical signs were maximum ($n = 9$). For UCP2 KO studies, UCP2 KO mice and their WT littermate in 129Sv background were infected with high dose ($n = 17$–19, resp.) or with a lower dose ($n = 4$–6) and survival and neuroinflammation were analyzed. In C57Bl/6 background, UCP2 KO and WT mice were infected with high dose ($n = 7$–9) and survival was analyzed.

2.3 In situ hybridization

Detection and localization of UCP2, TK, TLR-2, IκBα, TNF-α and iNOS transcripts were carried out on brain slices using specific cRNA probes for these genes with an in situ hybridization technique described in [34]. Radioactive riboprobes were synthesized with linearized plasmid as described before [34].

2.4 Co-localization experiments

Immunohistochemistry was combined with in situ hybridization to study the localization of UCP2 mRNA within particular immune cell types [34]. Microglial cells and infiltrating monocytes/macrophages in the brain of HSV-1-infected mice were immunolabeled with anti-ionized calcium binding adapter molecule 1 (*Iba1*—1/500, gratefully provided by Dr. Y. Imai (Institute of Neuroscience, Kodeira, Tokyo, Japan)) and anti-mouse neutrophil (1/500, Cedarlane).

2.5 Neuronal degeneration

Cell death was investigated using FluoroJade B (FJB) labeling. This polyanionic fluorescein derivative provides a sensitive marker of neurons undergoing degeneration [12, 31]. To perform the labeling, animals were perfused as described above, and their brains were cut and mounted as already described. Slide-mounted sections were immersed in absolute alcohol for 48 h. Prior to coloration, mounted brain sections were rehydrated through graded concentrations of alcohol (70%, 50%) and 1 min in distilled water, then transferred to a solution of 0.06% potassium permanganate for 10 min, rinsed and stained for 30 min in a solution prepared from a 0.01% stock solution of FJB (Chemicon Int.) containing 0.5% 4′-6-diamino-2-phenylindole-dilactate (DAPI) in 0.1% acetic acid. The sections were coverslipped without dehydration.

2.6 Brain reduced glutathione levels in 129Sv and C57Bl/6 mice

Total brain reduced GSH contents from control non-infected animals and infected animals were homogenized in phosphate buffer (50 mM, pH 7.4) as described in [11]. Brain GSH levels were measured 1 and 4 days after infection (mild symptoms for both 129Sv/C57Bl/6 strains) and day 5 (severe symptoms for 129Sv) or day 8 (ablation of symptoms for C57Bl/6). Non-infected mice were used as controls.

2.7 Data analysis

The relative intensity of mRNA signals in the brain was evaluated on an X-ray film and scored: 0, undetectable; 1, low; 2, moderate; 3, strong; and 4, very strong as reported in [10]. Processed slides were examined under darkfield and brightfield microscopy using an Olympus BX51 microscope (Olympus America). Images were acquired with an Evolution QEi camera and analyzed with ImagePro plus v6.0.1.11 (Media Cybernetics). The count of *Iba1* and FJB positive cells was completed in regions corresponding to viral replication clusters with photomicrographs at 40×

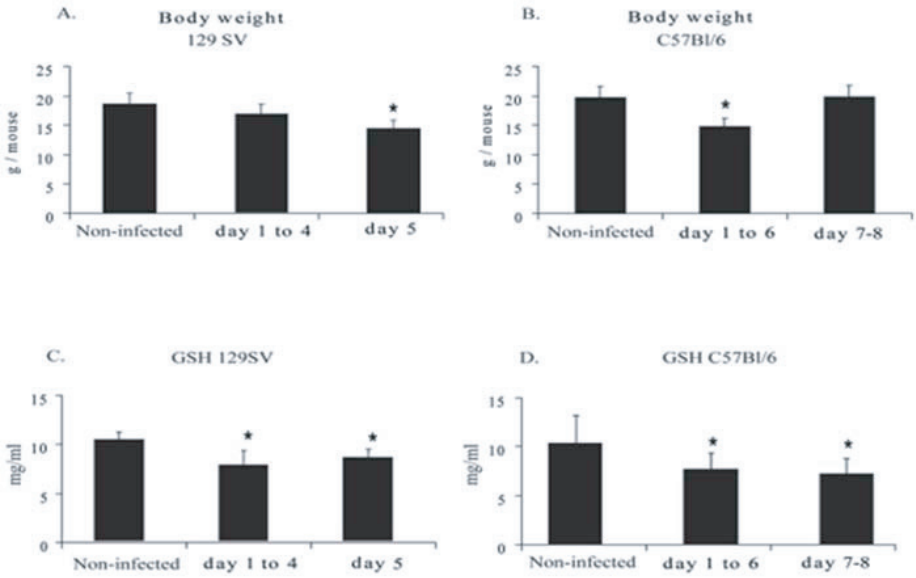

Figure 1: Effect of HSV-1 infection on body weight and brain GSH levels in 129Sv and C57Bl/6 mice, displayed as the mean ± SD.

