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REVIEW

John Cunningham virus: an overview on biology and disease of the etiological agent of the progressive multifocal leukoencephalopathy

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SUMMARY

John Cunningham virus (JCV), the etiological agent of progressive multifocal leukoencephalopathy (PML), is the first human polyomavirus described. After asymptomatic primary infection which occurs in childhood, the virus spreads by the hematogenous route from the primary site of infection to secondary sites including kidneys, lymphoid tissues, peripheral blood leukocytes, and brain to establish latent infection. During immunosuppression the virus undergoes molecular rearrangements that allow it to replicate in glial tissues causing PML.

PML occurs in people with underlying immunodeficiency or in individuals being treated with potent immunomodulatory therapies. Although the hypothesis that immune deficiency is a predisposing factor for PML, there are many unsolved issues including the pathogenic mechanisms related to the interaction of JCV infection/reactivation with the host. This is due to the difficulty of propagating the virus in human cell cultures and the absence of an animal model.

This review updates current understanding in the context of JCV and human disease.

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INTRODUCTION

John Cunningham virus (JCV) is a ubiquitous, hemagglutinating and species-specific virus belonging to the Polyomavirus family. The name Polyomavirus is derived from the Greek roots: poly- ("many") and -oma ("tumours") (see below).

JCV is the etiological agent of progressive multifocal leukoencephalopathy (PML), a rare but often fatal demyelinating disease of the central nervous system (CNS) (Koralnik *et al.,* 2006). PML was characterized in 1958 by Astrom *et al*. who described PML for the first time in three patients with Hodgkin's disease (Astrom *et al*., 1958). PML mainly affects adults, but rare instances of the disease have been reported in children.

JCV was the first human polyomavirus described and was isolated in 1971 from the brain of a patient with Hodgkin's disease (Padgett *et al*., 1971). In the same year, another polyomavirus, that later came to be known as BK virus, was isolated from a urine sample of a renal transplant patient (Gardner *et al*., 1971). Since then, several other human polyomaviruses have been identified: in 2007, KI polyomavirus (Allander *et al*., 2007) and WU polyomavirus (Gaynor *et al*., 2007) were isolated from respiratory samples of affected patients. Merkel cell polyomavirus was identified in 2008 and linked to the development of Merkel cell carcinoma in humans (Feng *et al*., 2008), while trichodysplasia spinulosa virus was described in 2010 (van der Meijden *et al*., 2010).

This review will summarize and analyze the current knowledge on JCV in the context of basic virology, pathogenesis and clinical diseases. There is still a need for studies to understand site(s) of viral latency, nature and mechanisms of virus reactivation, mode of virus transmission into the body or between individuals. This is due to the difficulty of propagating JCV in human cell cultures (Brew *et al.,* 2010), and few cell types support lytic infection in vivo or in vitro. The absence of an animal model hampers research on JCV, but several groups are working on this problem and some progress has been made (White *et al.,* 2015).

There have been major changes in the epidemiology and clinical presentation of PML since its initial description in 1958. Novel clinical entities caused by JCV infection have been discovered. These findings expanded the clinical features of JCV infection in the CNS although some issues are still intriguing.

JCV GENOME STRUCTURE

JCV is a small (~45 nm), non-enveloped, circular, double-stranded DNA genome**,** arranged in 72 viral capsomeres with icosahedral symmetry (Tan *et al*., 2009a). The genome (~5000bp) is divided into early and late regions that are separated by a non-coding control region (NCCR) containing the origin of replication (ORI), the promoter and the enhancer elements (White *et al.,* 2011).

Key words:
JCV; Immune response; JCV-associated diseases.

