

Crossing Barriers: How JC Virus Crosses the Blood Brain Barrier and Induces Progressive Multifocal Leukoencephalopathy in Patients on Disease-Modifying Therapies.

Maria-Elizabeth Baeva^a

^aDepartment of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY	69
INTRODUCTION	69
RESEARCH QUESTIONS	70
PROJECT NARRATIVE	
What are the genetic susceptibilities of JCV-induced PML?.....	70
How does JCV cross the blood brain barrier?.....	72
How do monoclonal antibody drugs aid in JCV reactivation?.....	73
SUMMARY & CONCLUSION	74
ACKNOWLEDGEMENTS	74
REFERENCES	74

SUMMARY Progressive multifocal leukoencephalopathy (PML) is a fatal demyelinating disease caused by the John Cunningham virus (JCV). While it is known to cause lytic infection in oligodendrocytes, many questions remain as to how this virus remains asymptomatic and dormant during initial infection throughout childhood and throughout most of the population's adulthood. JCV becomes neurotropic under very specific conditions such as autoimmune disease and the use of disease-modifying therapies, which are commonly monoclonal antibody drugs designed to suppress some part of the adaptive immune system. This paper addresses how JCV crosses the blood brain barrier, what host and viral genetics enable neurotropism and virulence and how monoclonal antibody drugs aid in the reactivation of the virus. Based on the current literature, the rearrangement of the non-coding control region and VP1 capsid protein are key determinants of whether the virus becomes neurotropic. Host immune factors such as permissive alleles and overexpression of Spi-B, a ubiquitous transcription factor, can also lead to immune evasion and increased viral replication by JCV. A model is proposed wherein JCV use B cells as a "Trojan Horse" to cross the blood brain barrier. Co-infection with Epstein-Barr virus (EBV) can result in B cell fusion with oligodendrocytes and transmission of JCV. Finally, disease-modifying therapies play a role by primarily suppressing the immune system and upregulating the migration of leukocytes, allowing the mobilization of JCV-infected cells to the blood brain barrier. Future studies should further elucidate the roles that Spi-B, hematopoietic stem cells and EBV co-infection play in transmission, pathogenesis, and genetic rearrangement of JCV.

INTRODUCTION

Progressive multifocal leukoencephalopathy (PML) is a demyelinating neurological disease and is characterized by rapid damage to the white matter of the brain [1]. PML is fatal in up to 50% of cases and survivors are left with severe neurological disability [2].

Accepted: 23 July 2018

Published: 01 August 2018

Citation: Baeva ME. 2018. Crossing Barriers: How JC Virus Crosses the Blood Brain Barrier and Induces Progressive Multifocal Leukoencephalopathy in Patients on Disease-Modifying Therapies. JEMI PEARLS 3:69-76

Editor: François Jean, University of British Columbia

Copyright: © 2018 Journal of Experimental Microbiology and Immunology. All Rights Reserved.

Address correspondence to Maria-Elizabeth Baeva mariaelizabeth.baeva@gmail.com

There is currently no cure for PML [2]. It has been known for over four decades that PML is caused by John Cunningham virus (JCV), a common and asymptomatic virus that over half of the population is infected with during childhood resulting in a chronic but benign infection [1]. The virus seems to primarily infect kidneys [3, 4], but can also infect tonsils [5, 6], B cells [5, 7], oligodendrocytes [8] and hematopoietic stem cells (HPSC) [5, 9]. The virus causes PML by infecting oligodendrocytes, the main myelinating cells of the central nervous system [8]. Once the oligodendrocytes are infected, the JCV T-antigen interacts with the oligodendrocyte myelin gene transcription factor, MEF-1/Puro, and this induces the lytic stage of the infection and the virus can infect nearby oligodendrocytes [10]. Without myelination, the neurons quickly degenerate. It is still unknown exactly how viral transmission occurs, although current hypotheses suggest it is transmitted via the oral route from parent to child [11]. Estimates suggest that 75-80% of the population worldwide are infected with this virus [1]. Once considered a rare disease, incidences of PML are now increasing [1]. This is in part due to the increased risk of JCV reactivation when patients with autoimmune disorders such as HIV [1], rheumatoid arthritis [9] and multiple sclerosis (MS) [12] are treated with disease-modifying therapies (DMT) [1]. The most common DMTs associated with PML are monoclonal antibodies such as rituximab [13, 14] and natalizumab [15, 16], which are designed to suppress parts of the adaptive immune system.

RESEARCH QUESTIONS

This paper will address what is currently known about the interactions between JCV, DMTs and PML and propose several models to explain this relationship. First, the genetics of both the host and the virus will be examined to determine whether mutations in the virus or genetic susceptibilities of the host increase risk of PML. Next, the question of how JCV crosses the blood brain barrier will be explored. Finally, how monoclonal antibodies such as rituximab and natalizumab reactivate JCV and allow the virus to become neurotropic will be addressed. Future directions will also be considered to suggest new areas of research with regards to JCV and PML. The main goal is to provide a hypothesis for how this common, asymptomatic virus can cause a destructive disease such as PML.

PROJECT NARRATIVE

What are the genetic susceptibilities of JCV-induced PML?