and 4× magnifications, respectively. Life expectancies between groups of mice were estimated using the Kaplan-Meier method and were compared using a log rank test. Body weight, GSH levels and count of *Iba1* and FJB positive cells were compared using an Anova test and represented with SD values. Statistical analyses were carried out with the Statview 5.0.1 software (SAS institute).

3 Results

3.1 Effect of HSV1 infection on body weight and survival in 129Sv and C57Bl/6 mice

Infection with HSV1 resulted in mild loss of body weight in both 129Sv and C57Bl/6 mice, although the time course of weight changes differed between the two strains. During the first 4 days of infection, 129Sv mice showed no significant weight loss but they developed at day 5 a drastic weight loss (Figure 1), associated with an increased onset in mortality. C57BL/6 mice showed a transient statistically significant body weight loss in response to infection (Figure 1) that occurred earlier than it did in 129Sv mice. After the loss, they showed total weight regain by day 8 and no mortality was observed.

3.2 Effect of HSV1 infection on brain glutathione levels

Our results show in 129Sv mice that the decrease in brain GSH preceded the decrease in body weight (Figure 1). In C57Bl/6 mice, both body weight drop and GSH loss occurred early in the infection from days 1–6. When weight regain occurred from day 7 to 8, mice stabilized at low brain GSH levels compared to non-infected controls.

3.3 Induction of UCP2 mRNA expression in susceptible mouse brain during HSV-1-infection

Representative rostro-caudal distributions of UCP2, TK and TLR2 mRNAs, as detected by in situ hybridization in the brain of susceptible mice (129Sv strain) infected with HSV-1, are shown in Figure 2. On day 4 p.i., TK and TLR2 mRNAs were barely detectable whereas UCP2 mRNA expression was unaffected and distributed according to the constitutive pattern [28]. Interestingly, on day 5, both TK and TLR2 mRNA levels and distributions were noticeably altered and were strongly detected in clusters located in the caudal brain, specifically in areas within and surrounding the trigeminal nucleus and in the pons and medulla. These changes in TK and TLR2 expressions, which reflect important viral replication and microglial activation processes [7, 19], were also reflected by changes in the expression of UCP2 with transcripts now distributed in these same areas (Figure 2). These changes in TK, TLR2 and UCP2 mRNA levels and distribution were very well correlated with the appearance of severe clinical signs (ocular swelling and uncontrolled shaking movements [10]).

3.4 Control of viral replication associated with UCP2 mRNA induction in C57Bl/6 resistant mice

Figure 3 presents the distributions of UCP2, viral TK and TLR-2 mRNAs 10 days p.i. in brains of C57BL/6 mice. Strong induction of UCP2 mRNA expression was exhibited despite a low TK signal. Compared to the susceptible 129Sv mice at day 5 (Figure 2), viral replication was less considerable. The increase in UCP2 expression levels was