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Early and late regions are transcribed in opposite directions from complementary strands of DNA (*Figure 1)*.
The early region encodes for large T antigen, small t antigen and three different T antigen splice variants: T'135, T'136 and T'165 (Trowbridge *et al*., 1995) (*Figure 1)*. Early proteins are non-structural but multifunctional proteins encoded by five transcripts alternatively spliced from viral early precursor mRNA. All these proteins are involved in viral transformation, gene regulation of the virus cycle and cell transformation (Assetta *et al*., 2017).
Large T antigen (TAg), perhaps the pivotal protein for all polyomaviruses, is produced prior to viral replication and it is a crucial DNA binding protein necessary for replication and transcription of viral DNA. In permissive cells, JCV entry into the nucleus is followed by transcription of the early genes and production of TAg. TAg represses early gene transcription and stimulates replication and late gene transcription. Since replication also requires host cell proteins, such as DNA polymerase α, TAg modulates cellular signaling pathways to induce quiescent cells to enter S phase in which cellular DNA is replicated. A key event in this process is the interaction of TAg with members of the retinoblastoma (Rb) protein family, which results in consequent progression of the cell cycle (Orba *et al*., 2010). In non-permissive cells, TAg has the ability to dysregulate several signaling pathways such as WNT/β-catenin that is responsible for the control of cell proliferation and the IGF-IR/IRS-1 pathway involved in DNA repair fidelity (Del Valle *et al.*, 2010).
Three distinct TAg domains contribute to oncogenic transformation. The J domain binds to the molecular chaperone Hsc70. The LxCxE domain releases transcription factors of the E2F family from their Rb partners. The C-terminal bipartite region directly binds and inactivates the tumor suppressor protein p53 (Bollag *et al*., 2010). Finally, it has been demonstrated that several lines of transgenic mice expressing JCV TAg developed a variety of tumors, once again verifying its oncogenic potential in the absence of viral replication (Del Valle *et al*., 2001).
Recently, it has been shown that JC virus contains micro-RNA in the distal part of the TAg gene. These microRNAs are predicted to target and downregulate the TAg mRNA and possibly the stress-induced ligand ULBP3 to help the virus escape immune elimination (Bauman *et al*., 2011).
T' proteins seem to influence cellular growth characteristics altering the phosphorylation status of cellular p107 and p130 proteins. They can be also found in the cell's nucleus and can be phosphorylated in a cell cycle-dependent manner (Bollag *et al.,* 2006).
Small t antigen (tAg) has recently become a focus of study: tAg plays a significant role in the replication of viral DNA altering the activity of cellular protein phosphatase 2. In addition, it binds to the viral agnoprotein and to the Rb proteins (pRB, p107, p130) influencing cell cycle progression (Bollag *et al*., 2010).
The late region encodes for three viral structural proteins: the capsid proteins VP1, VP2 and VP3. In addition there

