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HUMAN HERPUSVIRUS 6: A RISK FACTOR FOR MULTIPLE SCLEROSIS

ANALYSING THE POTENTIAL ROLE OF THE HUMAN HERPESVIRUS 6 IN THE DEVELOPMENT OF MULTIPLE SCLEROSIS.

Multiple sclerosis (MS) is very complex disease consistent of various phenotypes of which the exact origin is unknown. It is considered a combination of an inflammatory mediated auto immune disease with a neurodegenerative disease. Known risk factors for the development of MS are genetic predispositions and environmental factors such as smoking. More recently it is believed that viral infections such as human herpesvirus 6 (HHV6) can be an environmental risk factor for MS as well. Through the mechanism of molecular mimicry, HHV6 is considered to activate the autoimmune response that marks the pathogenesis of MS. Moreover, various studies suggest that HHV6 influences the concentration of TNF-α, a pro-inflammatory cytokine, which plays an important role in the development and progression of the disease. Other viruses such as the Epstein-bar virus (EBV) have been studied more extensively and have more proof substantiating the idea that they might contribute to the disease development. Based on evidence regarding EBV, it is believed that HHV6 can induce latent infections in the meninges of patients that will later contribute to the development of MS, possibly in combination with EBV infection. Although there have been many studies that presented evidence substantiating the idea that HHV6 is a risk factor for MS, the vast majority of that evidence is very circumstantial. It is because of this that many believe there to be no role for HHV6 and conducted meta-analysis in combination with alternative theories have tried to disprove the role of HHV6 in MS. Nevertheless, the admittingly circumstantial evidence does indicate a possible role for HHV6 in MS, but more research into a wide range of mechanisms is needed in order to definitely classify HHV6 as a risk factor.

JOERI ROLWES
SUPERVISOR: PROF. DR. ULRICH EISEL
University of Groningen
Immunology and infections

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Introduction

Multiple sclerosis (MS) is an autoimmune mediated disease of the central nervous system (CNS). It is a neuronal disease whereby myelin, used as a neuronal insulator, is degraded. Myelin is a lipid-rich material, surrounding nerve fibres. Myelin is produced by oligodendrocytes and allows for the conduction of axonal impulses. Degradation leads to various mental and physical incapacitations. Since almost the complete CNS can be affected by this disease, many different neurological symptoms can be experienced by patients. Symptoms that are commonly found in MS are weakness or numbness of limbs, optic neuritis, tremors, involuntary muscle movements, double vision, dysarthria, dizziness, and fatigue (Rolak, 2003).

The prevalence of MS has increased rapidly over the past years, with an astounding 10,4 percent between 1990 and 2016. In 2016, an estimate of 30.1 cases of MS was present per 100.000 inhabitants globally (Wallin et al., 2019). MS appears to be more frequent with increasing latitude, indicating a higher prevalence in the northern hemisphere. A United States population study substantiates this, showing a significantly higher prevalence between states above and below the 37th parallel, with a difference of approximately 30 cases per 100,000 inhabitants (Wallin et al., 2019). MS affects mostly young women between the ages of 20 and 40. The incidence ratio between men and women globally is 1:2.3-3.5, respectively. However, this predominance in women does differ with latitude. Nevertheless, MS is consequently one of the main causes leading to disability in young adults (Garg & Smith, 2015; Harbo et al., 2013).

MS has a range of different phenotypes that have a different disease progression and perspective. Initially, most cases of MS start with the development of clinically isolated syndrome (CIS). CIS is defined as an acute clinical attack of inflammation and demyelination in one or more places in the CNS, leading to an episode of neurological symptoms. Patients who develop CIS often progress to relapsing-remitting MS (RRMS) (Doshi & Chataway, 2016). RRMS is characterized by infrequent attacks of neurological symptoms. These attacks are followed by periods of remission whereby symptoms may disappear or become permanent (Lublin et al., 2014). After 10-15 years since diagnosis with RRMS, approximately 80 percent of patients develop secondary progressive MS (SPMS). In this stage, there is an accumulation of neurological symptoms without apparent clinical remission. The progression in SPMS can be either active, meaning worsening of neurological symptoms, or not active, meaning continuous neurological symptoms without remission or worsening. In some cases, MS can onset with the same phenotype as SPMS. This is seen in 10-15 percent of the cases and is called primary progressive MS (PPMS) (Baecher-Allan et al., 2018). Figure 1 shows the phenotype of the different subtypes of MS and the stage of neurological degradation and corresponding brain volume (Håkansson, 2019).

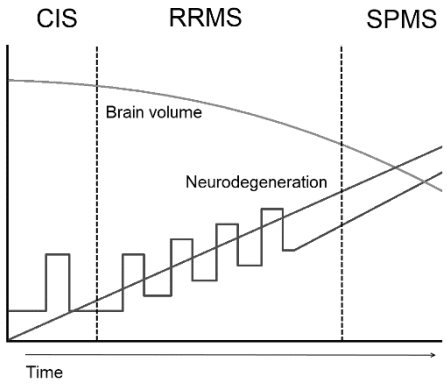

Figure 1: ***Phenotypes of MS subtypes*** *(Subtypes CIS, RRMS and SPMS over the course of the most common chronological progression of the disease. The corresponding brain volume and amount of neurodegeneration are also shown).*

The aetiology of the different subtypes of MS remains largely unclear. However, various studies have shown that there are many factors that can contribute to the development of the disease such as genetic predispositions and environmental factors. These environmental factors that contribute to a higher risk of developing MS are smoking, vitamin D deficiency and obesity. The role of vitamin D is hereby believed to play a major role in the increasing incidence by latitude (Ghasemi et al., 2016). Moreover, observations of patients with MS over the years have displayed some interesting

incidences. Many patients in various stages of MS showed reactivation of certain viral infections. This sparked the first idea that viral infections might also be an environmental trigger. Afterwards, increasing evidence that viral infections may also play a role in the pathogenesis of MS, thereby being the environmental trigger, came about. Over the past 30 years, several herpes viruses have gained an interest in the field of MS. An example is the Epstein-Barr virus (EBV), or human herpes 4 virus, which is nowadays a well-established risk factor for the development of MS (Guan et al., 2019). Another herpes virus that is more and more frequently linked to the development of MS, is the human herpes virus 6 (HHV6). HHV6 has been previously suspected to be associated with a variety of autoimmune diseases such as rheumatoid arthritis, and there has even been substantiating evidence for the role of this virus in the development of autoimmune thyroid gland diseases (Sultanova et al., 2017). Lately, the connection between HHV6 infections and MS development is being discussed. Evidence for the correlation between HHV6 and the development of MS is based on anti-HHV6 antibody levels in MS patients and HHV6 concentrations in MS plaques (Virtanen & Jacobson, 2012). Nevertheless, the evidence is often circumstantial and unreproducible. The role of HHV6 is a highly debatable subject and numerous studies submit different mechanisms that are supposedly affected by the virus in MS or disagree with the possible role all together (Fierz, 2017).