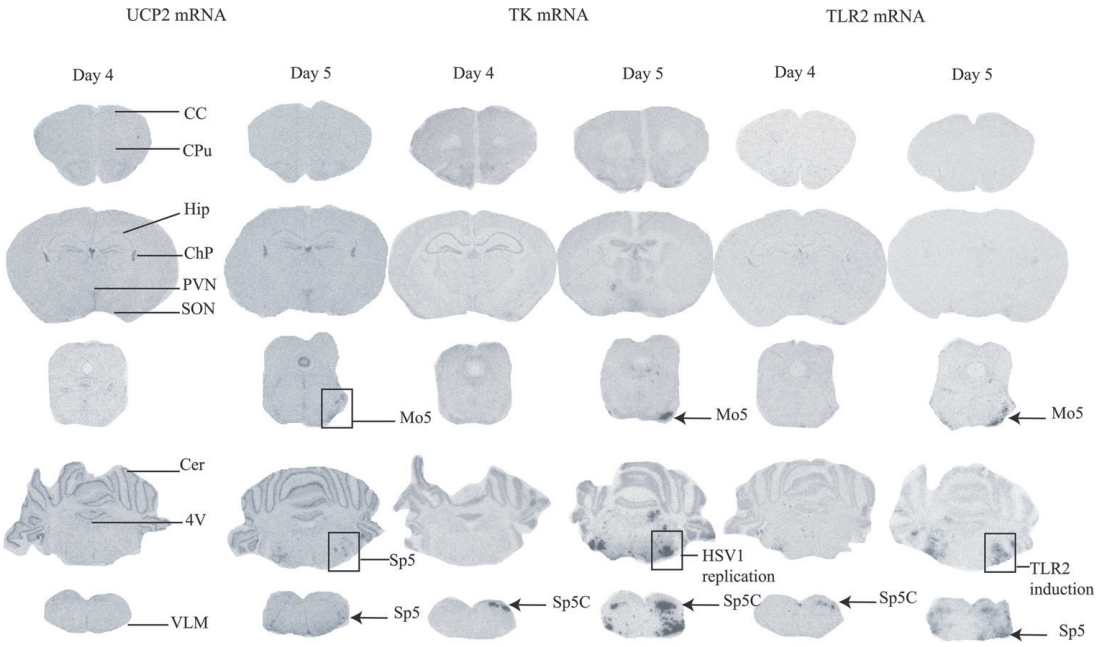

Figure 2: Representative rostrocaudal distributions of UCP2, TK and TLR2 mRNA in HSV-1-infected 129Sv brain on days 4 and 5. These distributions were prepared with digitized autoradiographs of coronal brain sections (25 μm) from HSV-1-infected mice hybridized with TK, TLR2, iNOS and UCP2 antisense cRNA probes labeled with ^{35}S. Cer, cerebellum; CC, cerebral cortex; CPu, caudate putamen; Hip, hippocampal formation; Mo5, motor trigeminal nucleus; PVN, paraventricular nucleus of hypothalamus; SON, supraoptic nucleus; Sp5, spinal trigeminal tract; Sp5C, spinal trigeminal nucleus; VLM, ventrolateral medulla.

however well correlated in time and space with the increased expression of TLR-2.

3.5 Phenotype of UCP2-expressing cells in HSV-1 infection

Using a double immunolabeling, we demonstrated that UCP2 mRNA was co-localized with *Iba1* immunopositive cells in brains of 129Sv and C57Bl/6 mice (Figures 4(A)–(D)). The presence of *Iba1* cells expressing UCP2 mRNA in the lumen of blood vessels (Figure 4(D)) demonstrates that at least some of the brain cells expressing UCP2 might originate from the periphery (macrophages) while others could likely be resident microglial cells. Neutrophils are known to be recruited in the brain during the course of HSV-1 infection [20], which were also shown (in a limited amount) to express UCP2 mRNA in susceptible mice (Figure 4(E)). Figure 4(F) depicts the neuronal death in 129Sv mice, by FJB staining. No neurodegeneration was noted in resistant C57Bl/6 mice (data not shown).

3.6 Role of proinflammatory cytokines in the induction of UCP2 mRNA expression

In C57BL/6 mice, inactivating either the IL-1β or TNF-α genes causes an increased sensitivity to HSV-1 infection, which in turn leads to the death of the majority of infected mice in 3 to 6 days p.i. [32]. We used IL-1β and TNF-α double KO mice in order to further delineate the cellular transduction pathways leading to UCP2 mRNA transcription following HSV-1 infection. Figure 5 demonstrates the distribution of UCP2 mRNA in the brain of HSV-1 infected wildtype C57BL/6 at day 10 following infection and double KO mice for IL-1β and TNF-α at day 6. Remarkably, UCP2 mRNA expression was still induced in the brain of IL-1β and TNF-α KO mice infected with HSV-1. As in the sensitive 129Sv mice, UCP2 mRNA expression was induced later in the double KO animals and was observed only in animals that survived for at least 5 and 6 days. Moreover, the expression pattern of UCP2 mRNA was modified and closely resembled that observed in the sensitive 129Sv strain (see Figure 2). The expression of the UCP2 mRNA appeared in the viral replication center located in the hindbrain, selectively in the pons and medulla. Our hypothesis was a common factor to these two different responses, such as inflammation and oxidative stress, which could be responsible of induction of UCP2.