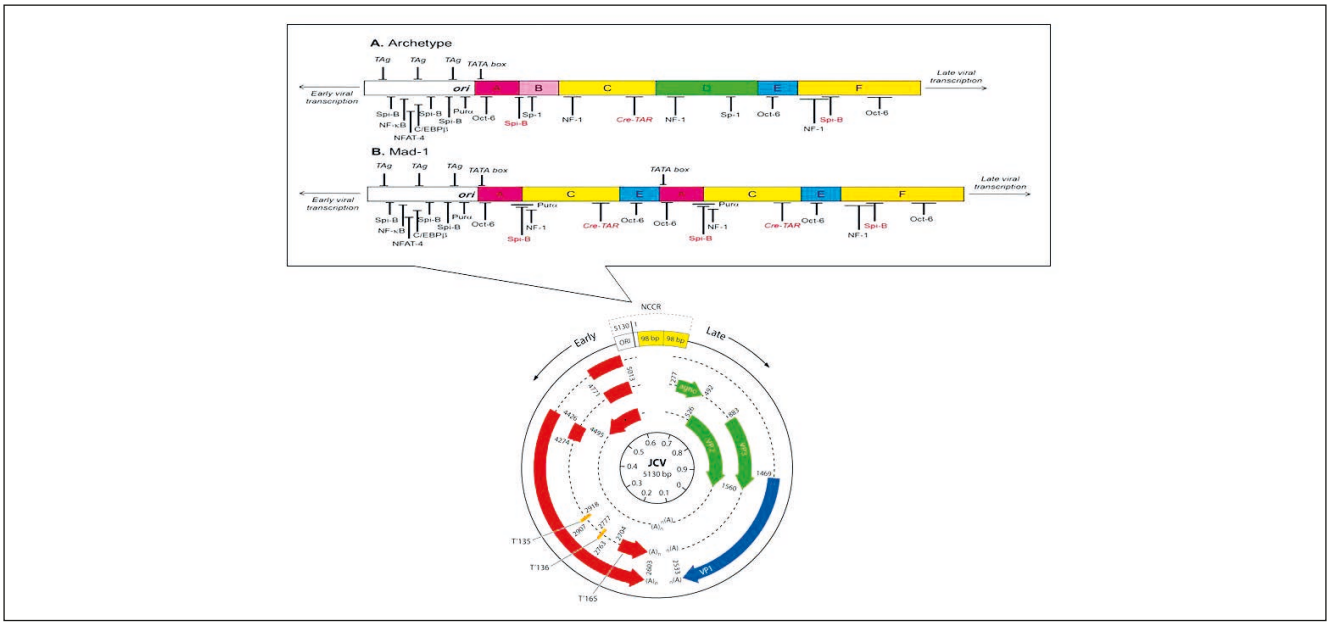

Figure 1 - *Schematic diagram of the JCV genome. The genome is 5,130 bp and is divided into early and late regions separated by a non-coding control region (NCCR) that contains the origin of replication (ORI). Early and late regions are transcribed in opposite directions from complementary strands of DNA and encodes respectively for non-structural proteins, large T antigen (T), small t antigen (t), T'135, T'136, T'165 (red), and for viral structural proteins VP1, VP2, VP3 and agnoprotein (green and blue). All transcripts are polyadenylated [(A)$_n$].*
On the top, two different NCCRs are shown, the non-rearranged archetype (A) and the prototype rearranged form Mad-1 (B). The archetype NCCR (CY strain) is divided into six regions named box A (36 bp), B (23 bp), C (55 bp), D (66 bp), E (18 bp), and F (69 bp). Each region contains binding sites for host transcriptional factors involved in viral transcription.
The JCV archetype sequence found in the kidney and urine is not associated with PML and is not infectious in tissue culture models. Prototype NCCR is a variant isolated from tissues of patients with PML and is identified in the hypothesis that the prototypes result from a rearrangement of the archetype sequence (Marshall et al., *2010). The original prototype is the Mad-1 isolate that contains a 98-bp tandem repeat (A-C-E-A-C-E-F) (Frisque, 1983). Adapted from Ferenczy* et al. *(2012).*

is the late protein named Agnoprotein (*Figure 1)*. VP1 is responsible for the icosahedral structure of JCV and contains the epitopes for antibody induction and recognition. VP2 and VP3 are minor structural components and are necessary for JCV propagation (Weissert, 2011). Agnoprotein directly interacts with TAg and contributes to the oncogenesis interfering with DNA repair and associated with several tumor suppressor proteins: the result is uncontrolled cell proliferation (Del Valle *et al.*, 2010). Finally, it has been demonstrated that agnoprotein is involved in virus propagation and release and acts as a viroporin (Suzuki *et al*., 2010).

Characterization of JCV species revealed that there is only one major VP1 serotype, but at least seven major genotypes. These genotypes, initially distinguished based on restriction site patterns and then on sequences from the intergenic region located between the distal early and late regions, were preferentially detected in different geographic areas of the world (Hirsch *et al*., 2013). Various ethnic groups around the world were shown to predominantly carry certain subtypes. Type 1 and type 4 are generally associated with Europeans and European-Americans, whereas types 2B and 2E were typical of Asians and Eurasians and of Western Pacific populations, respectively (Agostini *et al*., 2001). JCV subtype 2B has been associated with an increased incidence of PML, while type 4 has been related to a lower disease risk (Agostini *et al*., 2001). Interestingly, genotype 2B was found in Italian patients with chronic inflammatory rheumatic diseases (Rodio *et al*., 2016), suggesting a possible JCV genotype selection in response to immunomodulatory drugs.

Differing from the early and late coding regions that are well conserved, the NCCR is hypervariable and contains determinants for neurotropism and neurovirulence. Based on the NCCR structure, JCV can be referred to as having the designated archetype or prototype NCCR (Kim *et al.,* 2001). The archetype NCCR (CY strain) is divided into six regions named box A (36 bp), B (23 bp), C (55 bp), D (66 bp), E (18 bp), and F (69 bp) (*Figure 1)*. Each region contains binding sites for host transcriptional factors involved in viral transcription. These binding sites undergo deletion and enhancement processes that could generate variants that up-modulate viral expression in a specific anatomical site (Pietropaolo *et al*., 2003; Mischitelli *et al.,* 2005). The JCV archetype sequence, found in the kidney and urine, is not associated with PML (*Figure 1)*. Prototype NCCRs are variants isolated from tissues of patients with PML and are named on the hypothesis that the prototypes result from a rearrangement of the archetype sequence (Marshall *et al.*, 2010). The original prototype is the Mad-1 isolate that contains a 98-bp tandem repeat (A-C-E-A-C-E-F) (Frisque, 1983) (*Figure 1)*.

Based on the occurrence of deletions and duplications, JCV isolates are assigned to two classes: the class I viruses characterized by the 98-bp tandem repeat within the NCCR (i.e. Mad-1) and the class II viruses containing strains that display variations from the NCCR class I (White *et al.,* 2009).

LIFE CYCLE OF JCV AND TRANSCRIPTIONAL REGULATION

The JCV life cycle conceptually has been two levels of restriction: the first acts extracellularly on the cell surface through the presence or absence of virus receptors/coreceptors. The second acts intracellularly at any of the subsequent steps.

