The Role of PrPC in the Course of CMV Infection

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The graduation thesis contains 28 pages, 3 figure, 1 table and 49 references.

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List of Abbreviations

CMV	Cytomegalovirus
dpi	Days post Infection
GCV	Gancyclovir
HCMV	Human Cytomegalovirus
HE	Hematoxilin & Eosin
HHV	Human Herpes Virus
KO	Knock Out
MEDRI	Faculty of Medicine University of Rijeka
MCMV	Mice Cytomegalovirus
MEF	Mouse Embryonic Fibroblasts
PFU	Plaque-forming Unit
SNHL	Sensorineural Hearing Loss
VZV	Varicella Zoster Virus
WT	Wild-type

1. Introduction

The Herpesviridae family includes at least eight species (HHV-1-HHV-8) that can infect humans and are classified into three subfamilies: α-, β- and γ subfamily herpesvirinae (1). Among these, HHV1-HHV-5 (herpes simplex virus type 1 and 2, varicella-zoster virus (VZV), Epstein-Barr virus, and human cytomegalovirus (HCMV)) are extremely widespread among humans, and it is estimated that more than 90% of adults are infected with at least one of them. Infections are asymptomatic or unrecognized in most infected individuals but can pose a serious risk to immunocompromised patients, leading to severe complications or even death. In addition, apart from VZV, to date, there is no vaccine for other members of the herpesvirus subfamily, which together with the relatively frequent presence of virus strains resistant to classical antiviral drugs requires the identification of new potential targets for future antiviral treatments.

1.1 Cytomegalovirus Infection

HCMV, also known as human herpes virus 5 (HHV-5) is a double-stranded DNA virus that is part of the β-herpesvirinae subfamily (1). Its genetic material encodes 165 genes rendering it the virus with the largest genome among herpes viruses. The most common route of transmission is contact with body fluids such as saliva, blood, breast milk, urine and genital secretions of infected individuals.

In horizontal transmission the virus enters the host organism via mucosal cells, viral replication occurs in fibroblasts and then spreads to myeloid cells, where it establishes a latency state (2). Initial detection occurs by binding of so-called pathogen-associated molecular patterns (PAMP) as well as damage-associated molecular patterns (DAMP). Those are recognized by pattern recognition receptors (PRR), among which Toll-like receptors (TLR) play a central role (3,4).

CMVs are known to evade immune surveillance and for this purpose have an extremely large genome in which they ‘store’ genes for immunomodulation. These mechanisms ensure the virus’ survival in the host since CMV infection remains lifelong once a person has been infected. In fact, it is HCMV that is recognized as a representative of viruses that encode various proteins

and microRNAs capable of carrying out a whole series of immune evasion mechanisms, by manipulating innate natural killer (NK) cells, adaptive CD4+ and CD8+ T cells, neutralizing B cells and a whole series of soluble mediators of the immune reaction. As an example in terms of cytokines, CMV is known to influence the interleukin 10 pathway to suppress host response (5).

The cellular response triggers an extraordinary differentiation of T-cells in comparison to other human viruses. The proportion of the immune reaction is also reflected in the state of latency maintenance where CMV-specific T-cells dominate immunological memory compartments (6). It is however not established if this amount of memory cells is truly necessary to prevent reactivation. Virus-specific T-cells retain their cytotoxic potential and even continue to express pro-inflammatory cytokines such as INF-**γ**. Latent infection has further been linked to an increased risk of vascular damage (7). Additionally, the great effort of the immune system to maintain viral latency may contribute to the phenomenon of immune senescence, the decreased capability of the immune system usually seen in individuals above the age of 65 (8).

Primary infection in adults typically occurs asymptomatically, followed by the development of lifelong latency. Due to its asymptomatic course of infection, IgG seroprevalence ranges from 44.4 to 95.7% among the population of Europe. The IgG seroprevalence varies among the average populations of European countries (Germany 56.7%, Croatia 74.4%) and was found to be related to socioeconomic status (9,10). Even more concerning is the situation regarding IgM seroprevalence – indicating an active infection: European women of reproductive age (15–45 years) are serologically positive for CMV-IgM in 1.0-4.6% (11).

