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MINI REVIEW

Human polyomavirus JC reactivation and pathogenetic mechanisms of progressive multifocal leukoencephalopathy and cancer in the era of monoclonal antibody therapies

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Abstract Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the central nervous system caused by the neurotropic human polyomavirus JC (JCV) lytic infection of oligodendrocytes. PML was first described as a complication of lymphoproliferative disorders more than 50 years ago and emerged as a major complication of human immunodeficiency virus (HIV) infection in the 1980s. Despite the ubiquity of this virus, PML is rare and always seen in association with underlying immunosuppressive condition, such as HIV infection, autoimmune diseases, cancer, and organ transplantation. JCV remains quiescent in the kidneys, where it displays a stable archetypal non-coding control region (NCCR). Conversely, rearranged JCV NCCR, including tandem repeat patterns found in the brain of PML patients, have been associated with neurovirulence. The specific site and mechanism of JCV NCCR transformation is unknown. According to one model, during the course of immunosuppression, JCV departs from its latent state and after entering the brain, productively infects and destroys oligodendrocytes. Although the majority of PML cases occur in severely immunesuppressed individuals, PML has been increasingly diagnosed in patients treated with biological therapies such as monoclonal antibodies (mAbs) that modulate immune system functions: in fact, $CD4^+$ and $CD8^+$ T lymphopenia, resulting from this immunomodulatory therapy, are the primary risk factor. Furthermore, JCV reactivation in nonpermissive cells after treatment with mAbs, such as intestinal epithelial cells in Crohn's disease patients, in association with other host tumor-inducing factors, could provide valid information on the role of JCV in several malignancies, such as colorectal cancer.

Keywords Human polyomavirus JC · Monoclonal antibodies · Biological therapies · PML · Colorectal cancer

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Introduction

Progressive multifocal leukoencephalopathy (PML) is a demyelinating disease of the brain caused by the neurotropic human polyomavirus JC (JCV) lytic infection of oligodendrocytes. It was first described as a complication of chemotherapy in hematological patients in 1958 by Astrom and colleagues. The viral nature of PML was confirmed by the observation of

polyoma-like virions on electron microscopy by Zu Rhein in 1967, and the virus was named JCV in 1971 by Padgett and colleagues, based on the initials of the name of John Cunningham, the patient from whom the virus was first isolated (Åström et al. 1958; Padgett et al. 1971; Zu Rhein 1967). JCV has also been associated with central nervous system (CNS) tumors in patients with and without PML, as well as in non-CNS cancers, although this association remains under debate (Del Valle et al. 2008). Many elegant studies have been performed to investigate the association of JCV and human cancers, but a causative role for JCV in cancer has not been established. However, potential molecular and cellular mechanisms of oncogenesis are currently being solved, thanks to the observation that JCV is oncogenic in experimental animal models (Maginnis and Atwood 2009).

For a long time, PML remained a disease of patients with lymphoproliferative disorders treated with chemotherapy, with an estimated incidence of 0.07%. However, in the 1980s, PML emerged as a major complication of human immunodeficiency virus (HIV) infection. HIV infection accounts for 85% of all instances of PML, and overall, 5% of patients with AIDS develop the disease (Chan et al. 2008). Despite of JCV ubiquity, with a seroprevalence range from 66% to 92% in worldwide adult population, PML is rare and almost always seen in association with an underlying immunosuppressive condition, such as lymphoproliferative disorders, autoimmune diseases, cancer, and organ transplantation (Maginnis and Atwood 2009). The prognosis is still poor, with discouraging results from clinical trials of various therapeutic approaches, including immunomodulation and/or inhibition of viral replication (Focosi et al. 2010).