The disagreement and uncertainty in the literature about the role of HHV6 in the development of MS needs to be further addressed in order to determine the aetiology of MS. Currently, treatment for MS only consists of managing symptoms. It is based on slowing the progression of disease and speed up the recovery following attacks (Dargahi et al., 2017). Mapping the factors that can trigger the development of MS is therefore extremely important to expand the possible targets for therapy and prevention. Therefore, the aim of this article is to discuss whether the human herpes virus 6 is a risk factor for the development of multiple sclerosis.

Immunopathogenesis of MS

The primary cause in MS is the inflammation of the CNS. It is therefore that the CIS and RRMS stages are generally classified as the inflammatory phase driven by an autoimmune process while the progressive forms, SPMS and PPMS, are classified as neurodegenerative. The progressive forms are hereby characterized by scathing irreversible axonal loss (Sospedra & Martin, 2016). The direct cause for the inflammation and with that, the development of MS is, as mentioned before, still unknown. Many immunological studies have however examined various pathways that play a role in MS. These studies are often performed in animal models for human MS based on experimental autoimmune encephalomyelitis (EAE). Although this EAE model has proven to be adequate in studying diverse mechanisms resembling MS, the lack of knowledge about the differences between EAE and MS must be kept in mind (Loma & Heyman, 2011).

The immunopathogenesis of MS consists of attacks against the CNS due to a violation in self-tolerance to various antigens such as myelin basic protein (MBP), proteolipid protein (PLP) and myelin oligodendrocyte glycoprotein (MOG) (Garg & Smith, 2015). T-lymphocyte antigen diversity is generated by rearrangements in the V, D and J regions of the T-cell receptor (TCR) α and β genes. The production of TCR's takes place in the thymus and is an important mechanism for generating TCR variance (Qi et al., 2014). In the cortex of the thymus, positive selection reviews the compatibility of the TCR to the major histocompatibility complex (MHC). Afterwards, in the medulla, negative selection reviews the reaction to tissue specific antigens in order to eliminate autoreactive TCR's. This process is not completely efficient and avoidance of negative selection may contribute to the development of autoreactive T cells that are present in MS (Gonsette, 2012).

In animal models, EAE is induced by activating naïve $CD4^+$ T helper 1 (Th1) cells against myelin antigens. Autoreactive Th1 cells are hereby enough to create an inflammatory demyelinating disease mimicking MS. Nevertheless, effective treatments against EAE were not effective in MS, showing that MS is a more complex disease including more mechanisms than autoreactive Th1 cells (Lassmann & van Horssen, 2011). More recently, further research in the EAE model showed that not only Th1, but also Th17 and $CD8^+$ regulatory T-cells play a role in this disease (Kleinewietfeld & Hafler, 2014). This was considered applicable for MS as well when research on the immune response in MS patients themselves showed that T-cells, B-cells and antibodies contribute to the pathogenesis of the disease (Martin et al., 2016).

Activated autoreactive Th1 or Th17 express certain adhesion molecules that allow binding the endothelium-brain-barrier (EBB). The T-lymphocytes simultaneously express metalloproteinase (MMP) which are enzymes that disrupt the semi-permeability of the EBB by degrading parts of the extracellular matrix, allowing the infiltration of the lymphocytes. The autoreactive T-lymphocytes are reactivated by microglia which leads to the production of pro-inflammatory cytokines like IL-1, IFN-y and tumour necrosis factor α (TNF-α) causing further disruption of the EBB (Lazibat et al., 2018; Minagar & Alexander, 2003). The pro-inflammatory cytokines attract B -lymphocytes and macrophages as part of the inflammatory response, leading to the formation of inflammatory lesions called plaques. Plaques consist of an accumulation of cells surrounding white matter and appear in the visual neuron, basal ganglia, brain stem and spinal cord. The distribution of plaques in MS patients is an important factor that determines the phenotypical and prospective characteristics of the disease (Dargahi et al., 2017). In the plaques, the T-lymphocytes stimulate macrophages to internalize and degrade various myelin antigens, mediated by B-lymphocyte produced antibodies. This in combination with activation of the complement system, the release of free radicals and protease release leads to demyelination, axonal damage and oligodendrocyte destruction (Katsara & Apostolopoulos, 2018; Lazibat et al., 2018).

The inflammatory events that occur last about 2-14 days, after which a period of remission arises. Regulatory T-lymphocytes will mediate the inflammation by releasing cytokines such as IL-10, which inhibits inflammation, leading to a reduced inflammatory state. In RRMS, remaining oligodendrocytes will be restored and activated to remyelinate the unmyelinated axons. The axons will hereby regain some, but not full function, since the MBP isoform which used in the remyelination is functionally insufficient. Patients will experience reduced clinical symptoms during this period of remission. Further progression of MS will cause damage to the axons and the oligodendrocytes to become irreversible whereby the myelination stops. At this point, the disease is classified as SPMS since the phases of remission have stopped (Lazibat et al., 2018).

The role of B-lymphocytes in the development and progression of MS is very complex. B-lymphocytes can act as antigen presenting cells (APC) allowing stimulation of T-cells, produce autoantibodies that contribute to the destruction of myelin and oligodendrocytes and act as regulatory cells by affecting T-cell activity with cytokine production (Lazibat et al., 2018). Furthermore, B-lymphocytes can cause the formation of ectopic lymphoid structures (ELS) in the meninges of MS patients. These ELS consist of B-lymphocytes and plasma cells and there is increasingly more evidence that these structures can be a source of latent viral infections (Serafini et al., 2004).