Classical viral infection in the host cell is initiated by interactions between viral capsid or envelope proteins and cell surface receptors. JCV first attaches to the cell surface via an interaction between VP1 and the pentasaccharide LSTc. Viral particles then bind either α 2,3- and α 2,6- linked sialic acid receptors (Assetta *et al*., 2017). In addition to using sialic acid as a receptor, JCV has been shown to require the serotonin receptor 5HT2AR to infect astroglial cells *in vitro* and kidney epithelial cells, B lymphocytes, platelets, glial cells and neurons *in vivo* (Elphick *et al.*, 2004).

After binding to these viral-specific receptors, viral particles utilize a clathrin-dependent endocytosis mechanism to be transported into the cytoplasm. Once the virus has gained entry into the host cell, it travels to the endoplasmic reticulum and the VP1 protein facilitates entry into the host cell nucleus, where viral assembly occurs. The final viral products are released via host cell lysis (Ferenczy *et al*., 2012). Replication of the viral genome, viral protein synthesis, assembly of the viral particles and their release from the cell use the host cellular machinery (Bhattacharjee *et al.*, 2017).

The JCV life cycle is a relatively slow process: in fact, even in susceptible cells, in which TAg is already present, DNA replication is detectable only after some days (Major *et al*., 1985). Moreover, it has been demonstrated that cell-specific factors regulate the viral gene transcription, while the viral DNA replication is probably regulated by species-specific factors. These species-specific factors, which may be one or more components of the DNA polymerase, allow JCV DNA replication only in primates (Feigenbaum *et al*., 1987; Ferenczy *et al*., 2012).

There are numerous proposed binding sites for different transcription factors and repressors in the NCCR as reviewed in Ferenczy *et al.* (2012), and such factors influence both early and late transcription as well as replication.

The NF-I family is one of the best-studied groups of transcription factors modulating JCV replication and viral genes expression in vivo. The NF-I family contains four members: A, B, C, and X. The isoform NF-IX increases viral gene expression and is highly expressed in cells permissive to JCV replication (Monaco *et al.*, 2001). By contrast, NF-IA expressed in several JCV non-permissive cell types decreases viral late protein expression. NF-IX is overexpressed in the brain where it binds the JCV NCCR and affects early and late viral transcription. However, NF-I activity is not restricted to the brain: in fact, NF-I binds JCV genome in a variety of cell types (Shinohara *et al.*, 1997).

Other important players during both transcription and replication of JCV genes are Purα and YB-1. In the early stages of infection, Purα binds to the late strand of the NCCR and stimulates early viral gene transcription. When large T antigen accumulates in sufficient amounts it binds to the ORI and recruits the cellular DNA polymerase. It has been hypothesized that AgT/YB-1 binding to the NCCR and direct interactions between the two lead to Purα dissociation, so as to favor late gene transcription (Chen *et al*., 1995a; Chen *et al*., 1995b). The interaction between AgT/YB-1/Purα is considered a switch system to regulate early and late viral gene transcription (Assetta *et al*., 2017).

Different DNA-binding proteins have been implicated in the regulation of late viral transcription. In particular, NFAT4 has been shown to activate late-gene transcription, while C/EBPβ appears to function as a transcrip-

tional repressor. Both NFAT4 and C/EBPβ expression are under pro-inflammatory cytokine control, such as TNF-α (Manley *et al.*, 2006; Romagnoli *et al.*, 2009). Additionally, subunits of NF-κB have been shown to increase late-gene expression and can also increase viral expression in response to TNF-α stimulation (Wollebo *et al.*, 2011).
The higher number of transcription binding sites in the rearranged NCCR JCV often confers a transcriptional and replicative advantage compared to the archetype (Assetta *et al.*, 2017). In fact, a particular NCCR structure characterized by multiple duplications of upstream Tat-responsive DNA element (up-TAR) was observed in HIV-positive subjects with PML. Up-TAR is important for HIV-1 Tat stimulation of the JCV late promoter. Specifically, Tat enhances the ability of Purα to bind the up-TAR element and thus synergistically activates transcription (Wortman *et al.*, 2000; Daniel *et al.*, 2001). On this basis, it has been suggested that Tat may contribute to the pathogenesis of PML (Pietropaolo *et al.*, 2003; Mischitelli *et al.*, 2005).