3.7 Relationship between UCP2 and oxidative stress in infected brain

In order to establish a relationship between UCP2 expression and oxidative stress during HSV-1 infection, we measured expression levels of iNOS mRNA in susceptible

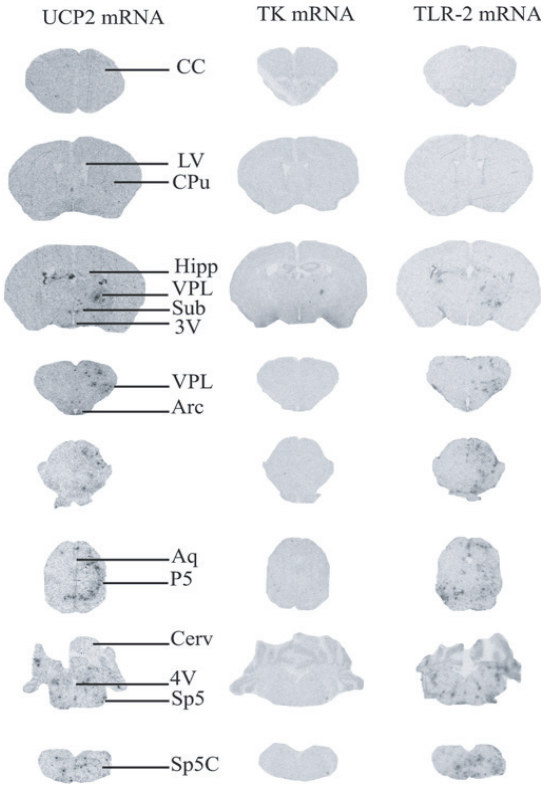

Figure 3: Representative rostrocaudal distributions of TK, TLR2 and UCP2 mRNA in HSV-1-infected resistant C57Bl/6 brain day 10 p.i. These distributions were prepared from digitized autoradiographs of coronal brain sections (25 μm) obtained from HSV-1-infected mice hybridized with UCP2, TK and TLR2 antisense cRNA probes labeled with ^{35}S-dUTP. TLR2 and UCP2 hybridization signals were detected throughout the brain of C57BL/6 without clinical signs at day 10 post infection. TK mRNA was barely detected in few neurons. IC, inflammatory cluster; LV, lateral ventricle; P5, peritrigeminal zone; Sub, submedius thalamic nucleus; VPL, ventral posterolateral thalamic nucleus; 3V, 3rd ventricle; 4V, 4th ventricle.

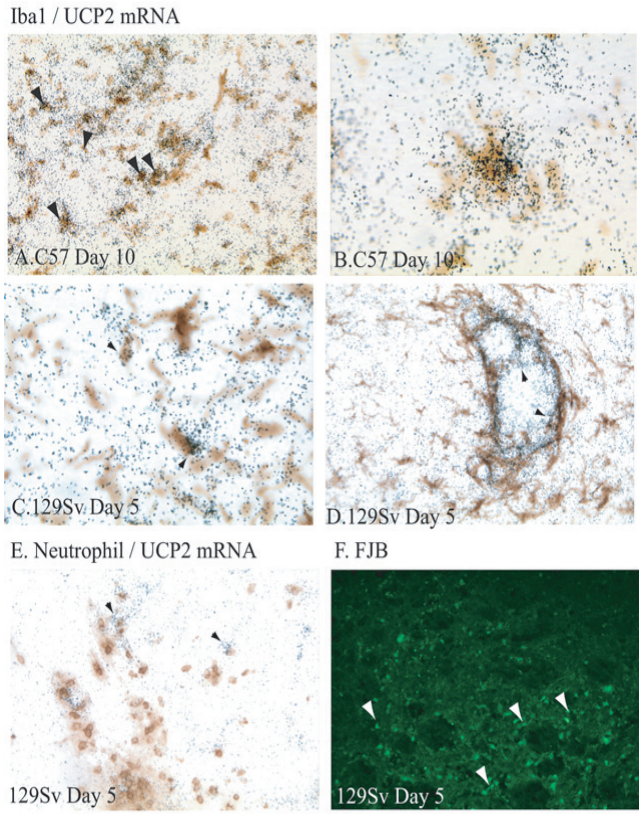

Figure 4: Neuronal death and phenotype of UCP2-expressing cells in HSV-1 infection in C57Bl/6 and 129Sv mice. Macrophage/microglia were labeled by immunochemistry with an antibody against ionized calcium binding adapter molecule 1 in C57Bl/6 mice at day 10 (*Iba1*, (A)–(B)) and in 129Sv mice at day 5 (*Iba1*, (C)–(D)) whereas an anti-mouse neutrophil antibody was used to label neutrophils in 129Sv (E). Note that macrophages from periphery express already UCP2 mRNA when they enter in brain (D). FluoroJade B staining was used to label apoptotic neurons at day 5 in 129Sv mice (F). Black arrowheads, double-labeled cells. Magnifications $40\times$: (A)–(F), $100\times$: (B).

mice, a marker of NO production by immune cells (Figure 6). iNOS mRNA became detectable only at day 5, and in regions also expressing TK and TLR2 mRNA. The hybridization signal left by UCP2 mRNA was light when compared to that of iNOS and distributed in a scattered pattern located around the apparently more localized iNOS expression.