The Virus JCV is a double-stranded, circular DNA virus [17] in which the genome is divergently transcribed with the non-coding control region (NCCR) acting as a promoter-binding site in between the two regions [18]. First, early proteins (large T antigen, small T antigen, and T' antigen) are transcribed and are involved in transcription and replication. Late proteins (agnoprotein and three viral capsid proteins, VP1, VP2, and VP3) are later transcribed and are used to exit the nucleus and build the capsid [18].

Two forms of JCV exist: archetypal JCV which infects kidneys and is asymptomatic, and neurotropic JCV which is found in PML patients. This could suggest that two separate infections occur, however it is more likely that intra-patient mutations are occurring [19]. Neurotropic and archetypal JCV are both found in B cells and in HPSCs, suggesting these cells are the site of genome rearrangement [9, 20].

The two major areas of interest with regards to the virus genome have been the VP1 protein and the NCCR. The VP1 capsid protein mediates the tropism of the virus. Archetypal JCV can bind to renal proximal tubular epithelial cells, B cells, T cells and peripheral blood mononuclear cells [19]. Mutations in the capsid gene can induce changes in cell tropism such as losing the ability to bind to sialylated molecules and to a variety of peripheral cell types [19]. However, it retains the ability to bind central nervous system glial cells. Therefore, it does not seem that rearrangement of VP1 causes the JCV to necessarily gain the ability to bind to oligodendrocytes, rather it loses the ability to bind to all other types of cells.

The Host The biggest change in the genome between the archetypal and neurotropic JCV is in the NCCR. JCV isolated from PML patients shows rearranged NCCRs (rr-NCCR) compared to archetypal JCV. While almost all cases have a deletion in the D segment and involve tandem repeats, the overall changes in sequences seem to be unique to each patient [21], presenting a challenge as to creating a therapeutic that could block this key region. As is

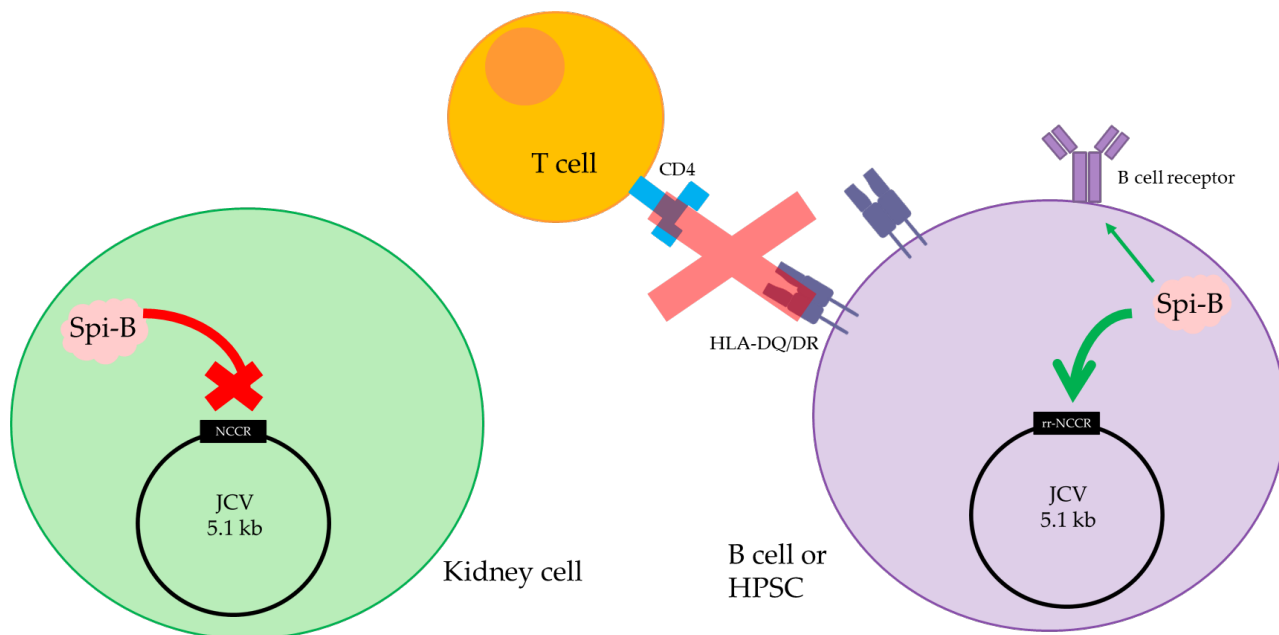


FIG. 1 Model for the sequestration of Spi-B and immune system evasion by neurotropic JCV. Inside kidney cells, Spi-B is unable to bind to the archetypal NCCR region on the JCV. In B cells or hematopoietic stem cells, Spi-B has increased expression and is sequestered to the JCV by re-arranged (rr-) NCCR. This retards the B cell receptor development and hinders the immune system's ability to respond to viral pathogens. As antigen-presenting cells, B cells with permissive MHCs to JCV such as DRB1*13, -DQA1*01:03, -DQB1*06:03 prevent the presentation of JCV to T cells, allowing further immune evasion.