Considering that primary infection in otherwise healthy adults most commonly does not evoke symptoms, no treatment is indicated. The clinical picture includes nonspecific symptoms such as myalgias, a non-icteric increase of liver enzymes, and headaches, but may also mimic other infections in the shape of colitis or splenomegaly. In contrast to mononucleosis inflicted by Ebstein-Barr virus (EBV or human herpes virus 8 [HHV8]), which is part of the gammaherpesvirinae subfamily, symptoms such as posterior cervical lymphadenopathy and tonsillar pharyngitis are less common (12). The differential diagnosis of EBV infection can be excluded by the heterophile antibody test (also Paul-Brunnel test) (13,14). CMV rarely evokes

life-threatening consequences in otherwise healthy individuals (15). However, central nervous system manifestations resembling the ones seen in immunocompromised patients (16) have been reported (17,18). Diagnostic tools to confirm infection include the presence of relative lymphocytosis (with a share of atypical lymphocytes of >10%), serological testing for IgG and IgM as well as polymerase chain reaction (PCR) amplification of body fluids such as urine or saliva (15,19).

Current treatment options include antimetabolites and polymerase inhibitors (Table: 1). The first-line drug for CMV disease is Ganciclovir (GCV) or its orally available derivate Valgancyclovir. Both are deoxyguanosine analogs that can terminate DNA replication. The development of resistance is possible in case of mutation of the CMV enzyme which the drug targets. Side effects are nephrotoxicity and myelosuppression. Second-line drugs are Foscarnet and Cidofovir in case of resistance to GCV. Both resemble GCV in terms of mechanism of action and side effects. A third alternative to GCV is Letemovir, which inhibits the virus' terminase, an enzyme that is involved in its structural assembly. Its side effects are described to be rather mild. One promising, recently approved drug is Maribavir, an antimetabolite of ATP that targets the virus' kinases (20).

1.1.2 Infection in Immunocompromised Individuals

An immunocompromised state, which may result from immunosuppression after organ transplantaion – either bone marrow or solid organs – or long-term infection with the human immunodeficiency virus (HIV), is associated with an increased risk of opportunistic infection.

For example, alemtuzumab is a monoclonal antibody that binds to CD52, causing profound lymphodepletion, allowing its use during solid organ transplantation to prevent graft rejection. However, since lymphocytes are the key cells that control viral infections, patients are necessarily at increased risk of opportunistic infections, such as CMV (21). CMV has – besides active infection – been proven to negatively influence other conditions such as cardiovascular disturbances and acute rejection and mortality (22). CMV reactivation has also been associated with acute graft versus host disease (aGVHD)(23).

Before the advent of highly active antiretroviral therapy (HAART), CMV was a common AIDS-defining opportunistic illness. Fortunately, today CMV has a significantly lower incidence in AIDS patients (24).

Table 1. Antiviral CMV Drugs.

Drug Name	Structure	Target
(Val-)Gancyclovir	nucleoside analog	DNA Polymerase
Foscarnet	nucleotide analog	DNA Polymerase
Cidofovir	nucleotide analog	DNA Polymerase
Letermovir	terminase inhibitor	CMV Terminase Complex
Maribavir	ATP analogs	Viral Kinase

CMV disease prevention in high-risk patients such as immunocompromised or transplant recipients follows two treatment principles, namely universal prophylaxis and pre-emptive therapy. The universal prophylactic approach includes prophylactic dose treatment, but its positive effects may be limited by the drugs' side effects. Pre-emptive therapy indicates weekly serum viral titer monitoring with drug administration only in case of an unexpected rise (13,25).

1.1.3 Congenital Infection

In terms of epidemiology, CMV-infection is present in about 0.5% of live births per year in the USA (26). Mothers may be infected via contact with body fluids, blood transfusions or sexual intercourse. In the majority of cases, these are mothers whose first child contracted the disease from, for example, the kindergarten, while they were pregnant. The fetus, however, is infected transplacentally. Positive maternal serostatus does not prevent placental transmission (27). The time of infection during gestation is a major determinant of clinical outcome: during the first and second trimester the transmission risk is estimated to be around 20% and reaches 70% until the

third one (28). Infection risk due to reactivation is estimated to be significantly lower. The most severe sequelae are seen in fetuses infected during the first trimester (29).

Among infected newborns, only around 10-15% present clinically detectable features. The most prominent symptoms/signs are small gestational birth weight, petechiae, jaundice, hepato- and splenomegaly as well as neurological manifestations such as ventricular calcifications, microcephaly, lethargy, and hypotonia.