3.8 HSV-1 infection in UCP2 KO mice

In order to clarify the potential role of UCP2 in HSV-1 pathogenesis, we compared the mortality rate of 129Sv WT and UCP2 KO mice ($n = 19$ and 17, resp., Figure 7). During these experiments, UCP2 KO mice exhibited signs of sickness after 4 days and had mean life expectancy of 5 days and 22 h (± 29.96, 142.58 h). The WT group exhibited clinical symptoms and had mean life of 6 days 3 h (± 32.26, 147.78 h). 89.5% of infected-WT mice and 94.2% of infected-KO mice died between day 5 and day 8 after the virus inoculation without any difference in the survival rate (Figure 7, $P = .7043$). Comparison of the survival rate between WT and UCP2 KO mice was also done with a non-lethal dose of 10^3 pfu of HSV1 ($n = 6$ and 4, resp.) and no symptom of encephalitis and no mortality were observed in either WT or UCP2 KO mice (data not shown). In C57BL/6 background, UCP2 KO mice were as resistant to HSV-1 as their wildtype counterparts ($n = 7$–9 per group).

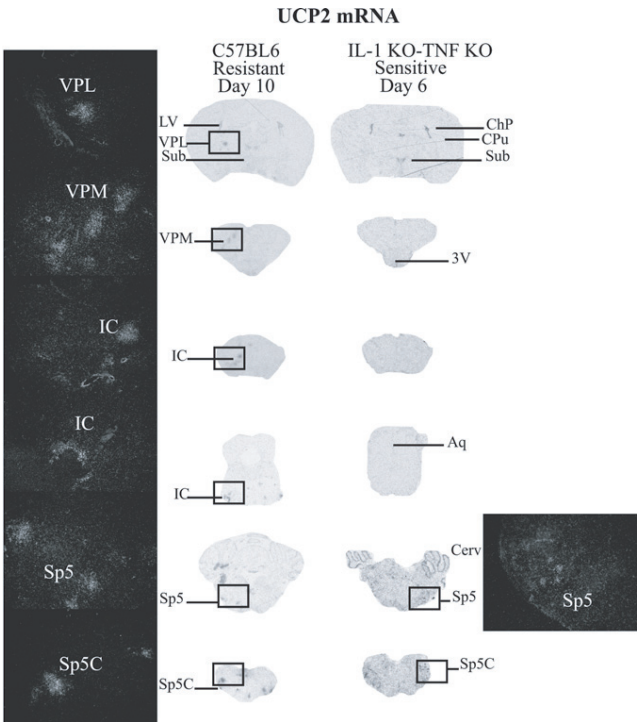

Figure 5: Distribution of the mRNA encoding UCP2 following an intranasal inoculation of HSV-1 in C57BL/6 resistant mice and TNF-α/IL-1β-deficient mice. Rostrocaudal distributions and corresponding darkfield photomicrographs were used to compare levels of UCP2 mRNA expression between the brains of resistant C57BL/6 wild type and susceptible TNF-α/IL-1β-deficient mice at time when clinical signs were at their highest levels. VPM, ventral posteromedial thalamic nucleus. Magnifications 40×.

3.9 HSV-1 replication and neuroinflammatory response of WT and UCP2 KO

The expression levels of TK, IκB-α, iNOS and TNF-α in 129Sv UCP2 KO and WT mice at days 5 and 6 were compared to further assess the function of UCP2 in viral replication and the host response to HSV-1 (Figure 7). We observed that the brain levels and distribution pattern of different hybridization signals for TK between WT and UCP2 KO mice was comparable in inflammatory clusters in the pons and medulla (Figure 7(A)). By comparing quantification of TK hybridization signal in specific hypothalamic regions of basal expression of UCP2 between UCP2 KO and WT mice with similar viral replication in hindbrain ($n = 2$–4), no significant difference was depicted in the viral propagation in hypothalamus without UCP2 (Figure 7). Accordingly, WT and UCP2 KO mice did not exhibit any significant difference in the expression of IκB-α (C-D), iNOS (E-F) and TNF-α (G-H) when compared with WT mice. The recruitment of microglia/macrophages in the