predictable, the host genetics may also influence JCV pathogenesis and the likelihood of developing PML. Interestingly, to the knowledge of this author, there have been no studies looking at major histocompatibility complex (MHC) I or CD8⁺ T cell specific genetic defects with regards to JCV infection. These are crucial components of the Type 1 immune response to pathogens. MHC I proteins are found on nearly all cells and are used to present intracellular pathogen antigens, such as viruses, to immune cells such as CD8⁺ T cells. Those T cells will then recognize the pathogen and eliminate the infected cell. This presents a gap in the current knowledge of JCV immunity as the Type 1 response is a vital aspect of how the immune system eliminates viruses, and it is known that CD8⁺ T cells can be activated in response to JCV [22]. However, there has been research investigating genetic polymorphisms of MHC II molecules and their role in JCV pathogenesis. These molecules are important as they are key to the role of antigen-presenting cells, which allows the adaptive immune system to create specific antibodies to the pathogen. It has been noted that certain MHC II alleles offer a protective effect against JCV, as was determined by measuring the production of anti-JCV antibodies. These include HLA-DRB1*15, -DQA1*01:02, -DQB1*06:02 [23]. Interestingly, these are dominant alleles. Alleles that are permissive to JCV have also been identified, mainly HLA-DRB1*13, -DQA1*01:03, -DQB1*06:03 [23]. In contrast to the protective alleles, these ones are additive.

Spi-B is a host transcription factor associated with RNA Polymerase that has garnered interest with regards to JCV. This transcription factor seems to be ubiquitously expressed, but it is highly expressed in immune cells [24]. Specifically, Spi-B seems to be involved in the development of the B cell receptor [24]. Archetypal JCV NCCR cannot bind Spi-B but the neurotropic rr-NCCR can [24]. Spi-B binding seems to increase viral replication. Additionally, Spi-B protein expression is upregulated 2-fold in CD19⁺ B cells in some patients on natalizumab and 100-fold in CD34⁺ hematopoietic precursors of patients on natalizumab [24]. Spi-B-binding JCV has been found in patients on other DMTs such as rituximab and highly active antiretroviral therapy [24].

Figure 1 presents a model outlining how the virus avoids immune detections via genetic susceptibilities in the host and viral rearrangements of the NCCR. When patients have genetically susceptible MHC II alleles, antigen-presenting B cells are unable to effectively present JCV on their MHC II molecules to elicit an immune response. Furthermore, in kidney

cells JCV is archetypical with a non-rearranged NCCR and is thus unable to bind to Spi-B, but in B cells and its precursors, the JCV rr-NCCR can bind and sequester the Spi-B, increasing viral replication. This will also have the synergistic effect of decreasing B cell receptor development and thereby again circumventing the immune system. This presents a potential model for how the host and viral genetics play a role in immune evasion of the virus.

How does JCV cross the blood brain barrier?

The primary site of JCV infection is thought to be the kidneys, as evidenced by anti-JCV antibodies being found in urine of children and adults without PML [25, 26]. The question has remained as to how JCV becomes neurotropic and infects the oligodendrocytes. Before infecting the central nervous system, JCV must cross the blood brain barrier. Since PML seems to occur in patients with autoimmune disorders, this could suggest that inflammatory cells and cytokines play a role in the increased permeability of the blood brain barrier [27]. It is known that blood brain barrier permeability increases in patients with MS [28, 29]. This may be related to why PML seems to occur primarily in patients with autoimmune disorders, as an increasingly permeable blood brain barrier could be more permissive to viral infections.

There are two models for how JCV most likely crosses this permeable barrier. The first suggests that free-circulating JCV passes endothelial cells lining the blood brain barrier transcellularly and subsequently bind to oligodendrocytes. This is supported by experiments demonstrating *in vitro* infection and replication of archetypal JCV in human brain microvascular endothelial cells [30]. However, this study used archetypal JCV and would suggest that some rearrangement of the JCV genome must occur just after crossing the blood brain barrier that enables them to enter and replicate inside the oligodendrocytes. The second hypothesis, which is becoming more widely accepted, is that JCV-infected B cells cross the blood brain barrier paracellularly via a “Trojan Horse” strategy and JCV are then able to escape and infect oligodendrocytes [7]. The current consensus regarding JCV infection supports this second theory as it has been shown that JCV can infect and replicate inside B cells and that JCV-infected B cells are able to transmit infection to naïve glial cells [20]. Therefore, this is the theory my model is based on.

At this point one more question remains, how would the typically dormant JCV exit the B cell, enter the oligodendrocyte and become lytic? A proposed explanation may not require