Although the majority of PML cases occur in severely immunesuppressed individuals, PML has been increasingly diagnosed in patients treated with biological therapies such as monoclonal antibodies (mAbs) that modulate immune system functions: in fact, $CD4^+$ and $CD8^+$ T lymphopenia seems to be the primary PML risk factor, following the use of natalizumab (Tysabri®; Biogen Idec, Elan Pharmaceuticals), efalizumab (Raptiva®; Genentech), and rituximab (Rituxan®/MabThera; Genentech, Biogen Idec) for the treatment of multiple sclerosis (MS), Crohn's disease (CD), severe forms of plaque psoriasis, hematologic malignancies, and rheumatoid arthritis (RA) (Major 2010). Currently, the issue of JCV reactivation in nonpermissive cells after treatment with biologics, such as intestinal epithelial cells in patients with CD treated with infliximab (Remicade®; Centocor Ortho Biotech) (Bellizzi et al. 2011), and its association with cancer is emerging. For this reason, this review will focus on (1) the available data regarding the risk of PML and the possible mechanism implicated in the pathogenesis of PML in association with biological therapies and (2) the downstream effect of mAbs and JCV reactivation on the development of malignancies, in order to provide valid information about the JCV role in several types of cancer, such as colorectal cancer (CRC).

JCV and PML pathogenesis

JCV is a circular double-stranded DNA virus belonging to the *Polyomaviridae* family. The viral genome is approximately 5.1 kbp in size and is packaged with cellular histones, forming the viral mini-chromosome, which has structural similarities to cellular host chromatin. The viral genome encodes six viral proteins, including two early regulatory proteins, small t antigen, and large T antigen (TAg), another late regulatory protein, *agnoprotein*, and three structural capsid proteins, VP1, VP2, and VP3 (Khalili and Stoner 2001). The presence of rearrangements within the highly variable non-coding control region (NCCR) characterizes the neurotropic and pathogenic Mad1-*type* strains, which probably derives from the non-pathogenic CY archetype, detected in the kidneys and tonsils of healthy individuals. It is not established whether the transmissible form of the virus is the CY archetype, Mad1-*type*, or both. Moreover, it is not known whether JCV superinfections can occur after initial childhood infection. It should be noted that the CY archetype and the Mad1-*type* of JCV differ only within the NCCR, and this will therefore not be reflected in any changes in the amino acid sequence and hence the antigenicity of viral proteins (White and Khalili 2011).

During primary infection, a critical role in virus binding and uptake is played by the cellular receptor for JCV, identified as terminal $\alpha(2,6)$- and $\alpha(2,3)$-linked sialic acid residues of *N*-linked glycoproteins (Dugan et al. 2008). In a series of elegant experiments, Elphick and colleagues (Elphick et al. 2004) identified also the serotonin receptor $5\text{-}HT_{2a}$ as a cellular receptor for infection of astroglial cells by the chimeric Mad1-SV40 JCV strain. JCV has a restricted cellular tropism, infecting oligodendrocytes, astrocytes, kidney epithelial cells, and peripheral blood cells, such as granulocytes and B lymphocytes. This tropism is, at least in part, defined by the cellular presence of both receptor components: in fact, recently Focosi and colleagues speculated that the $5\text{-}HT_{2a}$ receptor for JCV could be a *N*-linked glycoprotein, which would simplify the current understanding of JCV receptors (Focosi et al. 2010).

Primary infection, which is thought to occur in childhood, might occur through two routes: (1) the upper respiratory tract through inhalation and (2) the gastrointestinal (GI) tract through ingestion of contaminated food and water. The finding of JCV in the tonsils (Kato et al. 2004) seems to support the former route, but the hypothesis of GI tract, as initial site of viral infection, is also supported by the detection of JCV in intestinal epithelial cells (Laghi et al. 1999; Ricciardiello et al. 2001), enteroglial cells of the myenteric plexuses (Selgrad et al. 2009), and esophagus (Del Valle et

al. 2005). However, as JCV can infect circulating B lymphocytes, both tonsils and gastrointestinal tract may represent a site of latency rather than the entry route of the virus. After the primary asymptomatic infection, the virus remains latent in different sites, including the kidney (Markowitz et al. 1993), bone marrow (Tan et al. 2009), and B lymphocytes (Chapagain and Nerurkar 2010) (Fig. 1). There is a recent evidence that the virus enters the brain from the early phases of infection and establishes a nonproductive or low chronic infection of glial cells (Delbue et al. 2008; Perez-Liz et al. 2008). Under particular conditions, usually associated with severe immunosuppression, JCV can actively replicate into the brain, leading to PML. The site and modality of JCV reactivation are still poorly understood, but the most likely hypothesis is that the virus reactivates somewhere in the periphery and crosses the blood–brain barrier (BBB) through circulating infected cells, such as B lymphocytes and $CD34^{+}$ hematopoietic precursor cells, entering the CNS where it