Autoimmunity and molecular mimicry

HHV6 is the collective name for the HHV6-A and the HHV6-B viruses. These are double stranded DNA viruses that employ humans as their main host and niche for reproduction (Jaworska et al., 2010). HHV6 can utilize all nucleated cells as a host, but especially T-lymphocytes are highly infectable (Lusso et al., 1995). HHV6 can enter the cell due to the interaction of its gH-gL-gQ complex, consisting of three

glycoproteins with the transmembrane protein CD46 which is present on the host cell (Mori et al., 2003). After the initial interaction, the viral envelope merges with the cell membrane thereby releasing the virus in the cytoplasm of the cell. Cytosolic transport mechanisms that have not been identified for HHV6, transport the naked virus to the nuclear membrane. The virus fuses with the nuclear membrane and the viral genome is thereby released in the nucleoplasm. Cellular transcription and translation processes are then used to reproduce viral proteins, that can regulate the expression of other genes and replicate the viral DNA. Mature viruses are encapsulated by packaging proteins in the Golgi complex and eventually leave the host cell through exocytosis. The complete cycle of reproduction for the HHV6 virus takes approximately 72 hours (De Bolle et al., 2005).

Under normal circumstances, cells infected with a virus, such as HHV6, express microbial peptide fragments, typically 8 to 10 amino acids in length, using the MHC class 2 complex. Dendritic cells that are present in the tissue are activated due to the recognition of the protein and migrate to the local lymph nodes where they present the antigen to the relevant T-cells. Afterwards, activated T-cells differentiate and migrate to the specific tissue where they will react to the infected cells or co-stimulate other immune related responses causing the eventual clearance of the infection (Alberts et al., 2017). The selection of T and B cells is, as mentioned earlier, not completely effective. In some rare cases, an immune response is initiated against a presented peptide that is related to the host itself. In the bone marrow and thymus, where the initial selection occurs, only a limited number of self-antigens are present that can be used for selection. Therefore, the lymphocytes move to the periphery where a second selection process, called clonal anergy, takes place (Pelanda et al., 1997). It is hypothesised that genetic polymorphisms can result in defective regulation of lymphoid selection in the periphery, allowing certain lymphoid cells that are self-reactive, to escape control (Rosenblum et al., 2015).

Studies have shown that an immune reaction to self-peptides is one underlying factor in the development of MS. The first stages of MS are also classified as an autoimmune disease, and there are many studies that substantiate these claims. Over the last decade, more and more interest was placed on environmental factors such as viruses that are now believed to be a trigger for this autoimmunity. Viral infections such as EBV are believed to trigger autoimmunity by a process called molecular mimicry (Tengvall et al., 2019). Molecular mimicry is characterized as the cross-reactivity of B- and T-lymphocytes. Due to structural similarities between microbial and host peptides that are presented in the form of antigens, the lymphocytes will be able to react to both epitopes. Similarities do not need to be exact since only a part of the peptide will be presented by the APC. When part of a viral peptide is presented that has a similar glycosylated structure as a self-peptide, it can activate lymphocytes that are self-reactive and thereby initiate an autoimmune response (Shoenfeld et al., 2014).

The notion that molecular mimicry can cause the development of autoimmune diseases is not new. Currently, in many autoimmune diseases, a form or variant of the disease is often associated with a microbial infection. One example is rheumatoid arthritis, where one form concerning a group with nonpyrogenic conditions is usually associated with a preceding infection of the *proteus bacteria*. Moreover, ankylosing spondylitis has supporting data that its aetiology is related to subclinical bowel infections with the *Klebsiella bacteria*. (Rashid & Ebringer, 2012). These studies therefore show, that there is a possible role for microbial infections in the development of inflammatory and autoimmune diseases. More focussed on MS, EBV is nowadays a well-established risk factor for the development of the disease. Individuals with specific expression of the HLA gene, *HLA-DRb1*15:01*, who are infected with EBV have a significantly higher risk for MS than patients without the EBV infection (Rojas et al., 2018). Although it is not fully proven, most studies point towards molecular mimicry as the main mechanism for the increased risk of MS caused by EBV infections. In MS patients, increased concentrations of autoantibodies have been found against the chloride-channel protein anoctamin 2

(ANO2). The reactivity of these autoantibodies has been measured and compared against both ANO2 and EBV nuclear antigen 1 (EBNA1). Results show that the autoantibodies show cross-reactivity to the two antigens. This leads to the hypothesis that an infection with EBV can activate autoantibodies that are also reactive to a protein important in the development of MS. How an immune reaction towards ANO2 can affect MS is nevertheless unclear (Tengvall et al., 2019).

Similar to EBV, HHV6 has now also been linked to MS. Even though it is still debated whether HHV6 is a risk factor for MS, a meta-analysis study provided substantial evidence that HHV6 is indeed a risk factor. The meta-analysis, including 39 studies and over 2500 MS patients, concluded that there was a significant correlation between MS and HHV6 infection (Pormohammad et al., 2017). The first evidence for the implication of HHV6 in MS was already found a long time ago. A study performed by Derfuss et al. observed that in about 20 percent of the MS patients, intrathecal antibody production was present against HHV6. This, in contrary to the control group, where no patients showed an intrathecal immune response to HHV6 (Derfuss et al., 2005). This analysis was performed using oligoclonal bands (OCB), and reflects therefore only the B-cell reactivity towards HHV6. Later, a second study showed a strong increase in intrathecal EBV and HHV6 specific Th1 reactivity. Treatment with IL-2, known to decrease the disease activity of MS, leads to a strong decrease in the EBV and HHV6 specific Th1 reactivity. This was a clear suggestion that HHV6 reactive T-cells play a role in the disease activity of MS (Wuest et al., 2014).

One of the mechanisms that was suggested as a hypothesis for the role of HHV6 in the development of MS was, as for EBV, molecular mimicry. One of the viral proteins of HHV6, U24, shares a significantly homologous amino acid sequence with MBP. Seven amino acids within HHV6, ranging from 4 to 10, appeared to be identical to the range of amino acids 96-102 in MBP. This indicates that activation of MBP reactive T-cells ascribable to HHV6, is feasible (Tejada-Simon et al., 2003). Various studies have proven this concept and found that T cells in MS patients, recognizing MBP, were cross reactive and could be activated by epitopes of HHV6. More importantly even, the existence of $CD8^+$ cytotoxic T-cells that were cross reactive has been proven. This is especially important since these lymphocytes have the ability to directly induce harm to the oligodendrocytes (Cheng et al., 2012).