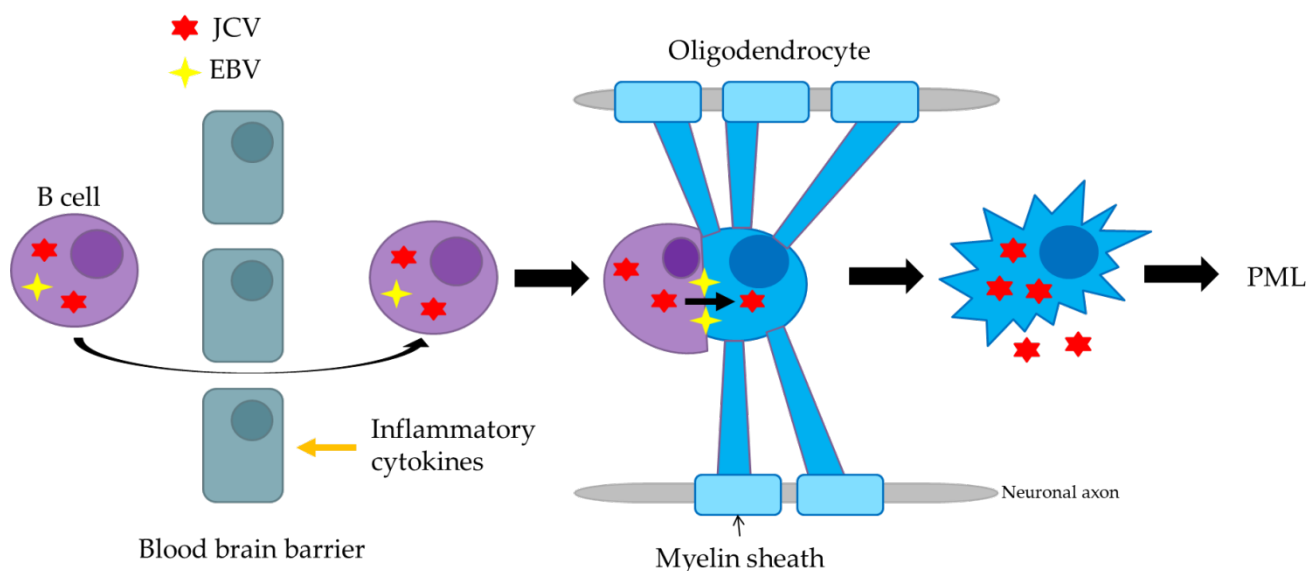


FIG. 2 Model for how JCV uses B cells to cross the blood brain barrier and infect oligodendrocytes. B cells co-infected with JCV and EBV cross the disrupted blood brain barrier. The permeability of the blood brain barrier can be increased due autoimmune diseases or disease-modifying therapies. Once the B cell has crossed the membrane, the EBV causes a fusion of the B cell to the oligodendrocyte. The JCV is able to cross into the oligodendrocyte, where due to its neurotropism, it enters a lytic state of infection. The death of oligodendrocytes causes the release of viable JCV able to bind and enter other oligodendrocytes and continue replication. The destruction of myelinating cells eventually induces PML.

the virus to exit the cell at all and involves co-infection with Epstein-Barr virus (EBV). EBV is another common, asymptomatic virus that infects B cells [31] and is implicated in MS pathogenesis [32, 33] and disease progression of rheumatoid arthritis [34]. An *in vitro* study demonstrated that EBV-transformed B cells are able to transmit archetypal JCV to primary human fetal glial cells [7]. Recent studies have also demonstrated the ability of EBV and JCV to undergo recombination and this recombined genome has been found in PML patients [35]. EBV has also been shown to cause cell fusion [36] and induce transfer of genetic material via cell-cell contact [37]. While this could be an interesting mechanism, further studies will need to be done to conclusively determine the role EBV plays in JCV pathogenesis.

My model (Fig. 2) proposes how all these findings can be used to describe entry of JCV into the blood brain barrier and infection of oligodendrocyte. First, an existing autoimmune dysfunction produces inflammatory cytokines and chemoattractants, which act on the blood brain barrier and induce disruption and permeability. Circulating B cells, which are co-infected with JCV and EBV, are then able to cross the blood brain barrier. EBV then causes a fusion of the B cell with the oligodendrocytes, allowing the neurotropic JCV to enter the oligodendrocytes. JCV will now establish a lytic infection and destroy the host cell. The death of oligodendrocytes prevents myelination of the neurons, causing PML.

How do monoclonal antibody drugs aid in JCV reactivation?

Two drugs most commonly associated with PML are natalizumab (Tysabri®) and rituximab (Rituxan®). Interestingly, the mechanism of action of each drug is quite different. Natalizumab are anti- $\alpha 4$ -integrin antibodies [38]. $\alpha 4$ -integrin are transmembrane receptors that bind to fibronectin and VCAM1 and are present on activated leukocytes [39]. The purpose of this antibody is to reduce the migration of pro-inflammatory leukocytes into the central nervous system through the blood brain barrier [38]. However, natalizumab has other effects on the immune system. Notably, there is an increase in CXCR3 on B cells [40], a receptor implicated in leukocyte migration. Natalizumab also causes an increase in expression of Spi-B in B cells [24] and an increase in migration of CD34+ progenitor cells (including HPSCs) to the peripheral blood [41]. This evidence suggests that natalizumab, while minimizing the binding of lymphocytes to the blood brain barrier, can increase the presence of JCV-carrying immune cells (such as B cells and HPSCs) in the peripheral blood. This can increase the chance that neurotropic JCV in one of those cells crosses the blood brain barrier.