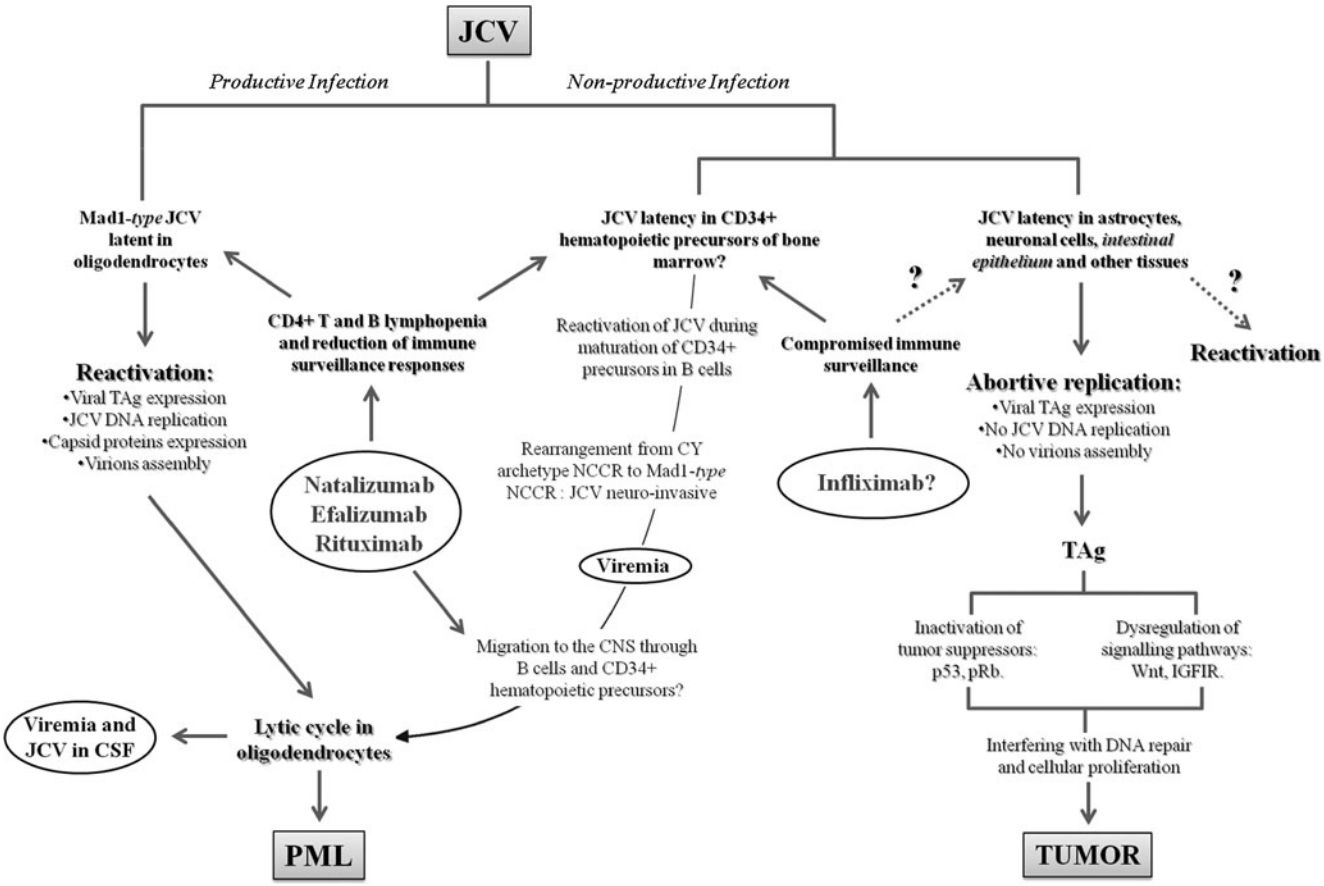

Fig. 1 Pathogenetic model for the development of either PML or JCV-associated cancer taking into account the compromised immunosurveillance following monoclonal antibody therapies. After the primary asymptomatic infection, the virus remains latent in different sites, including the kidney (Markowitz et al. 1993), bone marrow (Tan et al. 2009), B lymphocytes (Chapagain and Nerurkar 2010), and tonsils (Kato et al. 2004). There is recent evidence that the virus enters the brain from the early phases of infection and establishes a nonproductive or low chronic infection of glial cells (Delbue et al. 2008; Perez-Liz et al. 2008). Under immunocompromised state, such as following treatment with natalizumab, rituximab, and efalizumab, JCV can either productively replicate into oligodendrocytes, leading to PML, or reactivate somewhere in the periphery. After reactivation, the virus could cross the blood–brain barrier through circulating infected cells, such as B lymphocytes and CD34+ hematopoietic precursor cells, entering the CNS where it infects astrocytes and promotes lysis of oligodendrocytes with a consequent massive demyelination, predominantly involving white matter (Chapagain and Nerurkar 2010; Tavazzi et al. 2011). Recent introduction of infliximab in the management of autoimmune diseases, such as Crohn's disease (CD), has been immediately associated with the JCV reactivated infection. The TNF-α blocking results in a reduction in T cell activity with a decreased expression of interferon-γ, STAT-1, granzyme B, and other T cell inflammatory genes as well as a reduction in dendritic cell-mediated T cell activation that could unbalance the local immune surveillance and enhance the JCV reactivation from its several sites of latency (Bellizzi et al. 2010). In particular, JCV reactivation in nonpermissive cells after treatment with mAbs, such as intestinal epithelial cells in CD patients treated with infliximab, could lead to an abortive replication, characterized by the expression of the main viral ongonenic protein TAg that, in association with other host tumor-inducing factors, could result in cell transformation and cancer (White and Khalili 2004). *CNS* central nervous system, *CSF* cerebrospinal fluid, *IGFIR* insulin-like growth factor I receptor, *NCCR* non-coding control region, *PML* progressive multifocal leukoencephalopathy, *TAg* large T antigen

infects astrocytes and promotes lysis of oligodendrocytes with a consequent massive demyelination (Chapagain and Nerurkar 2010; Tavazzi et al. 2011) (Fig. 1).