Laboratory examination may support the diagnosis by displaying hepatobiliary system disturbances such as hyperbilirubinemia (conjugated/direct) and an increase of the liver aminotransferases. Both clinical findings commonly become apparent within the first two weeks after birth combined with thrombocytopenia (30). Long-term sequelae include severe psychomotor and perceptual handicaps such as bilateral sensorineural hearing loss (SNHL) and mental retardation. There is a significantly higher risk of developing SNHL in infants who are symptomatic at birth (31). The absence of predictive tools to identify newborns at risk renders the treatment difficult, bearing in mind the side effects of approved antiviral drugs. Diagnostic evidence of active infection can be established by confirmation of viral particles in the urine or saliva as well as presence of immunoglobulins in the blood. Postnatal infection is possible but bears no risk of development of pathologies of the central nervous system (32).

1.2 PrP^C

Since cytomegaloviruses are species-specific, the study of HCMV virus is not possible in animal models, but related animal viruses are used for this purpose, such as murine cytomegalovirus (MCMV) that infects mice (33). Studies of MCMV infections in adult mice played a significant role as a model of CMV pathogenesis, especially for the reason that an exceptional number of genes involved in the immunoregulation of the host's immune response are present in both viruses and are very similar. Animal models as such are necessary, given that there is no way to replicate the complexity of the immune system in vitro (34). In adult mice, the infection does not reach the brain, therefore MCMV infection in newborn mice is successfully used as a model of

congenital HCMV infection in newborns. This model was established and is in full use at the Faculty of Medicine University of Rijeka (MEDRI). It is shown that newborn mice infected with MCMV have high viral titers accompanied by a strong inflammatory response and that the inflammatory response itself is the basis of many damages, primarily to the central nervous system (35).

Given that the cellular prion protein PrP^C, which is strongly expressed in the CNS, has recently been identified as one of the molecules that could lead to a calming of the inflammatory response (36), a team of researchers from MEDRI is studying its role in the immune response during CMV infection in newborn mice, and a publication is in preparation. One of the key findings was that mice lacking PrP^C had lower viral titers. During the research, which was primarily focused on the CNS, a preliminary result indicated that MCMV could behave differently in the lungs of newborn mice, i.e. only in that tissue there was no difference in the viral titer of PrP KO and WT mice, or it was even opposite to that in other organs. For this reason, this thesis is focused on examining the behavior of the virus in the lungs of PrP KO and WT mice.

PrP^C is most strongly expressed in the central nervous system, followed by the immune system, and then the lungs (37,38). As a protein, it has been well researched, given its role in the fatal Creuft-Jacobson disease (39). Lungs were interesting for us to investigate also because currently the effect of PrP^C protein in influenza A virus is best investigated. In the lungs of mice lacking PrP^C, the titer of influenza A virus was very high. Therefore, the involvement of PrP^C in the course of infection has been demonstrated in mice infected with influenza A virus. It has been established that PrP^C KO mice are more susceptible to viral infection and experience higher rates of mortality. The protective mechanisms have been associated with PrP^C's influence on superoxide dismutase activity (37) and impact on macrophage polarization (40).

The PrP^C is encoded by the *PRNP* gene on band 20p13 on chromosome 20 (Chromosome 2 for mice). The protein's length before post-translational modification is 253 amino acids. At its N- and C-terminal it is framed by signal sequences that target the endoplasmatic reticulum (ER) and

thereafter the glycophosphatidylinostol anchor on the cell membrane. Both are removed during the processing course. The signal sequence is followed at the N-terminal by an intrinsically disordered domain that is believed to enable most interactions with other proteins. The N-terminal further contains four octapeptide repeats. In its high content of histidine, this domain is involved in Cu^{2+} metabolism. The C-terminal domain has a globular structure composed of three α-helix and two β-sheets as well as interconnecting loops. A disulfide bridge is formed between Cys-Cys residues 179 and 214, which gives the final shape to the structure of the mature protein. There are also two glycan binding sides coming into play during posttranslational modification by prolonging the protein's half-life: the first one lies within the second α-helix and the second one between helix numbers two and three.