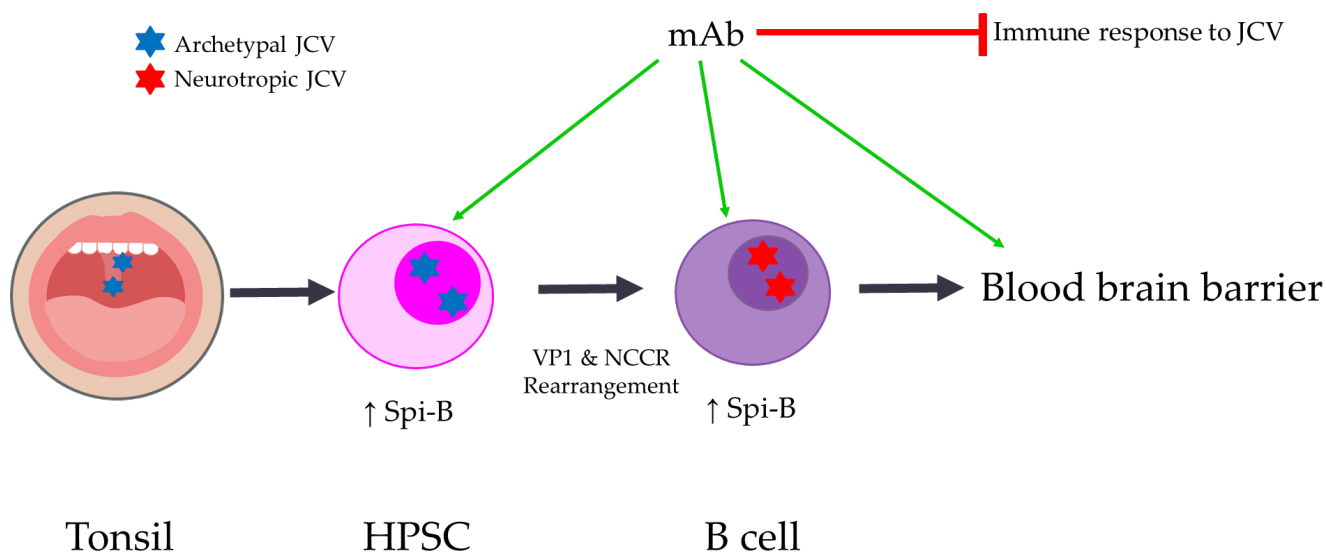


FIG. 3 Model for JCV infection of hematopoietic stem cells (HPSC), B cell precursors. Initial JCV infections occurs by archetypal virus. Inside the HPSC, the virus lays dormant until monoclonal antibodies are introduced into the host. The monoclonal antibodies (mAb) act on all aspects of the immune system, including increasing Spi-B expression, increasing immune cell migration into the periphery and suppressing the immune response to the virus. As the HPSC differentiates into the B cell, VP1 and NCCR rearrangement occurs and JCV becomes neurotropic but remains dormant. The B cell, now infected with neurotropic JCV, travels to the blood brain barrier.

On the other hand, rituximab is an anti-CD20 antibody [42]. CD20 is a receptor primarily located on maturing and activated B cells. As expected, this antibody decreases the expression of B cell receptor and can induce apoptosis in the B cells [43]. However, other immune side effects can occur such as induction of T regulatory lymphocytes and an increase in naïve and immature B cells [20]. This skewing of the immune system towards an immunosuppressive and anti-inflammatory state, especially with B cells unable to produce a strong humoral response to the virus, could be allowing JCV to become reactivated.

The proposed model for how JCV enters the immune system is summarized in Figure 3. This model suggests that archetypal JCV enters the tonsils and travels around the lymphatic circularity system. Eventually, JCV infect HPSCs and lays dormant. This part of the hypothesis is controversial, as there have been some studies that could not detect JCV DNA in MS patients on natalizumab or in PML patients [44], but there are other studies that have [45, 9]. Once the patient begins to express an autoimmune disorder and takes drugs which can further imbalance the immune system, several events occur. Spi-B expression and the circulation of progenitor immune cells in the peripheral blood are increased. At some point, the VP1 and NCCR undergo rearrangement in which some JCV are mutated and become neurotropic. The increased circulation of immune cells in the peripheral blood will eventually bring the infected cells to the blood brain barrier and thus the virus is able to cross the blood brain barrier via the mechanism explained in Figure 2. In addition, the immune-inhibitory nature of the monoclonal antibodies prevents the immune system from effectively recognizing and eliminating the virus.

SUMMARY AND FUTURE DIRECTIONS

The purpose of this paper was to hypothesize a big picture model for how JCV infects immune cells, avoids detection by the immune system and eventually crosses the blood brain barrier to infect oligodendrocytes. The evidence suggests that infection of B cells or HPSCs allows the virus to lay dormant in infected individuals. When monoclonal antibody drugs are taken that decrease mature B cell population and affect peripheral migration of immature immune cells, the increase in Spi-B causes a rearrangement of the NCCR followed by VP1 in the JCV genome. Several genetic factors increase the chance of JCV into developing into a neurotropic virus. The genetic predisposition of certain MHC alleles to be permissive to JCV, especially in patients with autoimmune diseases such as rheumatoid arthritis and MS, prevents the virus from being effectively contained. Once the HPSC matures into a B cell, the carrier B cell crosses the blood brain barrier. EBV co-infection seems to allow the B cells to fuse with oligodendrocytes, allowing the now neurotropic virus to establish lytic infection.