Viruses are known to play part in the aetiology of other autoimmune diseases, and in the case of EBV, in MS itself. Autoimmunity has a major role in the development of MS but its origin is ambiguous. Molecular mimicry by cross reaction of HHV6 and MBP sequences, in addition to a significant link between MS and HHV6, advocate for the role of HHV6 in the development of MS.

The role of Tumour necrosis factor

MS is a demyelinating inflammatory disease of the CNS. As previously mentioned, T-cells that are reactivated produce inflammatory cytokines such as IL-1, IFN-γ and TNF-α, to stimulate macrophage activation and further disruption of the EBB. One factor that is particularly important is the pleiotropic pro-inflammatory cytokine TNF-α. TNF-α plays an important role in the inflammatory state of MS and is produced by reactivated T-cells to induce the inflammatory state. In addition, activated macrophages will also produce TNF-α to stimulate the inflammatory response and induce injury to oligodendrocytes (Lock et al., 1999). TNF-α is found in either of two states, soluble TNF-α or transmembrane TNF-α. Soluble TNF-α predominantly activates the TNFR1 receptor, thereby inducing apoptosis and chronic inflammation. Transmembrane TNF-α, on the other hand, primarily activates TNFR2, thereby stimulating cell survival and anti-inflammatory effects. Low levels of TNF-α are associated with positive immune regulation and defence against pathogens, while high levels are associated with the induction of inflammation and cell damage (Göbel et al., 2018).

The specific role of TNF-α in MS is as of yet inconclusive. However, various studies on the role of TNF-α in EAE, an animal model often used to compare with MS, have shed some light on the function of this cytokine. Experiments showed that TNF-α KO mice developed even more severe inflammation and clinical symptoms in comparison to the wild type. In contrast, TNFR1 KO mice were entirely protected against the development of EAE while TNFR2 KO mice also developed more severe clinical symptoms in comparison to the wild type. Inducing a TNFR2 agonist in EAE mice thereby showed relief of inflammation and clinical symptoms. This substantiates the previously mentioned function of TNFR1 activation and indicates the role of this receptor, and soluble TNF-α, in the pathogenesis of MS (Fischer et al., 2019; Steeland et al., 2017). A study performed by Ribeiro et al., from 2019, corroborated these results by analysing over 150 patients and investigating the possible correlation between soluble TNF-α and the disease progression of MS. They discovered that the concentration of soluble TNF-α was positively related to the progression of disability in patients. Patients with RRMS had increased levels of soluble TNF-α in both blood serum and CSF (Ribeiro et al., 2019).

Knowing that TNF-α plays a role in the development and pathogenesis of MS, it is important to determine whether HHV6 has the potential to alter the production of TNF-α and thereby induce MS or aid in its development. An *In vitro* study with peripheral blood mononuclear cells infected with HHV6 was performed to observe the concentration of various cytokines. Using polymerase chain reactions (PCR), the concentrations of IL-1, TNF-α and IL-6 were determined. Conclusions were that HHV6 has an enhancing effect on the gene expression of TNF-α and the production rate of TNF-α was 8.5 times higher in infected cells compared to uninfected cells (Flamand et al., 1991).

HHV6 is a virus belonging to the β-Herpesviridae family. This subfamily of viruses contains a total of three human herpesviruses. Alongside HHV6, this subfamily contains the cytomegalovirus (CMV) and HHV7. HHV6 and CMV are known to have many biological and molecular similarities. Interestingly, many studies have described CMV and its mechanisms, and have thereby established that CMV is able to interfere with TNF-α production. This, in combination with *In vitro* studies showing that HHV6 has the ability to upregulate TNF-α, corroborates the hypothesis that HHV6 can also upregulate TNF-α *in vivo*. A study including six patients, performed by Yoshikawa et al. further substantiates these claims when they concluded that the serum concentration of TNF-α was increased in all patients after infection with HHV6 (Yoshikawa et al., 2006) The patients used in this study were claimed to be healthy and not affected by underlying diseases apart from the HHV6 infection. This evidence, combined with earlier mentioned substantiated claims, contributes to the idea that HHV6 affects MS via TNF-α.

Ectopic lymphoid structures

The lymphatic system is a very important player in the immune system and specifically in the activation of the immune response. Throughout the human body, secondary lymphoid organs can be found in local tissues, hereby attached to lymphatic vessels. They play a role in the activation of the immune response and specifically recruit activated APCs and naïve lymphocytes. The construction of secondary lymphatic structures is a pre-programmed human system and does not require external activation. This, contrary to tertiary or ectopic lymphatic structures (ELS), requiring some form of inflammation in order to develop. These structures are hereby formed in almost all tissues, such as the meninges as can be seen in figure 2, and do not need to be near a lymphatic vessel. Although their function has not been as extensively studied, it

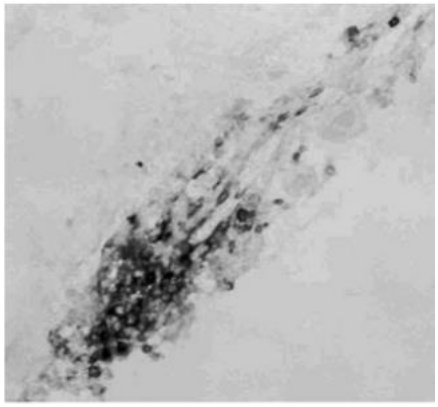

Figure 2 ***ELS in patient meninges*** *(Follicles containing B-cells, in the meninges of patients with SPMS (Serafini et al., 2004).)*

is believed that it is relatively similar to the function of secondary lymphoid structures (Carragher et al., 2008).