PATHOGENESIS AND IMMUNE RESPONSE TO JCV

Infection with JC virus is widespread in the general population. Blood samples taken from healthy individuals indicate that 50-90% of adults have been exposed to this virus with 20% urinary shedding (Wollebo *et al.*, 2015). Most people are infected with JCV in childhood with no clinically evident or pathological consequences and for the vast majority the infection is self-limiting to a point where viremia is undetectable.
Nevertheless, the mode of transmission is not yet well defined although the presence of JCV DNA in stromal cells of the tonsils and oropharynx supports the proposal that the tonsils may serve as an initial site of viral infection (Monaco *et al.*, 1998).
Virus might enter the mouth or nose by close interpersonal contact or via fomites and presumably spread by the hematogenous route from the primary site of infection to secondary sites such as kidneys, bone marrow, lymphoid tissues, and brain to establish focal areas of infection or persistence (Chapagain *et al.,* 2010) (*Figure 2).* Potential alternative modes of transmission include urino-oral, transplacental and transmission by blood transfusion, semen and organ transplantation (Wiedinger *et al.*, 2014). Archetype virus has also been isolated from sewage samples from different geographical areas suggesting a possible transmission by contaminated food and water (White *et al.*, 2011).
Since humoral immunity to JCV does not protect against the development of PML, as demonstrated by the detection of high anti-JCV antibody titers in advanced stages of the disease, it is assumed that immune control of JCV is focused on cellular immunity. Cytotoxic T cells (CTLs) recognize the epitopes of viral proteins presented on the class I HLA molecules preventing further spread of the virus. The virus is not eliminated but remains in the body in a state referred to as latency *(Figure 2)*. The dissemination of the virus which is going to be latent in different tissues around the body is thought to take place during primary viremia. The nature of primary viremia is not well understood but current hypotheses speculate that the virus may exist as free virions and/or as a white blood cell-associated virus (White *et al.*, 2011).
Many tissues have been reported to harbor latent JCV: kidney (Yogo *et al.*, 1990; Yogo *et al.*, 2008), where archetype virus may persist with occasional shedding when low level replication occurs in tubular epithelial cells (Wollebo *et al.*, 2015) (*Figure 2)*, tonsil (Monaco *et al.*, 1998; Kato *et al.*, 2004) and peripheral blood leukocytes (Dorries *et al.*, 1994), including B lymphocytes. In fact, B cells possess the Rag1 and Rag2 enzymes for immunoglobulin gene rearrangements. This hypothesis is sustained by the observation that different viral NCCRs have been found in the blood and bone marrow (Marzocchetti *et al.*, 2008; Tan *et al.*, 2009b).
The mechanism through which latent JCV is reactivated is not well understood in detail. Before JCV can cause PML, several requirements need to be met: virus must enter the blood; neurotropic forms of the virus must be present or emerge; neurotropic virus must migrate to the brain; active viral gene expression must occur in the glial cells of the brain; immunosuppression must be present (*Figure 2)*. All of these points are necessary for JCV reactivation to cause PML, but non specific steps appear to be sufficient (White *et al.,* 2011).
Since JCV DNA has been detected in the brains of healthy individuals who do not have PML (Tan *et al.*, 2010a), some studies have pointed to a model where latent JCV is reactivated in the brain (Perez-Liz *et al.*,

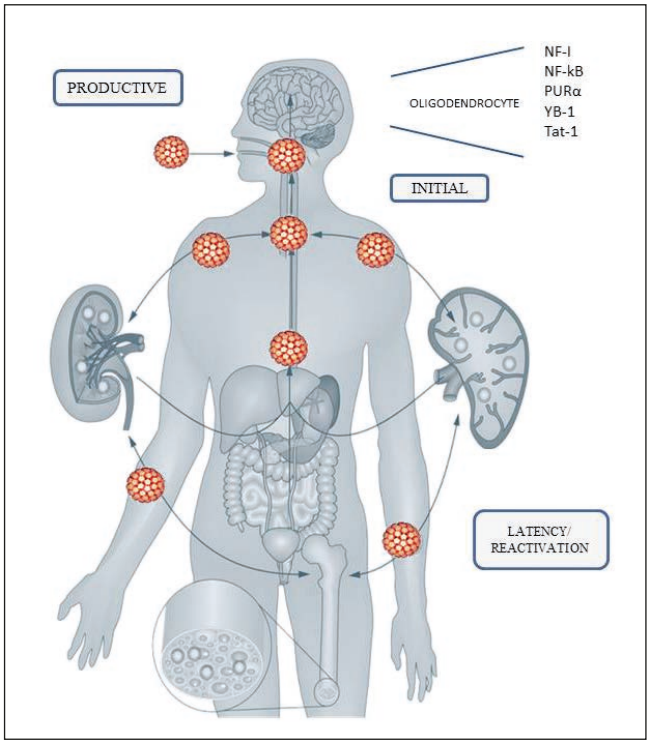

Figure 2 - *Steps of JCV infection. JCV infection is at first asymptomatic and occurs in childhood. Virus might enter the mouth or nose by close interpersonal contact and presumably spread by hematogenous route from the primary site of infection to secondary sites including kidneys, lymphoid tissues, peripheral blood leukocytes, and brain to establish latent infection. Whether JCV infects and remains latent in the CNS is controversial. During immunosuppression, the virus undergoes molecular rearrangements that allow it to replicate in glial tissues causing PML. Adapted from Calabrese* et al. *(2015).*