2. Methods

2.1 Hematoxylin & Eosin Staining

The process of Hematoxylin & Eosin (H&E) staining is a commonly applied method to differentiate tissue structures in histological slides. It is a two-dye technique, which facilitates tissue analysis by highlighting the intrinsic differences in the acidophilic and basophilic properties of cellular structure, ground substance, and fibrillary elements. Hematoxylin, being a basic color, stains acidic structures such as the DNA or RNA-rich components. Eosin, on the other hand, stains acidophilic structures such as collagen fibers (41). The staining process begins with deparaffinization: the slide is immersed two times for 3 minutes in 100% xylol, followed by two immersions in 100% ethanol for two minutes. Thereafter, two more 2 min baths in decreasingly concentrated ethanol (90% and 70%) are applied. The slide is thereafter immersed in distilled water for 2 minutes. The procedure's next step is a five-minute bath in hematoxylin, staining basophilic structures such as the nucleus. Excess dye is removed under running tap water for 5 minutes. Thereafter, the slide is immersed in eosin for one minute. Excess dye is removed under running tap water for 30 seconds. The slide is then dehydrated by immersions in increasingly concentrated ethanol. After one minute in 96% alcohol and two one-minute baths in 100% alcohol to remove the remaining water, the slide is placed in two xylol baths for 3 minutes. Entellan as a rapid mounting medium is applied to ensure durability. The slide is now ready for microscopic examination.

2.2 Titer Determination

Viral titer was determined using standard titration protocol: One day before titration, mouse embryonic fibroblast (MEF) cells are seeded in 48-well plates in Minimum Essential Medium (MEM) supplemented with 3% fetal calf serum (FCS). MEF cells are adherent and attach to the bottom of the well where they form a 'film' on which it is later possible to monitor virus-induced clearings (so-called plaques). On the day of titration, the virus-infected tissue is defrosted from -20°C to room temperature. Methylcellulose too is defrosted from - 4 °C to room temperature (25°C). Titer dilutions are carried out for $1:10^2$ to $1:10^5$. The tubes are filled with 900 µL of 3%

DMEM (Dulbecco's Modified Eagle Medium) via a dispenser. Steel beads are added to tubes containing media and tissue for homogenization. Homogenization is carried out at 2/S (Svedberg) for 2 minutes followed by centrifugation at 4000 rpm for 5 minutes. Thereafter, 100 µL of the homogenate is transferred to the first $1:10^2$-dilution and the solution is thoroughly mixed. Accordingly, further tenfold dilutions are prepared by transferring 100 µL to the subsequent dilution step with fresh pipetting tips. Before the dilutions are transferred to the wells, the medium is removed from the MEF monolayer by a vacuum pump. 100 µL of infected organ homogenate is transferred to the wells using a multi-tip dispenser. Centrifugal enhancement is thereafter carried out at 2100 rpm in order to increase viral infectivity (42). The plate is then placed into the incubator at 37°C and 5% CO_2 for 60 minutes. 500 µL of methylcellulose is added from a sterilized graduated cylinder. It serves both as a nutritive medium as well as a physical restriction for viral progeny. The plate is placed in an incubator (37°C, 5% CO_2) for 3-4 days. Finally, the formed plaques are counted using a light microscope. The number of plaque-forming units (PFU) is quantified according to the step of dilution in relation to the extracted organ weight, determined earlier (34).

2.3 Cytokine Performance Assay

Cryopreserved organs were lysed using Procartaplex™ buffer (Thermo Scientific) and homogenized (3 x 30s at frequency 2.5). Tissue proteins were quantified using a ProcartaPlex™ Mouse Immune Monitoring Panel 48-Plex (EPX480-20834-901, Thermo Scientific). We acquired this kit to determine differences in the inflammatory process in mouse tissues. For its analysis, a device is used that we do not own, so the prepared tissues were sent to our colleagues on dry ice, and the tissue analysis was performed at the Institut Pasteur in Paris. In short, DropArray 96-well-plates (Curiox Biosystems) were blocked with 1% bovine serum albumin (BSA) for 30 min. After blocking, 10 µL of premixed beads, then 10 µL of samples, controls, or standard were added per well and incubated for 2 h. The plates were incubated on the shaker, with 5 µL of detection antibody for 30 min, with 10 µL of streptavidin-PE for 30 min, and with 15 µL reading buffer per well, before being transferred to reading plates. Between incubation steps, plates were washed three times (PBS, 0.1% BSA, 0.05% tween) and DropArray LT210 MX washing station (Curiox Biosystems). The samples were read by the Bio-Plex200™

instrument (Bio-Rad) in 70 μL of reading buffer. We performed quantification and statistical processing of data on cytokines and other soluble molecules, obtained from Paris.