The formation of ELS, in the meninges of MS patients, is called neolymphogenesis. These ELS often form at sites of inflammation in tissues targeted by autoreactive immune cells. ELS are identified as a collection of B-lymphocytes and plasma cells and were found in 66 percent of MS patients diagnosed with SPMS (Serafini et al., 2004). It is believed that ELS can harbour latent viral infections such as EBV, and thereby contribute to the progression of the disease. A latent EBV infection, present in the ELS, can cause repeating infections that can trigger an inflammatory response (Lazibat et al., 2018). Meningeal inflammation, expected to be caused by an ELS associated latent infection, seems to be related to increased neurodegeneration and demyelination (Magliozzi et al., 2010). One study describes the hypothesis that EBV and HHV6 both play a role in the formation of ELS, and state that HHV6 causes the aggregation of lymphoid cells, leading to the initial formation of ELS (Eriksen, 2017). This possible link between ELS and HHV6 is very obscure. Furthermore, a single case study about drug reaction with eosinophilia and systemic symptoms (DRESS) syndrome describes the presence of HHV6 in secondary lymphoid structures and insinuates the feasibility that HHV6 is associated with the inflammation present (Mine et al., 2014). This is a farfetched, but only, indication thus far that HHV6 can induce a latent infection in ELS and thereby contribute to MS. Latent HHV6 infection in ELS might contribute to the pathogenesis of MS, but the role of latent viruses in general, is still unexplained.

Disputable correlation

As has been mentioned a couple of times already, the role of HHV6 in the development and progression of MS is very ambiguous. Although many believe there to be a role for this virus in MS, absolute certainty has not yet been achieved. Various reports show different prevalence rates of HHV6 in MS patients, ranging from 0 to 100 percent. Thereby, similar prevalence rates can often be found in patients with other neurological diseases or even in healthy individuals. Earlier, a large meta-analysis performed by Pormohammad et al., described the correlation between MS and HHV6. They determined that there was in fact a significant correlation. Other studies show contradictory results, reporting a 67 percent prevalence in MS patients, and a 60 percent prevalence in healthy individuals (Ablashi et al., 2000). Many studies contradict results of other MS related studies and a research group by Hon et al. was intrigued by the widespread of differences in prevalence and conducted a clinical analysis with 30 MS patients and compared their results with 30 healthy individuals that matched the age, sex and race of the patients. For the screening of HHV6, standard PCR was used. The results showed that none of the control group patients were infected with HHV6 and just 3 percent of the MS patient group. These data therefore suggest that HHV6 might not be a risk factor MS (Hon et al., 2014).

Another theory that is provided, disproving the role of HHV6, is the relationship between HHV6 and EBV. Both HHV6 and EBV can cause immune suppression and would be able to reactivate each other. It is thought that EBV plays a major role in MS, and can reactive latent HHV6. The mentioned upregulation and reactivation of HHV6 are hereby believed to be caused by EBV and only the reactivation by EBV explains therefore the increased concentrations of HHV6 (Eriksen, 2017). However, this hypothesis is up until thus far unproven.

Finally, the most important argument that is often mentioned, describing why HHV6 might not be a risk factor for MS, is the lack of evidence. Research on the link between HHV6 and MS has started at the beginning of the 20th century and yet most, if not all, evidence that substantiates the correlation is highly circumstantial. A clear example of this is the link between HHV6, TNF-α and MS. Meta studies conclude that an infection with HHV6 induces upregulation of TNF-α. Moreover, a higher concentration of TNF-α is found in patients diagnosed with MS. This evidence is often used as proof of

theory even though it is very presumptive. Direct evidence for the role of HHV6 and the mechanism(s) it utilizes is not present. Although *In vitro* studies and animal EAE models corroborate the theory to some extent, the main reason HHV6 is believed to have no influence in MS is the circumstantial and inconclusive evidence present nowadays.

Discussion

When reviewing the various studies presented in this article, it becomes clear that MS is a very complex disease with parts of its pathogenesis and aetiology that have yet to be discovered. Factors that influence the risk of acquiring MS, are genetics and environmental factors. It is highly debated whether these environmental factors include viral infections such as EBV and HHV6, although the role of EBV is more widely accepted than that of HHV6. There is much controversy noticeable between studies, attempting to substantiate different hypotheses on the relationship between MS and HHV6. Comparison of the different studies is often extremely difficult due to the variety of methods, the different patients in various stages of MS and the control groups that are used. Nevertheless, evidence supporting the theory that HHV6 is at least in some way engaged in the development of MS is seemingly undeniable. Meta-analysis often involve some form of PCR, whereby a specific primer has to be selected. The selection of different primers can explain the vast range of outcomes in these studies. Still, in spite of the fact that the outcome of meta-analysis studies might be debatable, other results are evident. Similarities in amino-acid sequences between MBP and U24 are clear evidence that substantiates the hypothesis. Moreover, the role of TNF-α in MS, in combination with *In vitro* upregulation of TNF-α after an infection is more fundamental proof of this concept.

Much of the controversy that is found might find its origin in the unknown. Since there are so many undetermined aspects of MS and the role environmental factors play in it, experimental results can be hard to interpret. Contradicting results can be influenced by mechanisms that have so far not been discovered. One example is a paper published in 2004, by Dietrich et al., which reported that HHV6 is able to infect primary human glial precursor cells directly. Infection of the cells lead to impairment of cell replication and alteration of the cell morphology. This is important since the glial precursor cells have an indirect role in the remyelination process. Regardless, research into this particular mechanism and the part it can possibly play in the development of MS has not been performed yet (Dietrich et al., 2004). Furthermore, most research is focused on one particular aspect of MS, or its origin. Often the role of HHV6 is observed unaccompanied, even though some studies have suggested some form of interplay between different viruses.

Overall, it seems arguable to say that HHV6 participates in some extent, to the development and progression of MS. However, gaining more insight in this entanglement of factors that play a role, is extremely important for the understanding and possible treatment of this disease. Therefore further research should aim to clarify the various possible mechanisms that HHV6 adopts. An important start is to examine the feasibility of coaction between numerous possible factors, such as the interplay between HHV6 and EBV. Moreover, it is clear that regulatory T-cells play an important role in MS, specifically in the progression. The effect of HHV6 on these regulatory T-cells has not been determined yet. It is therefore of great value to study this potential effect. Finally, it has been briefly mentioned that HHV6 can infect precursor glial cells. This could possibly have tremendous effects on the development of MS, and should definitely be further examined as well. So, although HHV6 plays at least some role in MS, further research is required to definitely state that HHV6 is a risk factor for the development of MS.