2008; White *et al.*, 2011). The JCV NCCR contains binding sites for transcription factors regulated downstream of cytokine signal transduction pathways. In this case virus reactivates transcription in response to extracellular cytokines initiating a "flare-up" of viral replication and it might spread forming a PML lesion, owing to a lack of immuno-surveillance associated with the underlying immunosuppression (White *et al.*, 2011). In PML occurring with human immunodeficiency virus type 1 (HIV-1) or AIDS, HIV-1 trans-activator protein Tat can stimulate JCV transcription (Tada *et al.*, 1990) and replication (Daniel *et al.*, 2004).

Numerous models on JCV reactivation emphasize the role of the immune system hypothesizing two main functions. The first concerns B lymphocytes, that may play an important role in PML pathogenesis since this latent virus may be PML-type (see above). These cells are not only serving as a source of latent virus but may also allow virus to circulate around the body and enter the brain (Berger *et al.*, 2009; Major, 2010; White *et al.*, 2011). Second, as stated, JCV-specific CTL responses are crucial to contain viral replication and to explain the role of immunosuppression in PML pathogenesis. Cytotoxic $CD8^+$ T cell responses specific to JCV are important to control JCV (Wuthrich, *et al.*, 2006; Lima *et al.*, 2007; Lima *et al.*, 2010), and during chronic viral infections $CD4^+$ T cells are required to maintain a $CD8^+$ T cell response (Matloubian *et al.*, 1994). Moreover, increased circulation of B cells may favor JCV crossing of the blood brain barrier (BBB).

In the CNS, one of the main roles of microglia is to be the first line defense against infectious agents and injury-related products in the CNS parenchyma. The molecular markers of antigen presentation and activation, such as MHC II, CD80, CD86, CD40 and CD11a, are rapidly increased on microglia in response to pathological changes in the CNS, and these cells are capable of presenting antigens to and activating T cells (Shrikant *et al.*, 1996). In addition to antigen presentation, microglia have all the machinery necessary to detect most microbes that access the CNS parenchyma and can rapidly mount a potent inflammatory response (Bellizzi *et al.*, 2013).

Taking into account this scenario, upon suppression of $CD4^+$ and $CD8^+$ T cell mobilization, as occurs with HIV infection, during chemotherapy or immunosuppressive therapy, JCV enters the brain, either within B cells or as cell-free virus, where it infects and kills oligodendrocytes, leading to demyelination (Bellizzi *et al.*, 2013). The decrease in $CD4^+$ T cells is considered the major determining factor for developing PML because they are necessary for maintenance of $CD8^+$ T cells, important in the control of JCV infection (Jelcic *et al.*, 2016).

Therefore, it is assumed that JCV pathogenesis is under the control of immune surveillance, as the transcription of early viral genes is finely regulated by cellular transcription factors under pro-inflammatory cytokine control (Bellizzi *et al.*, 2013).

Recently, it has been reported that human polyomaviruses encode microRNAs that downregulate large T expression and target host factors, helping the virus to escape immune elimination. Consequently, it is tempting to speculate that viral microRNAs may also have a role in the induction of polyomavirus reactivation, but further studies are required to understand their role in the success of persistence or replication and the development of diseases (Martelli *et al.*, 2017).

JCV AND PML

PML is a fatal neurodegenerative disease characterized by productive infection of JCV. JCV lytically infects the oligodendrocytes of the brain which are responsible for myelin production and maintenance for neuronal axons and, to a lesser extent, astrocytes which in turn are responsible for a wide variety of activities in the CNS (Aksamit *et al.*, 1987; Houff *et al.*, 1989; Wollebo, 2015).

Destruction of oligodendrocytes produces multiple expanding regions of demyelination that eventually coalesce to form larger lesions containing inclusion bodies and bizarre astrocytes constitute the triad of histopathological features that are characteristic of PML (Wollebo, 2015).