Based on this model, JCV-induced PML seems to require immune dysregulation through a combination of genetic defects of MHCs and immune disorders, infection of the lymphoid cells or lymphoid precursors, the rearrangement of VP1 and NCCR of JCV and disease-modifying therapies with B cell effects. Future studies will need to investigate MHC I genetic predisposition to JCV infection, analyze the JCV sequence in HPSCs at various stages of differentiation and maturity and conclusively determine whether HPSCs can act as the latent reservoir. Other studies should focus on elucidating the mechanism for the upregulation of Spi-B in patients on disease-modifying therapies and how this plays into NCCR rearrangement. The mechanism for NCCR rearrangement is a particularly large gap into how archetypal JCV becomes neurotropic. Finally, analysis of EBV and JCV coinfection in B cells and oligodendrocytes found in brain biopsy of PML patients should be performed to determine how JCV enters oligodendrocytes and whether any other mechanism exists that could explain how JCV exits the B cell and infects oligodendrocytes.

ACKNOWLEDGEMENTS

I would like to thank Dr. Francois Jean for his contributions and advice regarding this research. I would also like to thank my colleagues in MICB 406, specifically Shivani Mysuria, for their critiques and suggestions as well.

REFERENCES

1. Saribaş AS, Özdemir A, Lam C, Safak M. JC virus-induced Progressive Multifocal Leukoencephalopathy, *Future Virology* 2010, 5, 13–323. doi: 10.2217/fvl.10.12

2. Progressive Multifocal Leukoencephalopathy (PML). [Internet] Multiple Sclerosis Society of Canada. Retrieved from <https://mssociety.ca/managing-ms/treatments/medications/disease-modifying-therapies-dmts/progressive-multifocal-leukoencephalopathy-pml>. Cited on 25 Feb 2018.
3. Cornelissen CN, Harvey RA, Fisher BD. (2012) "X. Opportunistic Infections of HIV: JC Virus (JCV)". Microbiology. 3rd edition, Lippincott Williams & Wilkins. p. 389. ISBN 978-1-60831-733.
4. DuShane JK, Wilczek MP, Mayberry CL, Maginnis MS. ERK is a Critical Regulator of JC Polyomavirus Infection. *Journal of Virology*, 2018. doi: 10.1128/JVI.01529-17.
5. Monaco MC, Atwood WJ, Gravell M, Tornatore CS, Major EO. JC virus infection of hematopoietic progenitor cells, primary B lymphocytes, and tonsillar stromal cells: implications for viral latency. *Journal of Virology*, 2016, 70, 7004–7012.
6. Monaco MCG, Jensen PN, Hou J, Durham LC, Major EO. Detection of JC Virus DNA in Human Tonsil Tissue: Evidence for Site of Initial Viral Infection. *Journal of Virology*, 1998, 72, 9918–9923.
7. Chapagain ML, Nerurkar VR. JCV infection of human B lymphocytes: A possible mechanism for JCV transmigration across the blood-brain barrier. *Journal of Infectious Diseases*, 2010, 202, 184–191. doi: 10.1086/653823
8. Cavanagh JB, Greenbaum D, Marshall AH, Rubinstein LJ. Cerebral demyelination associated with disorders of the reticuloendothelial system. *The Lancet*, 1959, 274, 524-529.
9. Marzocchetti A, Wuthrich C, Tan CS, Tompkins T, Bernal-Cano F, Bhargava P, Ropper AH, Korálnik IJ. Rearrangement of the JCV regulatory region sequence in the bone marrow of a patient with Rheumatoid Arthritis and Progressive Multifocal Leukoencephalopathy. *Journal of NeuroVirology*, 2008,14, 455–458. doi: 10.1080/13550280802356837
10. Tretiakova A, Otte J, Croul SE, Kim JH, Johnson EM, Amini S, Khalili K. Association of JC Virus Large T Antigen with Myelin Basic Protein Transcription Factor (MEF-1/Purα) in Hypomyelinated Brains of Mice Transgenically Expressing T Antigen. *Journal of Virology*, 1999, 73, 6076–6084.
11. Berger JR, Miller CS, Mootoor Y, Avdiushko SA, Kryscio RJ, Zhu H. JC Virus Detection in Bodily Fluids: Clues to Transmission. *Clinical Infectious Diseases*, 2006, 43, e9–e12. doi: 10.1086/504947