The prevalence of JCV genome and antibodies has been investigated in different patient cohorts and in different biological fluids. JCV is commonly found in urine, regardless of the immune status of the individual, and the prevalence of JC viruria increases linearly with age. The virus reactivates in kidneys, and increased urinary excretion occurs under different conditions of immunesuppression. Interestingly, there is no relation between urinary load and the occurrence of hemorrhagic cystitis associated with JCV (Focosi and Kast 2007). In contrast to BK virus, another human polyomavirus which is commonly found in the peripheral blood of transplant recipients (Drachenberg et al. 2007), JCV is very rarely found in peripheral blood in immunocompetent people, and the prognostic value of JC viremia and antibody titer in relation to the development of PML is currently under investigation (Pineton de Chambrun et al. 2008; Gorelik et al. 2010). However, high rates of JC viremia have been reported in multiple sclerosis patients, and it is currently not known what enables JCV to enter the bloodstream (Focosi et al. 2008). Deletions in certain genome regions of JCV and amino acid substitutions in the outer loop of the VP1 capsid protein have been shown in brain isolates, but whether these are required for JCV entry into the bloodstream or just for infection of glial cells is currently not understood (Gorelik et al. 2011).

Biological therapy, reactivation of JCV and PML

The observation of PML after using of mAbs therapies has raised concerns about the safety profile of these agents, but has also provided a window into the pathogenesis of PML. The mAbs that target (1) cell adhesion molecules, such as very late antigen-4 (VLA-4) (natalizumab for multiple sclerosis and Crohn's disease) or lymphocyte function-associated antigen-1 (LFA-1) (efalizumab for severe forms of plaque psoriasis) to prevent extravasation of inflammatory T cells into tissues, or (2) the cell surface marker CD20 (rituximab for hematologic malignancies and RA) to deplete peripheral circulating B cells, have all been associated with PML. Thus, these mAbs currently carry US Food and Drug Administration (FDA)-mandated "blackbox" warnings (Carson et al. 2009a).

Furthermore, anti-tumor necrosis factor alpha (anti-TNF-α) agents, such as adalimumab (Humira®; Abbott) and infliximab, are used in several autoimmune diseases, mainly in CD and severe forms of plaque psoriasis. Several cases of demyelinating events of the nervous system have been reported, althought the incidence of such events is relatively low (Keene et al. 2011).