Symptoms of PML are characterized by motor dysfunction, visual defects and speech impairment. Such symptoms are not specific to PML and magnetic resonance (MR) imaging of the brain is needed to visualize the characteristic multifocal lesions. The presence of JCV DNA in the cerebrospinal fluid (CSF) or of JCV proteins and DNA in brain biopsies is required to complete an accurate diagnosis. The detection of anti-JCV antibodies or excretion of JCV in the urine is not predictive of disease outcome, as healthy individuals can satisfy such criteria without developing PML. Nevertheless, the presence of anti-JCV antibodies is important for risk stratification (Assetta *et al.*, 2017).

PML was originally recognized as a rare complication of hematological malignancies or systemic inflammatory disorders. However, a dramatic 50-fold increase in incidence occurred with the HIV epidemic. With the advent of the AIDS pandemic, PML became more prevalent with an incidence of ~3% in all patients with HIV (Major, 2010; Assetta *et al.*, 2017). The introduction of highly active antiretroviral therapy (HAART) increased the life expectancy of individuals with PML but the mortality rate in AIDS-associated PML cases is still approximately 50% (Garvey *et al.*, 2011; Assetta *et al.*, 2017). A synergistic role between JCV and HIV at molecular level has been proposed and it is possible that it might increase the chances of developing PML (Tada *et al.*, 1990; Assetta *et al.*, 2017).

PML can be observed after organ and stem cell transplantations and recently in patients under treatment with immunomodulatory compounds like therapeutic monoclonal antibodies (mAbs) (Bellizzi *et al.*, 2013; Calabrese *et al.*, 2015). There are four mAbs that have been associated with PML: natalizumab (Tysabri), efalizumab (Raptiva), rituximab (Maptera) and infliximab (Remicade).

Natalizumab is a humanized monoclonal antibody that is able to inhibit alpha-4 beta-1 integrin (VLA-4), preventing transmigration of lymphocytes across the BBB. Natalizumab interferes with lymphocyte trafficking through the BBB by blocking the interaction of very late antigen 4 (VLA-4) with adhesion molecule type 1 on vascular cells (VCAM-1). This blocking seems to result in decreased immuno-surveillance and JCV reactivation from latency (Wuthrich *et al.*, 2006). It is used in the treatment of multiple sclerosis (MS) and Crohn's disease and it is believed to work by reducing the ability of inflammatory immune cells to attach to and pass through the cell layers lining the intestines and BBB. In the past, two patients with MS treated with natalizumab and a patient with Crohn0's disease developed PML (Kleinschmidt-DeMasters *et al.*, 2005; Langer-Gould *et al.*, 2005; Van Assche *et al.*, 2005) so the drug was taken off the market. It was re-introduced in

2006 in the USA and subsequently in Europe, carrying US Food and Drugs Administration agency (FDA) mandated "black box" warnings for the disorder. At present, the risk of developing PML under treatment with natalizumab is 1.35 cases per 1000 for patients treated for more than one year and 1.78 per 1000 for patients treated more than two years (Weissert *et al*., 2011).

Efalizumab is a mAb directed against CD11a or lymphocyte function-associated antigen 1 (LFA-1). It has been used for the treatment of psoriasis (Lebwohl *et al*., 2003). Probably, the mechanism by which it causes PML is connected to the lack of immune surveillance from the block of T cell trafficking and activation (Assetta *et al*., 2017). Due to the emergence of PML cases, Efalizumab was taken off the market (Carson *et al*., 2009; Seminara *et al.,* 2010).

Rituximab is a mAb directed against CD20 in B cells and PML has been observed as a side-effect of treatment with this drug (Carson *et al*., 2009). It is used in the treatment of lymphomas and of various autoimmune conditions (i.e. rheumatoid arthritis) (Rastetter *et al*., 2004; Hauser *et al.*, 2008). Lymphoproliferative diseases are also predisposing factors for PML, so it is difficult to establish the causative role of rituximab. However, a rise in the risk of PML development has been noted in patients treated with rituximab and PML is enumerated as a potential side-effect of the treatment (Steiner *et al*., 2012, Assetta *et al*., 2017).

Infliximab was originally developed in mice, nevertheless, since humans have immune reactions to mouse proteins, it was later developed into a humanized antibody. Infliximab works by blocking the action of tumor necrosis factor alpha and it was approved for the treatment of autoimmune diseases such as Crohn0's disease. However, it was demonstrated that although infliximab reduced clinical symptoms, it could also unbalance the local immune-surveillance promoting JCV virus reactivation and NCCR rearrangements (Bellizzi *et al*., 2010; Bellizzi *et al*., 2011).