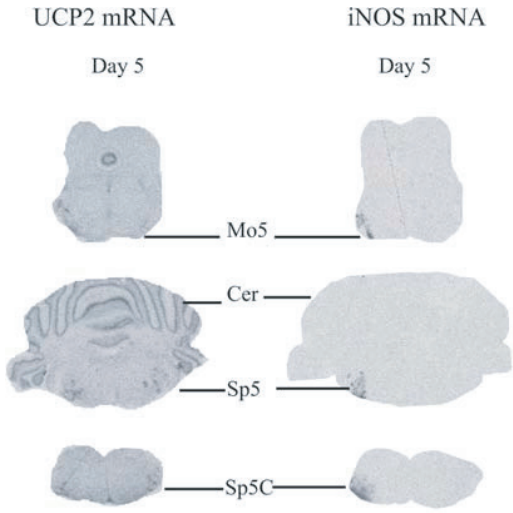

Figure 6: Representative caudal distributions of UCP2 and iNOS mRNA in 129Sv mouse brain at day 5 following HSV-1 infection.

infected brain was also apparently not different between WT and UCP2 KO mice (Figure 7(K), 1.2 *Iba1*-positive cells per mm^2 $\pm$ 0.48 versus 1.12 *Iba1*-positive cells per mm^2 $\pm$ 0.42, $P = .67$). Moreover, neuronal death was also similar to WT mice in the UCP2 KO mice (Figure 7(L), 0.56 FJB-positive cells per mm^2 $\pm$ 0.19 versus 0.52 FJB-positive cells per mm^2 $\pm$ 0.15, $P = .72$).

4 Discussion

Our studies show that after intranasal HSV-1 infection, both resistant (C57BL/6) and susceptible (129SV) mice strains show a rapid decrease in brain antioxidant GSH. This is in accordance with previous studies which demonstrated that HSV-1 induces oxidative stress/ROS and this may be involved in the pathology of HSV-1 infection. Since mitochondrial UCP2 has been shown to play a major role in the regulation of ROS and in particular during infection, we proceeded to characterize UCP2 temporal expression in the brain of HSV-1 infected mice. We observed that during the initial phase of infection (days 1–4) the virus was detectible at a low level in the brain. Also TK was spatially and temporally associated with TLR2 induction. From day 5 onwards, we and others detected by in situ hybridization a strong induction of other markers of cerebral inflammation such as TNFα, IκB [10], iNOS and UCP2 at the mRNA levels. In both mouse strains, UCP2 induced by infection was always associated with phagocytes, in primarily in microglia but also infiltrating macrophages and neutrophils. Temporal UCP2 expression in the brain is similar to that we have previously observed with *T. gondii* infection, in that UCP2 was detected in the brain of infected mice only