3. Aims and Objectives

The aim of the thesis is to examine the influence of PrP^C on the immune response against MCMV in the lungs in a model of congenital HCMV infection. Also, to correlate the results with findings in other tissues of mice inoculated with MCMV. The objective is to examine the preliminary observation that a different phenomenon occurs in lung tissue, that is, that there is no difference in virus titer in the lungs between strains or that PrP KO mice have even more MCMV virus in that tissue than the equivalent WT strain. Further, we will search for an explanation for this phenomenon.

4. Results

4.1 Newborn PrP KO mice inoculated with MCMV virus have milder histopathological changes

We decided to examine the organs of animals from experiments in which titers in the salivary glands, brain, and spleen were shown to be consistent with the phenomenon that PrP KO mice have significantly less MCMV virus in the organs (Figure 1 A). The observation that PrP KO mice have a statistically significantly lower titer than their WT counterparts was not published but is available as an abstract at the Sneak Peek link for previews of papers (43). In Figure 1A, we therefore show a schematic representation of the result we refer to.

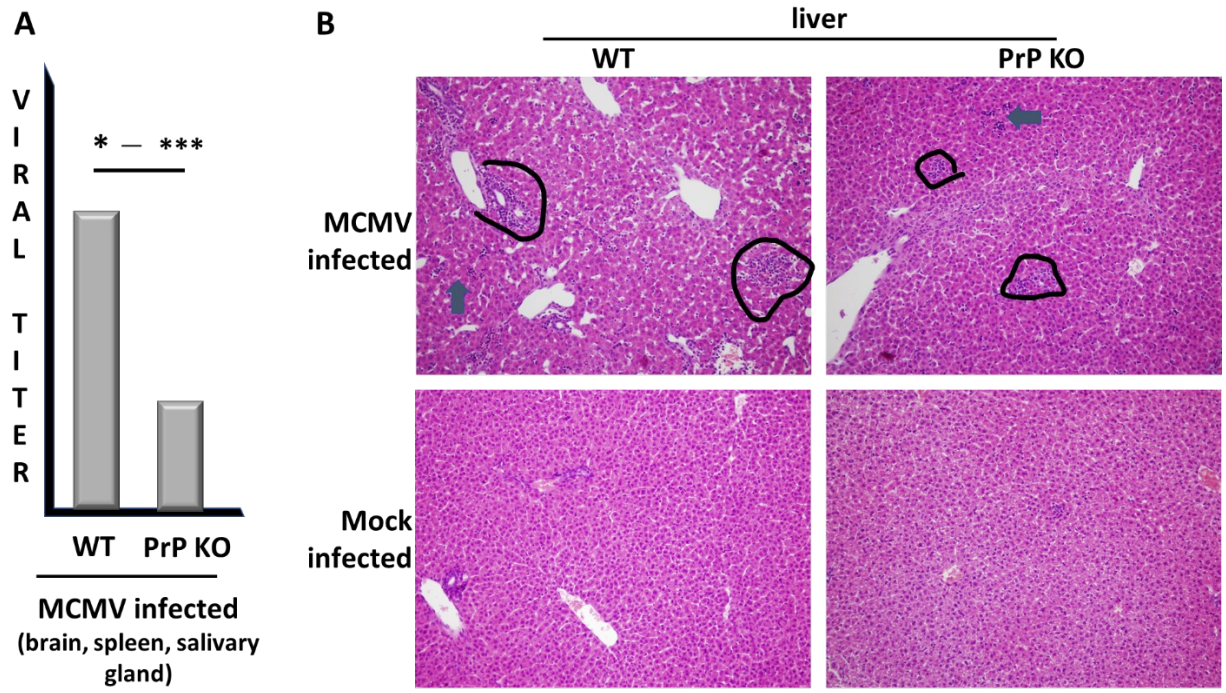

Figure 1. PrP KO mice better control MCMV infection. A) Schematic representation which shows that PrP KO mice have lower virus titers in tissues (except lungs). B) Light microscope analysis of HE-stained CMV- and mock- infected liver. HE = hematoxylin & eosin staining. asterisks indicate statistically significant differences.