Other therapeutic agents, such as rentuximab-vedotin, Alemtuzumab, Eculizumab, Anti-tumor necrosis factor biologicals, Dimethylfumarate and fumaric acid esters, Fingolimod and brutinib may also be associated with PML risk (Misbah *et al*., 2017*)*.

Another group of individuals that can manifest PML are solid organ or bone marrow transplant patients. All transplant recipients follow an immunosuppressive regime with one or more drugs and it is difficult to establish a direct link between a specific treatment and PML onset. However, it appears that prednisone, cyclosporine and mycophenolate mofetil treatment carry a higher risk (Mateen *et al*., 2011; Assetta *et al*., 2017).

AN UNFAVOURABLE OUTCOME: FROM PML TO IRIS

Immune Reconstitution Inflammatory Syndrome (IRIS) is a disease condition commonly observed in HIV patients treated with HAART (French *et al*., 2004). It is due to an immune reconstitution which leads to infiltration of lymphocytes into the PML lesions. In particular, IRIS is thought to result from resumption of immune surveillance in the CNS and might be associated with an initial worsening of neurological symptoms. IRIS probably corresponds to CD8-mediated inflammatory changes and can often be identified by gadolinium enhancement on MR (Gray *et al*., 2005). In fact, clinical diagnosis is based on MR and an elevation of the $CD8^+$ and $CD4^+$ T cell counts that also represent the most predictive factors for survival from PML (counts over 200) (Marzocchetti *et al*., 2009). The diagnostic utility of CSF JCV DNA for distinguishing between IRIS PML and PML progression is unknown and requires additional study.

OTHER JCV-ASSOCIATED DISEASES

In addition to PML, other JCV-associated diseases have been reported: JC virus granule cell neuronopathy (GCN), JC virus encephalitis, JC virus meningitis and JCV associated nephropathy (Tan *et al.*, 2010b; Assetta *et al.,* 2017).

Some patients develop a cerebellar syndrome without classical PML lesions but with detectable JCV DNA load in CSF or on biopsy. The infected cells are granule cell neurons in the cerebellum; this clinical entity has been named JCV granule cell neuronopathy (JCV GCN) (Du Pasquier *et al*., 2003; Koralnik *et al*., 2005). Destruction of granule cell neurons in the cerebellum leads to symptoms including gait ataxia, dysarthria and incoordination (Tan *et al*., 2010b). The virus strains found in these patients are variants characterized by small deletions in the C-terminal portion of the VP1 capsid protein. *In vitro*, these mutants show decreased replication ability in glial cells (SVG cells) and low levels of replication in cerebellar neuronal tumor-derived cells (Dang *et al*., 2012). Recently, a clinical entity associated with cortical neuron infection by JCV, JCV encephalopathy (JCVE), was recognized (Wuthrich *et al*., 2009b). The infection started from cortical pyramidal neurons and propagated to astrocytes causing necrosis. The disease was fulminant (Assetta *et al*., 2017).

There have been several case reports of meningeal symptoms where JCV was present in the CSF and all other neurotropic viruses were absent. The cases were reported in both immunocompromised and immunocompetent individuals and it is not clear whether they resulted from primary infection or reactivation. No focal lesions in the white matter were detected (Tan *et al*., 2010b; Assetta *et al*., 2017). Finally, the possibility that JCV could be associated with nephropathy (JCVAN) was considered. The literature reports a few cases of JCVAN during immunosuppression (Kazory *et al*., 2003; Wen *et al.*, 2004; Kantarci *et al*., 2011; Lautenschlager *et al*., 2014).

CONCLUSIONS

JCV is a human virus that rarely causes disease. Immunosuppression appears to be paramount in JCV reactivation. In some individuals with underlying immunosuppressive disease or under treatment with immunomodulatory compounds, several finely regulated pathways determine JCV reactivation from its latent state and JCV NCCR recombination, leading to the emergence of neurovirulent variants. However, the host immune system plays a decisive role in facilitating or diminishing the expression of particular cellular transcription factors, which are essential to the virus for its reactivation and subsequent productive infection in permissive cells. PML remains a significant public health problem due to the use of immunosuppressant therapy in autoimmune diseases and organ transplantation, the occurrence of individuals with no obvious cause of immunosuppression (including the elderly) and patients with chronic liver or kidney disease or those with idiopathic or transient lymphocytopenia.

Since JCV identification, important advances have been

made in the understanding of the biology of the virus and the pathogenesis of PML but there are still important areas where our knowledge is incomplete. Further studies are needed to elucidate the exact mechanisms involved in the disease pathogenesis.

Competing interests

The authors declare that they have no competing interests.

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