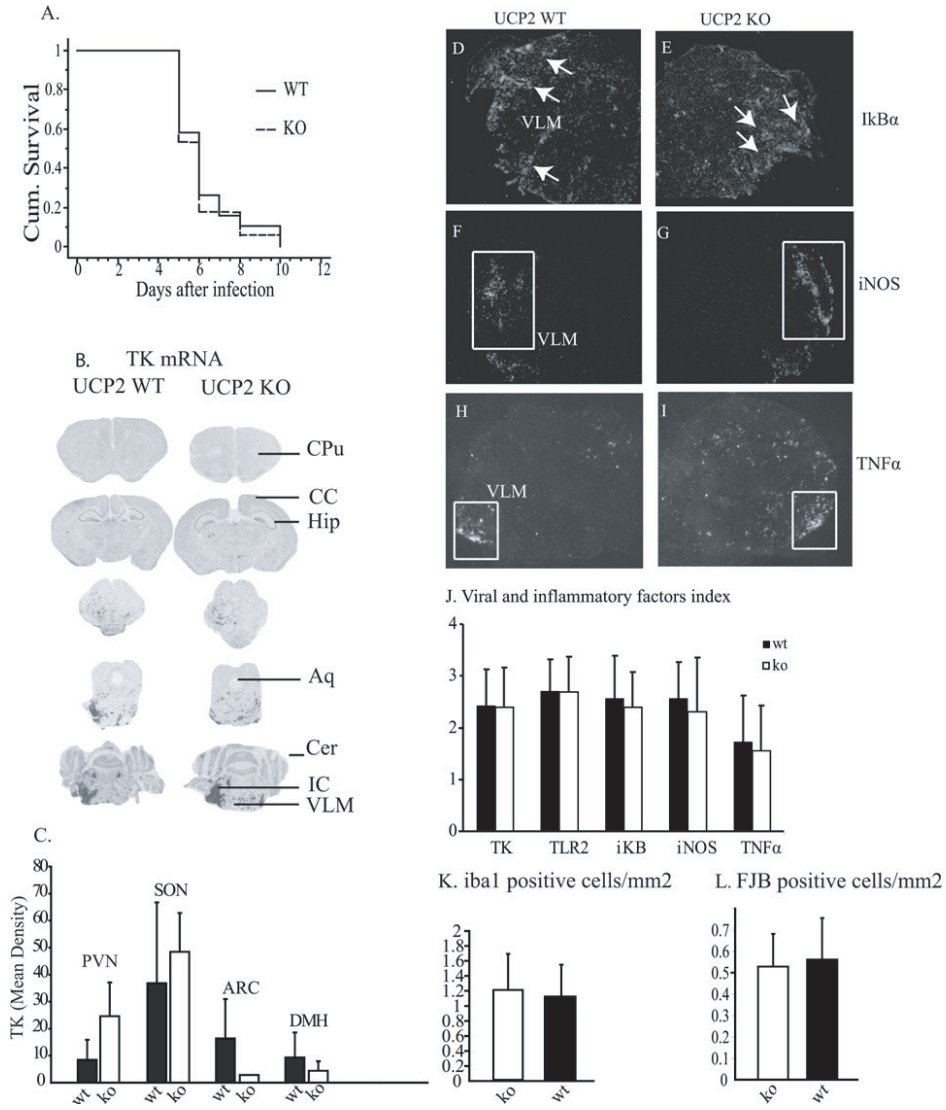

Figure 7: Susceptibility and immune response of UCP2 KO mice and UCP2 WT mice in 129Sv background following HSV-1 infection. An amount of 17 UCP2 KO mice and 19 UCP2 WT mice four weeks old were used in 2 independent experiments. Similar lethal phenotype was found in these genotypes ((A), $P = .7043$). Representative examples of TK hybridization signal (B), quantification of TK levels in PVN, SON, arcuate nucleus (ARC) and dorsomedial hypothalamus (DMH) (C), darkfield photomicrographs and expression score of neuroinflammatory response ((D)–(J)), Iba1-positive cells (K) and neurodegenerescence (L) in 129Sv UCP2 WT and UCP2 KO mice following HSV-1 inoculation. Coronal sections were hybridized using Iκ-Bα ((D)–(E)), iNOS ((F)–(G)) and TNF-α cRNA probe ((H)–(I)). Iba1-positive cells represent macrophages/microglia cells (J). By FJB staining, apoptotic neurons appeared and were counted (K). Magnifications $4\times$: (D)–(I).

after day 7 of infection and remained elevated in the chronic phase of infection. It was also observed that resistant and susceptible mouse strains showed different patterns of UCP2 expression; susceptible mice showed more UCP2 expression in the mid- and hindbrain, whereas resistant mice showed more UCP2 expression in the forebrain. What causes these differences in brain UCP2 expression due to HSV-1 is currently unknown. It was additionally observed that HSV-1 decreased the antioxidant GSH in the brain and that this preceded induction of brain UCP2. UCP2 brain expression appears therefore to be a marker of resistance/susceptibility to HSV1 infection but it does not play a role in survival or regulation of brain viral load. In conclusion, resistance to HSV-1 is associated with differential temporal body weight changes and changes in brain GSH, compared to susceptible mouse strain.

Following *T. gondii* infection, we demonstrated that UCP2 regulated mitochondrial macrophage ROS levels and that was associated with an enhanced microbicidal effect on the parasite in the acute phase of the infection [5]. UCP2 KO mice were shown to be more resistant to toxoplasmosis infection than their wildtype counterparts. Resistance to HSV-1 was associated with decreased viral loads in the brain. Somewhat surprisingly in this study, the UCP2 KO mice, be they on a resistant (C57BL/6) or susceptible (129SV) background, did not differ from the WT counterparts in terms of both survival and inflammatory response to HSV-1. This was surprising since we (Figure 1) and others [24,36] have observed that alterations in oxidative stress occur in response to HSV-1 infection.

Deficiencies in proinflammatory cytokines such as TNFα and IL-1β were demonstrated to decrease the innate immune reaction such as TLR-2 induction, suggesting a protective role for neuroinflammation in this viral model [32]. In that respect, C57BL/6 mice deficient in TNFα/IL-1β were used to demonstrate the impact of the loss of resistance and dysfunctional innate immune response on UCP2 expression. TNFα/IL-1β KO mice presented an induction of UCP2 with and altered spatial distribution (similar to the UCP2 pattern seen in susceptible 129SV brain) and had elevated virus load [32]. Therefore, susceptibility is associated with regulation of viral load in the brain. Furthermore, a possible explication for the different patterns of UCP2 expression in the brain of resistant and susceptible mice is that peripheral organs such as liver and lung do not eliminate the virus efficiently and that there is retrograde transport from the organs to the brain since nerves from the organs are linked to brain stem/hindbrain and thus we could expect to find HSV-1 virus in these sites.