In order to examine the differences in the lungs, we selected an experiment in which the difference titer phenomenon was confirmed (Figure 1A). Livers were available from this experiment, which were used for histopathological staining, as a positive control that PrP KO mice have a slightly better histological picture in the organs (Figure 1B). First, we took livers from WT and PrP KO mice, uninfected and infected to confirm that WT mice have a stronger infection in liver tissue. Light microscope analysis in search of hematological centers in the liver serves as a semi-quantitative measurement of the state of damage suffered from infection. We opted for HE staining and found that the pattern was similar in terms of the amount of residual hematopoiesis due to infection (gray arrows), while in WT mice we found a slightly higher number and an increased size of immune infiltrates (indicated by black circles in the affected area). The histological analysis of liver tissue indicates a more effective viral clearance in liver tissue in PrP KO mice.

4.2 Viral Titer in lungs are not influenced by PrP^C

As displayed by the schematic representation (Figure 1A) in brain-, spleen- and salivary gland tissue the number of viral particles decreased on day 14 days post-infection in PrP KO mice. The most apparent difference was found in brain tissue. However, in lung tissue, viral titers behaved differently from expectations inferred from the aforementioned findings. There was no statistically significant difference between infected mice on 14 dpi. In fact, a trend was visible indicating increased viral titers in PrP KO mice compared to WT animals.

At 8 dpi viral titers show no significant difference comparing KO and WT mice (Figure: 2A). This is in line with the expectations and findings in other tissues, since the difference in viral

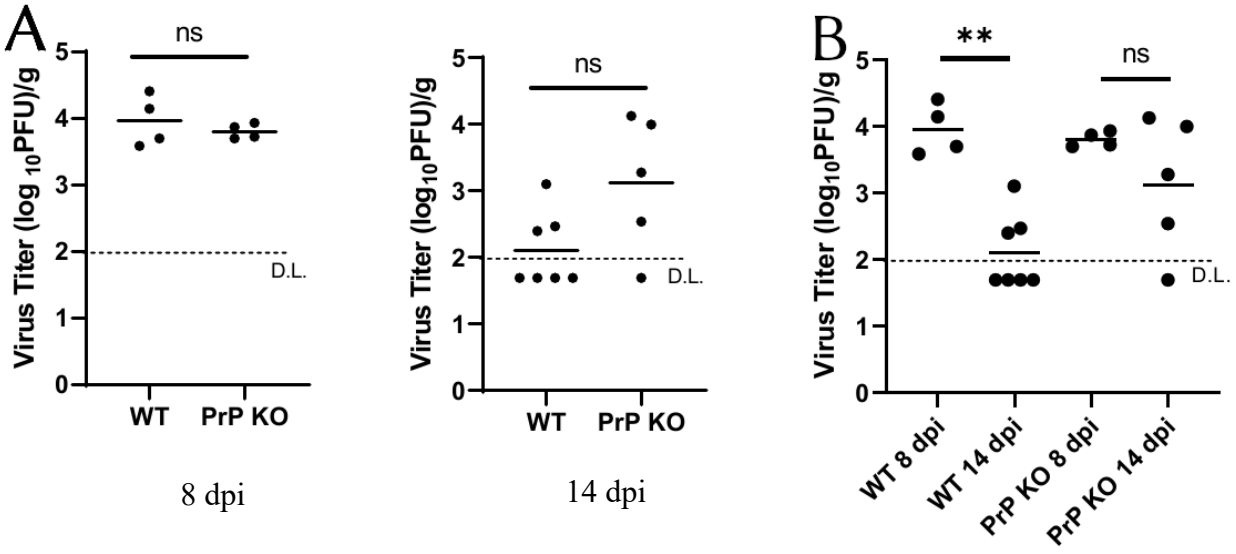

Figure 2: viral titers do not follow the trend seen in other tissues A) viral titers in lung tissue examined at 8 dpi (left) and at 14 dpi (right). B) Comparison of the same viral titers shown in each strain as a function of time. PFUlog/g = log of plaque forming unit per gram of infected lung tissue; asterisks indicate statistically signifiant differences. ns = not statistically significant.

titers is significant only on day 14 post infection. The determined decrease in viral titer comparing 8 and 14 dpi in WT mice is however statistically significant, indicating a successful induction of the adaptive immune response in lungs. Interestingly, the viral reduction appears to be somewhat slower in the lungs of PrP KO mice, which further indicates that this tissue might have a specific mechanism of viral control (Figure 2B).