12. Mills EA, Mao-Draayer Y. Understanding Progressive Multifocal Leukoencephalopathy Risk in Multiple Sclerosis Patients Treated with Immunomodulatory Therapies: A Bird’s Eye View. *Frontiers in Immunology*, 2018, 9, 138. doi: 10.3389/fimmu.2018.00138.
13. Clifford DB, Ances B, Costello C, Rosen-Schmidt S, Andersson M, Parks D, Perry A, Yerra R, Schmidt R, Alvarez E, Tyler KL. Rituximab-Associated Progressive Multifocal Leukoencephalopathy in Rheumatoid Arthritis. *Archives of Neurology*, 2011, 68, 1156–1164.
14. Sikkema T, Schuiling WJ, Hoogendoorn M. Progressive multifocal leukoencephalopathy during treatment with rituximab and CHOP chemotherapy in a patient with a diffuse large B-cell lymphoma. *BMJ Case Reports*, 2013. doi: 10.1136/bcr-2012-008142.
15. McGuigan C, Craner M, Guadagno J, Kapoor R, Mazibrada G, Molyneux P, Nicholas R, Palace J, Pearson OR, Rog D, Young CA. Stratification and monitoring of natalizumab-associated progressive multifocal leukoencephalopathy risk: recommendations from an expert group. *Journal of Neurology, Neurosurgery, and Psychiatry*, 2016, 87, 117–125. doi: 10.1136/jnnp-2015-311100.
16. Perkins MR, Ryschkewitsch C, Liebner JC, Monaco MCG, Himelfarb D, Ireland S, Roque A, Edward HL, Jensen PN, Remington G, Abraham T, Abraham J, Greenberg B, Kaufman C, LaGanke C, Monson NL, Xu X, Frohman E, Major EO, Douek DC. Changes in JC Virus-Specific T Cell Responses during Natalizumab Treatment and in Natalizumab-Associated Progressive Multifocal Leukoencephalopathy. *PLoS Pathogens*, 2012, 8, e1003014. doi: 10.1371/journal.ppat.1003014.
17. White MK, Bellizzi A, Ibba G, Pietropaolo V, Palamara AT, Wollebo HS. The DNA damage response promotes polyomavirus JC infection by nucleus to cytoplasm NF- kappaB activation. *Virology Journal*, 2017, 14, 31. doi: 10.1186/s12985-017-0707-7
18. Safak M, Barrucco R, Darbinyan A, Okada Y, Nagashima K, Khalili K. Interaction of JC Virus Agno Protein with T Antigen Modulates Transcription and Replication of the Viral Genome in Glial Cells. *Journal of Virology*, 2001, 75, 1476–1486. doi: 10.1128/JVI.75.3.1476–1486.2001
19. Gorelik L, Reid C, Testa M, Brickelmaier M, Bossolasco S, Pazzi A, Bestetti A, Carmillo P, Wilson E, McAuliffe M, Tonkin C, Carulli JP, Lugovskoy A, Lazzarin A, Sunyaev S, Simon K, Cinque P. Progressive Multifocal Leukoencephalopathy (PML) Development Is Associated With Mutations in JC Virus Capsid Protein VP1 That Change Its Receptor Specificity. *Journal of Infectious Disease*, 2011, 204, 103–114. doi: 10.1093/infdis/jir198
20. Durali D, de Goër de Herve MG, Gasnault J, Taoufik Y. B Cells and Progressive Multifocal Leukoencephalopathy: Search for the Missing Link. *Frontiers in Immunology*, 2015, 6, 241.
21. Gosert R, Kardas P, Major EO, Hirsch HH. Rearranged JC Virus Noncoding Control Regions Found in Progressive Multifocal Leukoencephalopathy Patient Samples Increase Virus Early Gene Expression and Replication Rat. *Journal of Virology*, 2010, 84, 10448–10456. doi: 10.1128/JVI.00614-10
22. Gheuens S, Bord E, Kesari S, Simpson DM, Gandhi RT, Clifford DB, Berger JR, Ngo L, Korálnik IJ. Role of CD4+ and CD8+ T-Cell Responses against JC Virus in the Outcome of Patients with Progressive Multifocal Leukoencephalopathy (PML) and PML with Immune Reconstitution Inflammatory Syndrome. *Journal of Virology*, 2011, 85, 7256-7263. doi: 10.1128/JVI.02506-10
23. Sundqvist E, Buck D, Warnke C, Albrecht E, Gieger C, Khademi M, Lima Bomfim I, Fogdell-Hahn A, Link J, Alfredsson L, Söndergaard HB, Hillert J, International Multiple Sclerosis Genetics Consortium, Oturai AB, Hemmer B, Kockum I, Olsson T. JC Polyomavirus Infection Is Strongly