Afterword

The process of writing this thesis has been an extremely interesting and educative process. I am very grateful to have had the opportunity to further develop my scientific writing skills and acquaintance myself with the process of a literary research. Furthermore, I am pleased to have gained more insight into the very interesting and controversial topic that is MS.

I would hereby like to sincerely thank my thesis supervisor prof. dr. Ulrich Eisel of the University of Groningen, for his advice on the principles of writing a thesis and for the feedback that he has provided me with throughout the writing process. I am very grateful for his guidance throughout the process.

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References

Ablashi, D. ., Eastman, H. ., Owen, C. ., Roman, M. ., Friedman, J., Zabriskie, J. ., Peterson, D. ., Pearson, G. ., & Whitman, J. . (2000). Frequent HHV-6 reactivation in multiple sclerosis (MS) and chronic fatigue syndrome (CFS) patients. *Journal of Clinical Virology*, *16*(3), 179–191. https://doi.org/10.1016/S1386-6532(99)00079-7

Alberts, Johnsen, Lewis, Morgan, Raff, Roberts, & Walter. (2017). *Molecular biology of the cell* (6th ed.). Garland Science.

Baecher-Allan, C., Kaskow, B. J., & Weiner, H. L. (2018). Multiple Sclerosis: Mechanisms and Immunotherapy. In *Neuron* (Vol. 97, Issue 4, pp. 742–768). Cell Press. https://doi.org/10.1016/j.neuron.2018.01.021

Carragher, D. M., Rangel-Moreno, J., & Randall, T. D. (2008). Ectopic lymphoid tissues and local immunity. In *Seminars in Immunology* (Vol. 20, Issue 1, pp. 26–42). NIH Public Access. https://doi.org/10.1016/j.smim.2007.12.004

Cheng, W., Ma, Y., Gong, F., Hu, C., Qian, L., Huang, Q., Yu, Q., Zhang, J., Chen, S., Liu, Z., Chen, X., Zhou, T., & Zhang, D. (2012). Cross-reactivity of autoreactive T cells with MBP and viral antigens in patients with MS. *Frontiers in Bioscience*, *17*(5), 1648–1658. https://doi.org/10.2741/4010

Dargahi, N., Katsara, M., Tselios, T., Androutsou, M. E., De Courten, M., Matsoukas, J., & Apostolopoulos, V. (2017). Multiple sclerosis: Immunopathology and treatment update. In *Brain Sciences* (Vol. 7, Issue 7). MDPI AG. https://doi.org/10.3390/brainsci7070078

De Bolle, L., Naesens, L., & De Clercq, E. (2005). Update on human herpesvirus 6 biology, clinical features, and therapy. In *Clinical Microbiology Reviews* (Vol. 18, Issue 1, pp. 217–245). American Society for Microbiology (ASM). https://doi.org/10.1128/CMR.18.1.217-245.2005

Derfuss, T., Hohlfeld, R., & Meinl, E. (2005). Intrathecal antibody (IgG) production against human herpesvirus type 6 occurs in about 20% of multiple sclerosis patients and might be linked to a polyspecific B-cell response. *Journal of Neurology*, *252*(8), 968–971. https://doi.org/10.1007/s00415-005-0794-z

Dietrich, J., Blumberg, B. M., Roshal, M., Baker, J. V., Hurley, S. D., Mayer-Pröschel, M., & Mock, D. J. (2004). Infection with an endemic human herpesvirus disrupts critical glial precursor cell properties. *Journal of Neuroscience*, *24*(20), 4875–4883. https://doi.org/10.1523/JNEUROSCI.5584-03.2004

Doshi, A., & Chataway, J. (2016). Multiple sclerosis, a treatable disease. *Clinical Medicine*, *16*(6), 53–59.

Eriksen, W. (2017). The spread of EBV to ectopic lymphoid aggregates may be the final common pathway in the pathogenesis of ME/CFS. *Medical Hypotheses*, *102*, 8–15. https://doi.org/10.1016/j.mehy.2017.02.011

Fierz, W. (2017). Multiple sclerosis: an example of pathogenic viral interaction? In *Virology journal* (Vol. 14, Issue 1, p. 42). BioMed Central. https://doi.org/10.1186/s12985-017-0719-3

Fischer, R., Padutsch, T., Bracchi-Ricard, V., Murphy, K. L., Martinez, G. F., Delguercio, N., Elmer, N., Sendetski, M., Diem, R., Eisel, U. L. M., Smeyne, R. J., Kontermann, R. E., Pfizenmaier, K., & Bethea, J. R. (2019). Exogenous activation of tumor necrosis factor receptor 2 promotes recovery from sensory and motor disease in a model of multiple sclerosis. *Brain, Behavior, and Immunity*, *81*, 247–259. https://doi.org/10.1016/j.bbi.2019.06.021

Flamand, L., Gosselin,' Mario D'addario, J., Hiscott, J., Ablashi, D. V, Gallo, R. C., & Menezesl, J. (1991).

Human Herpesvirus 6 Induces Interleukin-l and Tumor Necrosis Factor Alpha, but Not Interleukin-6, in Peripheral Blood Mononuclear Cell Cultures. In *JOURNAL OF VIROLOGY* (Vol. 65, Issue 9).

Garg, N., & Smith, T. W. (2015). An update on immunopathogenesis, diagnosis, and treatment of multiple sclerosis. *Brain and Behavior*, *5*(9), 362. https://doi.org/10.1002/brb3.362

Ghasemi, N., Razavi, S., & Nikzad, E. (2016). Multiple Sclerosis: Pathogenesis, Symptoms, Diagnoses and Cell-Based Therapy. *Cell*, *19*(1), 1–10. https://doi.org/10.22074/cellj.2016.4867

Göbel, K., Ruck, T., & Meuth, S. G. (2018). Cytokine signaling in multiple sclerosis: Lost in translation. *Multiple Sclerosis (Houndmills, Basingstoke, England)*, *24*(4), 432–439. https://doi.org/10.1177/1352458518763094

Gonsette, R. E. (2012). Self-tolerance in multiple sclerosis. In *Acta Neurologica Belgica* (Vol. 112, Issue 2, pp. 133–140). Springer-Verlag Italia s.r.l. https://doi.org/10.1007/s13760-012-0061-x