Somewhat enigmatic in our findings is that the rapid decrease in brain GSH levels and body weight in both resistant and susceptible (GSH only) strains of mice. This could not be explained by cytokine TNFα mRNA induction in the brain of infected mice. However, a previous study has shown that increases in brain TNFα can be detected after intranasal infection of infiltrating immune cells found in blood vessels and cells lining the brain but not inside the brain during the early stages of infection (days 1–3) [36]. Another possible explanation for the decrease in GSH is that responsible factors do not originate in the brain. Our studies with *T. gondii* infection have also shown a similar behavior; during the early phase of infection there was an increase in lipid peroxidation (increased oxidative stress) in the brain of *T. gondii* infected mice, at the time cytokines such as TNFα, IL1β and IFNγ were not detected in the brain of infected mice. However, an elevation in serum IFNγ was detected. Furthermore, we showed in the *T. gondii* study that IFNγ was a vital mediator in the infection induced by oxidative stress in several tissues including the brain [3,4]. The observed transient change in body weight that is reported here in C57BL/6 mice has previously been described and shown to correlate with lung immune activation and peak body weight loss that was associated with increased natural killer cell activity and IFNγ levels [26]. It is possible that cytokines from another infected organ have an effect on the brain GSH levels. IFNγ has been shown to be important in mediating ROS during infection and is also a key cytokine in microglia UCP2 expression during chronic toxoplasmosis [3,4]. Changes in brain parameters prior to detection of virus in the brain could be due to peripheral tissue responses to virus. The brain is not the only organ that can be affected after HSV-1 infection. Some studies have shown that other organs such as the liver could also be affected [16]. The liver is a major source of GSH for peripheral and central tissue and infection could therefore increase pro-oxidant cytokines (IFNγ) and cause toxic effect on liver. This could reduce GSH pools and therefore increase oxidative stress. GSH has been suggested to play an important role in HSV infection [24,36]. It must be remembered that GSH requires appropriate levels of NADPH and ATP for its synthesis. Since HSV-1 virus has been shown to increase ROS by reducing ATP, this could also be a mechanism of increasing indirectly, by depleting GSH levels. Moreover, HSV-1 induces ROS production by depleting ATP by glycoprotein J [6] concealing a potential hyperproduction of ROS in UCP2-KO macrophages. This process involves mitochondrial ROS production. It would be interesting to compare the immunologic response as well as the oxidative stress of UCP2 KO mice to the HSV-1 deficient in glycoprotein J.

In viral pathology, oxidative stress is considered as a double-edged sword [1,33] and could be the link between UCP2 and mortality in susceptible mice or resistance in C57BL/6. Remarkably, UCP2 mRNA was detected only on day 5 after infection, that is at a time coinciding with the appearance of the oxidative stress marker iNOS. This coordinated expression was possibly of a certain importance in the local regulation of oxidative stress mediated by NO during HSV-1 encephalitis. NO is well known to play a major functional role in the immunity and it has been shown that UCP2 deficiency might enhance the local production of NO by macrophages [8]. Given this relationship between UCP2 and NO, one can also argue that the induction of UCP2 mRNA expression occurs as a late event during the pathogenesis of HSV-1 to blunt the innate immune system activation in order to reduce local production of NO to protect the brain parenchyma from excessive damage [14]. Moreover, the expression of UCP2 by neutrophils (which can be a major producer of ROS) is another indication suggesting a function for UCP2 in regulating the cellular adaptation to these highly toxic molecules. Unexpectedly, the results obtained in UCP2 KO mice in this study suggest

that the survival rate of the animals after HSV-1 replication does not depend on UCP2. UCP2 KO mice on either background did not show a decrease rate in the spreading of the HSV-1 unlike what was observed following *T. gondii* infection [5] and *L. monocytogenes* [29] but it is noteworthy that UCP2 induction appeared to manifest itself as a late event likely occurring after the onset of oxidative stress. In this study, we showed that resistant mice (C57BL/6) had a lower viral load than susceptible mice (SV129). Interestingly C57BL/6 mice rendered susceptible by deletion of IL-1β/TNFα were shown to have an increased brain viral load. Finally, we suggest that UCP2s, effect on host survival to infection may be pathogen specific and largely subordinate to the direct effect of the toxicity of oxidative stress on the infectious agent itself.

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