4.3 MCMV related inflammation induces IL-17A expression in lung- but not in brain tissue

To investigate the determined difference, we compared the concentrations of 40 cytokines and other soluble molecules in lung and brain tissue to discover a reason for the divergent viral titers described earlier.

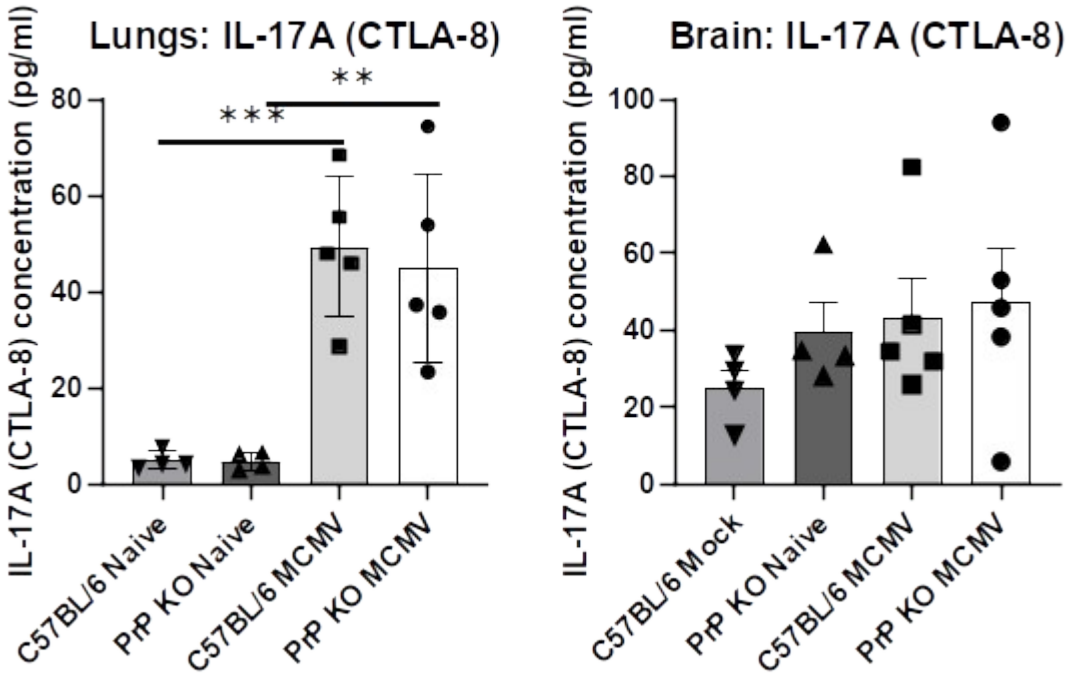

Figure 3: IL-17A in lung tissue does not correspond with brain cytokine levels. **L**evels measured at 9 dpi in C57BL/6 (WT) and PrP KO mice in left) lungs and right) brain. asterisks indicate statistically significant differences. only statistically significant differences are indicated.

In this comparison, IL-17A raised our attention. The interleukin's role in infection has proven complex, but it is generally considered pro-inflammatory. Cytokine concentrations of IL-17A or CTLA-8 (cytotoxic T-lymphocyte-associated serine esterase 8) measured at 9 days post-infection in mice lungs and brain displayed a significant difference. A significant increase of IL-17A was seen in lung tissue following infection both in WT and PrP KO animals (Figure 3, left).

In brain tissue, there was no significant increase of IL-17A following infection neither in KO nor WT animals (Figure 3, right). Our hypothesis is that in the lungs, the increased inflammatory effect through IL-17A may overshadow the inhibitory effect of the PrP^C itself. Namely, the inhibitory effect of PrP^C on the immune response is proposed by MEDRI researchers as an explanation for frequently encountered higher titers in other organs of WT mice, such as the brain.