Guan, Y., Jakimovski, D., Ramanathan, M., Weinstock-Guttman, B., & Zivadinov, R. (2019). The role of Epstein-Barr virus in multiple sclerosis: From molecular pathophysiology to in vivo imaging. In *Neural Regeneration Research* (Vol. 14, Issue 3, pp. 373–386). Wolters Kluwer Medknow Publications. https://doi.org/10.4103/1673-5374.245462

Håkansson, I. (2019). *Biomarkers and Disease Activity in Multiple Sclerosis : A cohort study on patients with clinically isolated syndrome and relapsing remitting multiple sclerosis* (Vol. 1697). Linköping University Electronic Press. https://doi.org/10.3384/diss.diva-160762

Harbo, H. F., Gold, R., & Tintora, M. (2013). Sex and gender issues in multiple sclerosis. *Therapeutic Advances in Neurological Disorders*, *6*(4), 237–248. https://doi.org/10.1177/1756285613488434

Hon, G. M., Erasmus, R. T., & Matsha, T. (2014). Low prevalence of human herpesvirus-6 and varicella zoster virus in blood of multiple sclerosis patients, irrespective of inflammatory status or disease progression. *Journal of Clinical Neuroscience*, *21*(8), 1437–1440. https://doi.org/10.1016/j.jocn.2013.10.027

Jaworska, J., Gravel, A., & Flamand, L. (2010). Divergent susceptibilities of human herpesvirus 6 variants to type I interferons. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(18), 8369–8374. https://doi.org/10.1073/pnas.0909951107

Katsara, M., & Apostolopoulos, V. (2018). Multiple Sclerosis: Pathogenesis and Therapeutics. *Medicinal Chemistry*, *14*(2), 104–105.

Kleinewietfeld, M., & Hafler, D. A. (2014). Regulatory T cells in autoimmune neuroinflammation. *Immunological Reviews*, *259*(1), 231–244. https://doi.org/10.1111/imr.12169

Lassmann, H., & van Horssen, J. (2011). The molecular basis of neurodegeneration in multiple sclerosis. *FEBS Letters*, *585*(23), 3715–3723. https://doi.org/10.1016/j.febslet.2011.08.004

Lazibat, I., Majdak, M. R., & Župani ć, S. (2018). Multiple sclerosis: New aspects of immunopathogenesis. In *Acta Clinica Croatica* (Vol. 57, Issue 2, pp. 352–361). Klinicka Bolnica Sestre Milosrdnice. https://doi.org/10.20471/acc.2018.57.02.17

Lock, C., Oksenberg, J., & Steinman, L. (1999). The role of TNFalpha and lymphotoxin in demyelinating disease. *Annals of the Rheumatic Diseases*, *58*(Supplement 1), i121–i128. https://doi.org/10.1136/ard.58.2008.i121

Loma, I., & Heyman, R. (2011). Multiple Sclerosis: Pathogenesis and Treatment. *Current Neuropharmacology*, *9*(3), 409–416. https://doi.org/10.2174/157015911796557911

Lublin, F. D., Reingold, S. C., Cohen, J. A., Cutter, G. R., Sørensen, P. S., Thompson, A. J., Wolinsky, J. S., Balcer, L. J., Banwell, B., Barkhof, F., Bebo, B., Calabresi, P. A., Clanet, M., Comi, G., Fox, R. J., Freedman, M. S., Goodman, A. D., Inglese, M., Kappos, L., … Polman, C. H. (2014). Defining the clinical course of multiple sclerosis: The 2013 revisions. In *Neurology* (Vol. 83, Issue 3, pp. 278–286). https://doi.org/10.1212/WNL.0000000000000560

Lusso, P., Garzino-Demo, A., Crowley, R. W., & Malnati, M. S. (1995). Infection of γ/δ T lymphocytes by human herpesvirus 6: Transcriptional induction of CD4 and susceptibility to HIV infection. *Journal of Experimental Medicine*, *181*(4), 1303–1310. https://doi.org/10.1084/jem.181.4.1303

Magliozzi, R., Howell, O. W., Reeves, C., Roncaroli, F., Nicholas, R., Serafini, B., Aloisi, F., & Reynolds, R. (2010). A Gradient of neuronal loss and meningeal inflammation in multiple sclerosis. *Annals of Neurology*, *68*(4), 477–493. https://doi.org/10.1002/ana.22230

Martin, R., Sospedra, M., Rosito, M., & Engelhardt, B. (2016). Current multiple sclerosis treatments have improved our understanding of MS autoimmune pathogenesis. In *European Journal of Immunology* (Vol. 46, Issue 9, pp. 2078–2090). https://doi.org/10.1002/eji.201646485

Minagar, A., & Alexander, J. S. (2003). Blood-brain barrier disruption in multiple sclerosis. *Multiple Sclerosis Journal*, *9*(6), 540–549. https://doi.org/10.1191/1352458503ms965oa

Mine, S., Suzuki, K., Sato, Y., Fukumoto, H., Kataoka, M., Inoue, N., Ohbayashi, C., Hasegawa, H., Sata, T., Fukayama, M., & Katano, H. (2014). Evidence for human herpesvirus-6B infection of regulatory T-cells in acute systemic lymphadenitis in an immunocompetent adult with the drug reaction with eosinophilia and systemic symptoms syndrome: A case report. *Journal of Clinical Virology*, *61*(3), 448–452. https://doi.org/10.1016/j.jcv.2014.08.025

Mori, Y., Yang, X., Akkapaiboon, P., Okuno, T., & Yamanishi, K. (2003). Human Herpesvirus 6 Variant A Glycoprotein H-Glycoprotein L-Glycoprotein Q Complex Associates with Human CD46. *Journal of Virology*, *77*(8), 4992–4999. https://doi.org/10.1128/jvi.77.8.4992-4999.2003

Pelanda, R., Schwers, S., Sonoda, E., Torres, R. M., Nemazee, D., & Rajewsky, K. (1997). Receptor editing in a transgenic mouse model: Site, efficiency, and role in B cell tolerance and antibody diversification. *Immunity*, *7*(6), 765–775. https://doi.org/10.1016/S1074-7613(00)80395-7

Pormohammad, A., Falah, F., & Faghihloo, E. (2017). *Relationship of human herpes virus 6 and multiple sclerosis: A systematic review and meta-analysis*. https://doi.org/10.1002/jcp.26000