- Controlled by Human Leucocyte Antigen Class II Variants. *PLoS Pathogen*, 2014, 10, e1004084. doi: 10.1371/journal.ppat.1004084
24. Marshall LJ, Ferenczy MW, Daley EL, Jensen PN, Ryschkewitsch CF, Major EO. Lymphocyte Gene Expression and JC Virus Noncoding Control Region Sequences Are Linked with the Risk of Progressive Multifocal Leukoencephalopathy. *Journal of Virology*, 2014, 88, 5177-5183. doi: 10.1128/JVI.03221-13
 25. Dominguez-Mozo MI, Garcia-Montojo M, De Las Heras V, Garcia-Martinez A, Arias-Leal AM, Casanova I, Arroyo R, Alvarez-Lafuente R. Anti-JCV antibodies detection and JCV DNA levels in PBMC, serum and urine in a cohort of Spanish Multiple Sclerosis patients treated with natalizumab. *Journal of NeuroImmune Pharmacology*, 2013, 8, 1277-1286. doi: 10.1007/s11481-013-9496-y.
 26. Polo C, Pérez JL, Mielnichuck A, Fedele CG, Niubo J, Tenorio A. Prevalence and patterns of polyomavirus urinary excretion in immunocompetent adults and children. *Clinical Microbiology and Infection*, 2004, 10, 640-644. doi: 10.1111/j.1469-0691.2004.00882.x
 27. Chai Q, He WQ, Zhou M, Lub H, Fu ZF. Enhancement of Blood-Brain Barrier Permeability and Reduction of Tight Junction Protein Expression Are Modulated by Chemokines/Cytokines Induced by Rabies Virus Infection. *Journal of Virology*, 2014, 88, 4698-4710. doi: 10.1128/JVI.03149-13.
 28. Cramer SP, Modvig S, Simonsen HJ, Frederiksen JL, Larsson HB. Permeability of the blood-brain barrier predicts conversion from optic neuritis to multiple sclerosis. *Brain*, 2015, 138, 2571-2583. doi: 10.1093/brain/awv203.
 29. Cramer SP, Simonsen H, Frederiksen JL, Rostrup E, Larsson HB. Abnormal blood-brain barrier permeability in normal appearing white matter in multiple sclerosis investigated by MRI. *NeuroImage: Clinical*, 2014, 4, 182-189. doi: 10.1016/j.nicl.2013.12.001
 30. Chapagain ML, Verma S, Mercier F, Yanagihara R, Nerurkar VR. Polyomavirus JC infects human brain microvascular endothelial cells independent of serotonin receptor 2A. *Virology*, 2007, 364, 55-63. doi: 10.1016/j.virol.2007.02.018.
 31. Amon W, Farrel PJ. Reactivation of Epstein-Barr virus from latency. *Reviews in Medical Virology*, 2005, 15, 149-156. doi: 10.1002/rmv.456
 32. Pender MP, Burrows SR. Epstein-Barr virus and multiple sclerosis: potential opportunities for immunotherapy. *Clinical & Translational Immunology*, 2014, 3, e27. doi:10.1038/cti.2014.25
 33. Ascherio A, Munger KL. Epstein-barr virus infection and multiple sclerosis: a review. *Journal of Neuroimmune Pharmacology*, 2010, 5, 271-277. doi: 10.1007/s11481-010-9201-3.
 34. Draborg AH, Duus K, Houen G. Epstein-Barr Virus in Systemic Autoimmune Diseases. *Clinical & Developmental Immunology*, 2013, 535738. doi: 10.1155/2013/535738
 35. Wortman MJ, Lundberg PS, Dagdanova AV, Venkataraman P, Daniel DC, Johnson EM. Opportunistic DNA Recombination With Epstein-Barr Virus at Sites of Control Region Rearrangements Mediating JC Virus Neurovirulence. *Journal of Infectious Disease*, 2016, 213, 1436-1443. doi: 10.1093/infdis/jiv755.
 36. Chesnokova LS, Ahuja MK, Hutt-Fletcher LM. Epstein-Barr virus glycoprotein gB and gHgL can mediate fusion and entry in trans, and heat can act as a partial surrogate for gHgL and trigger a conformational change in gB. *Journal of Virology*, 2014, 88, 12193-12201. doi: 10.1128/JVI.01597
 37. Nanbo A, Terada H, Kachi K, Takada K, Matsuda T. Roles of Cell Signaling Pathways in Cell-to-Cell Contact-Mediated Epstein-Barr Virus Transmission. *Journal of Virology*, 2012, 86, 9285-9296.
 38. Sellebjerg F, Cadavid D, Steiner D, Villar LM, Reynolds R, Mikol D. Exploring potential mechanisms of action of natalizumab in secondary progressive multiple sclerosis. *Therapeutic Advances in Neurological Disorders*, 2016, 9, 31-43. doi: 10.1177/1756285615615257
 39. Rice G, Hartung H, Calabresi P. Anti-alpha4-integrin therapy for multiple sclerosis: mechanisms and rationale. *Neurology*, 2005, 64, 1336-1342.
 40. Saraste M, Penttilä TL, Airas L. Natalizumab treatment leads to an increase in circulating CXCR3-expressing B cells. *American Academy of Neurology*, 2016, 3, e292. doi: 10.1212/NXI.0000000000000292
 41. Monaco MCG, Major EO. Immune System Involvement in the Pathogenesis of JC Virus Induced PML: What is Learned from Studies of Patients with Underlying Diseases and Therapies as Risk Factors. *Frontiers in Immunology*, 2015, 6, 159. doi: 10.3389/fimmu.2015.00159.
 42. Weiner GJ. Rituximab: mechanism of action. *Seminars in Hematology*, 2010, 47, 115-123. doi: 10.1053/j.seminhematol.2010.01.011
 43. Kheirallah S, Caron P, Gross E, Quillet-Mary A, Bertrand-Michel J, Fournié JJ, Laurent G, Bezombes C. Rituximab inhibits B-cell receptor signaling. *Blood*, 2010, 115, 985-994.
 44. Warnke C, Smolianov, Dehmel T, Andrée M, Hengel H, Zohren F, Arendt G, Wiendl H, Haas R, Hartung HP, Adams O, Kieseier BC. CD34Q progenitor cells mobilized by natalizumab are not a relevant reservoir for JC virus. *Multiple Sclerosis Journal*, 2011, 17, 151-156
 45. Frohman EM, Monaco MC, Remington G, Ryschkewitsch C, Jensen PN, Johnson K, Perkins M, Liebner J, Greenberg B, Monson N, Frohman TC, Douek D, Major EO. JC virus in CD34+ and CD19+ cells in patients with multiple sclerosis treated with natalizumab. *JAMA Neurol*. 2014 May;71(5):596-602. doi: 10.1001/jamaneurol.2014.63.