5. Discussion

Preliminary data has found an influence of PrP^C on the extent of MCMV infection in terms of viral titers in the brain, the salivary gland, and the spleen (43). In these tissues, viral titers were decreased in PrP KO mice at 14 dpi.

Our histological analysis of liver tissue too suggests decreased damage in infected KO mice. To corroborate this deduction, it may be of further interest, to determine viral titers in liver tissue on 14 dpi. When investigating lung tissue, viral titers of infected animals behaved differently in that KO mice did not display lower viral titers on the same day past infection. Viral titers displayed no significant difference comparing WT and KO groups on 14 dpi with even a visible trend indicating higher levels of viral particles in KO mice.

Other authors have found the presence of PrP^C to influence the outcome of viral infection in favor of survival and decreased extent of lung damage in the course of IAV pneumonia associated with higher viral titers (37,40). Experiments in the aforementioned publications were however performed on a virus whose course of infection is very different from cytomegalovirus. Nevertheless, they instructed us to pay attention to the behavior of the CMV virus in the lungs, especially when we noticed an inconsistency with other tissues.

These contrasting viral titers let us search for differences in cytokine expressions between brain and lung tissue that may explain these findings. The rise of IL-17A levels measured in mice lungs and its apparent absence in CNS tissue after infection raised our attention. IL-17's role in the course of viral infection has proven complex. It has both been found to act pro- and anti-inflammatory (44). Its involvement in the recruitment of B-cells in IAV infection indicates its importance in the mechanism of anti-viral defense (45). On the other hand, interleukin 17 A has been found to promote viral infection in other conditions by suppressing the immune response (46,47).

Increased levels of expression of IL-17A in CMV infection have been investigated in liver transplant recipients, where an increase was encountered after infection (48). Further, increased

levels of IL-17A in CMV-complicated renal transplants have been associated with acute rejection (49).

6. Conclusion

The results of our study indicate that the lack of PrP^C has varying effects on the immune response to CMV in different tissues. Our findings demonstrate that the absence of PrP^C does not have the same impact on the immunological response to CMV in lung tissue as it does in brain, spleen, and salivary gland tissue (43). The comparison of IL-17A levels between lung and brain tissue suggests that the regulation of this cytokine is not related to PrP^C's immunomodulatory function in lung tissue during CMV infection. It should be noted that although our findings propose one possible factor contributing to the observed differences, it does not rule out the influence of other factors on these variations in lung and brain responses, which may or may not be related to PrP^C.

7. Summary

This thesis investigates the influence of PrP^C on the immune response against MCMV in lung tissue. Histological investigations support the preliminary claim that the absence of PrP^C influences viral clearance in multiple tissues such as in the liver leading to less recorded tissue insult.

Further, viral titration of lung tissue demonstrated no significant difference between wild type mice and those lacking PrP^C. Searching for an explanation for this finding, we analyzed differences in cytokine levels in the brain and lungs. We found IL-17A levels to behave differently in the lungs compared to brain tissue. Only in the lungs did we record a significant increase of IL-17A levels in PrP^C KO mice following infection while in brain tissue a comparable finding was absent. Concisely, our findings indicate the MCMV inflicted immune response to be differently affected by the absence of PrP^C in comparison to other tissues and the expression of IL-17A to be unrelated to PrP^C.

Keywords: cytomegalovirus, immunology, PrP, histology, lung

8. Curriculum Vitae

Benjamin Herrlich was born in Künzell, Germany on May 31st, 1998. He received his high school diploma from the Eduard Stieler Schule in Fulda. In 2017, he began his integrated undergraduate and graduate studies at the Medical Faculty in Rijeka, Croatia. His studying efforts were honored with a scholarship for special student achievements by the University of Rijeka for the academic year 2018/2019.

Benjamin acquired scientific working experience during his one-year lasting diploma research at the Center of Proteomics at the Faculty of Medicine, where he conducted experiments such as cell culturing and viral titration of cytomegalovirus in organs. He also developed a scientific approach to research in medicine and a critical approach to analyzing results.

During the COVID-19 pandemic in 2020/2021, he volunteered at the emergency department in Sušak, Rijeka for two months, where he assisted in the care and treatment of some of the most critically ill COVID-19 patients. Additionally, he volunteered at multiple departments during his summer breaks. Throughout his studies, Benjamin gained proficient skills in the Croatian language (B1 level).

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