Qi, Q., Liu, Y., Cheng, Y., Glanville, J., Zhang, D., Lee, J.-Y., Olshen, R. A., Weyand, C. M., Boyd, S. D., & Goronzy, J. J. (2014). Diversity and clonal selection in the human T-cell repertoire. *Proceedings of the National Academy of Sciences*, *111*(36), 13139–13144. https://doi.org/10.1073/pnas.1409155111

Rashid, T., & Ebringer, A. (2012). Autoimmunity in Rheumatic Diseases Is Induced by Microbial Infections via Crossreactivity or Molecular Mimicry. *Autoimmune Diseases*, *2012*, 1–9. https://doi.org/10.1155/2012/539282

Ribeiro, C. M., Sayonara, ·, Oliveira, R., Alfieri, D. F., Flauzino, T., Damacio, ·, Kaimen-Maciel, R., Andréa, ·, Simão, N. C., Maes, M., Maria, E., & Reiche, V. (2019). Tumor necrosis factor alpha (TNF-α) and its soluble receptors are associated with disability, disability progression and clinical forms of multiple sclerosis. *Inflammation Research*, *68*, 1049–1059. https://doi.org/10.1007/s00011-019-01286-0

Rojas, M., Restrepo-Jiménez, P., Monsalve, D. M., Pacheco, Y., Acosta-Ampudia, Y., Ramírez-Santana, C., Leung, P. S. C., Ansari, A. A., Gershwin, M. E., & Anaya, J. M. (2018). Molecular mimicry and autoimmunity. In *Journal of Autoimmunity* (Vol. 95, pp. 100–123). Academic Press.

https://doi.org/10.1016/j.jaut.2018.10.012

Rolak, L. A. (2003). Multiple sclerosis: it's not the disease you thought it was. In *Clinical medicine & research* (Vol. 1, Issue 1, pp. 57–60). Marshfield Clinic. https://doi.org/10.3121/cmr.1.1.57

Rosenblum, M. D., Remedios, K. A., & Abbas, A. K. (2015). Mechanisms of human autoimmunity. *Journal of Clinical Investigation*, *125*(6), 2228–2233. https://doi.org/10.1172/JCI78088

Serafini, B., Rosicarelli, B., Magliozzi, R., Stigliano, E., & Aloisi, F. (2004). Detection of Ectopic B-cell Follicles with Germinal Centers in the Meninges of Patients with Secondary Progressive Multiple Sclerosis. *Brain Pathology*, *14*(2), 164–174. https://doi.org/10.1111/j.1750-3639.2004.tb00049.x

Shoenfeld, Y., Luigi, P., Gershwin, M., & Gershwin, E. (2014). *Polylysine - an overview | ScienceDirect Topics* (3rd ed.). https://www.sciencedirect.com/topics/medicine-and-dentistry/molecular-mimicry

Sospedra, M., & Martin, R. (2016). Immunology of Multiple Sclerosis. *Semin Neurol*, *36*, 115–127. https://doi.org/10.1055/s-0036-1579739

Steeland, S., Van Ryckeghem, S., Van Imschoot, G., De Rycke, R., Toussaint, W., Vanhoutte, L., Vanhove, C., De Vos, F., Vandenbroucke, R. E., & Libert, C. (2017). TNFR1 inhibition with a Nanobody protects against EAE development in mice. *Scientific Reports*, *7*(1), 1–17. https://doi.org/10.1038/s41598-017-13984-y

Sultanova, A., Cistjakovs, M., Gravelsina, S., Chapenko, S., Roga, S., Cunskis, E., Nora-Krukle, Z., Groma, V., Ventina, I., & Murovska, M. (2017). Association of active human herpesvirus-6 (HHV-6) infection with autoimmune thyroid gland diseases. *Clinical Microbiology and Infection*, *23*(1), 50.e1-50.e5. https://doi.org/10.1016/j.cmi.2016.09.023

Tejada-Simon, M. V, Zang, Y. C. Q., Hong, J., Rivera, V. M., & Zhang, J. Z. (2003). Cross-Reactivity with Myelin Basic Protein and Human Herpesvirus-6 in Multiple Sclerosis. In *Ann Neurol* (Vol. 53).

Tengvall, K., Huang, J., Hellström, C., Kammer, P., Biström, M., Ayoglu, B., Bomfim, I. L., Stridh, P., Butt, J., Brenner, N., Michel, A., Lundberg, K., Padyukov, L., Lundberg, I. E., Svenungsson, E., Ernberg, I., Olafsson, S., Dilthey, A. T., Hillert, J., … Kockum, I. (2019). Molecular mimicry between Anoctamin 2 and Epstein-Barr virus nuclear antigen 1 associates with multiple sclerosis risk. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(34), 16955–16960. https://doi.org/10.1073/pnas.1902623116

Virtanen, J. O., & Jacobson, S. (2012). Viruses and Multiple Sclerosis. *CNS Neurol Disord Drug Targets*.

Wallin, M. T., Culpepper, W. J., Nichols, E., Bhutta, Z. A., Gebrehiwot, T. T., Hay, S. I., Khalil, I. A., Krohn, K. J., Liang, X., Naghavi, M., Mokdad, A. H., Nixon, M. R., Reiner, R. C., Sartorius, B., Smith, M., Topor-Madry, R., Werdecker, A., Vos, T., Feigin, V. L., & Murray, C. J. L. (2019). Global, regional, and national burden of multiple sclerosis 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *The Lancet Neurology*, *18*(3), 269–285. https://doi.org/10.1016/S1474-4422(18)30443-5

Wuest, S. C., Mexhitaj, I., Chai, N. R., Romm, E., Scheffel, J., Xu, B., Lane, K., Wu, T., & Bielekova, B. (2014). A complex role of herpes viruses in the disease process of multiple sclerosis. *PLoS ONE*, *9*(8). https://doi.org/10.1371/journal.pone.0105434

Yoshikawa, T., Fujita, A., Yagami, A., Suzuki, K., Matsunaga, K., Ihira, M., & Asano, Y. (2006). Human herpesvirus 6 reactivation and inflammatory cytokine production in patients with drug-induced hypersensitivity syndrome. *Journal of Clinical Virology : The Official Publication of the Pan American Society for Clinical Virology*, *37 Suppl 1*.