Natalizumab

Natalizumab is a humanized mAb which binds to the α chain of α4β1 (or VLA-4) and α4β7 integrins on the hematopoietic cells. Binding of natalizumab to α4β1 integrins prevents adhesion and transmigration of activated lymphocytes through the BBB. As the blockade of α4β7 integrins prevents activated lymphocytes from crossing the epithelium of the intestinal barrier, natalizumab has been approved for the treatment of moderate-to-severe CD in the USA (Edula and Picco 2009).

Unfortunately, the efficacy of natalizumab was overshadowed by the occurrence of PML in two MS patients (Kleinschmidt-DeMasters and Tyler 2005; Langer-Gould et al. 2005) and one CD patient (Van Assche et al. 2005). Thus, in 2005, Biogen Idec (Cambridge, MA, USA) and Elan (Gainesville, GA, USA) voluntarily suspended their marketing. After a large assessment of clinical, MRI, and laboratory data of patients treated with natalizumab, the US FDA and the European Medicines Agency (EMEA) reintroduced its market with a close postmarketing surveillance (Yousry et al. 2006). Between July 2006 and November 2009, there were 28 cases of confirmed PML in patients with MS treated with natalizumab, of which eight were fatal (Clifford et al. 2010). MedWatch reports from Biogen Idec were reviewed, comprising all 42 cases of natalizumab-related PML since its reintroduction until March 2010 (Tan et al. 2011). To date, 102 cases of PML have been reported among 82,732 patients treated with Tysabri worldwide through February 28, 2011, and this number seems expected to rise. The risk of PML increases with duration of exposure to natalizumab over the first 3 years of treatment, and median treatment duration to onset of symptoms was 25 months (range, 6–80 months) (FDA U.S. Food and Drug Administration 2011).

Since natalizumab has not been consistently associated with opportunistic infections other than PML, it cannot be considered as a classical immunosuppressant drug. Thus, there must be a specific mechanism that causes PML mainly in patients affected by autoimmune diseases. By binding to integrins on $CD34^+$ hematopoietic precursor cells, natalizumab prevents them from attaching to vascular cell adhesion molecule (VCAM) and then forces them to migrate out of the bone marrow. Therefore, an increase in $CD34^+$ cells in peripheral blood is evident immediately after natalizumab injection (Major 2009; Neumann et al. 2009), as well as an upregulation of genes involved in B cell maturation (Lindberg et al. 2008). This dynamic creates a favorable environment for JC virus, which can reside in a latent state in the bone marrow for long periods before the development of PML and which can use B cells and their DNA-binding proteins to initiate viral replication. However, Warnke and colleagues support the hypothesis that $CD34^+$ progenitor cells mobilized

by natalizumab are not a relevant reservoir for JC virus (Warnke et al. 2011).

Interestingly, JCV-specific T cell response seems to increase in natalizumab-treated patients. Nevertheless, since T cells specific for other viral and myelin antigens seem also to be increased in the same patients, we can hypothesize that antigen-specific activated T cells could be trapped in the peripheral blood. Thus, by preventing autoimmune T cells from reaching the brain, natalizumab is very efficient, but, on the other hand, this drug may also impair the immune surveillance against foreign antigens such as JCV (Lysandropoulos and Du Pasquier 2010). Moreover, natalizumab decreases dramatically the number of dendritic and $CD4^+$ T cells in the cerebral perivascular space, as well as B cells and T cells in cerebrospinal fluid (CSF). However, JCV-specific $CD8^+$ cytotoxic T lymphocytes can be detected in the CSF of patients with a better prognosis for PML after reconstitution of the immune system, suggesting that the protective effect of these JCV-specific $CD8^+$ cytotoxic T cells is mediated at the CNS level (del Pilar Martin et al. 2008). Nevertheless, how would JCV reach the brain if precisely the BBB is blocked? One possibility is that JCV is already present in the brain of some individuals before natalizumab treatment: in fact, Perez-Liz and colleagues have detected JCV DNA fragments in normal brain tissue (Perez-Liz et al. 2008). Moreover, JCV could also use some infected B lymphocytes and $CD34^+$ hematopoietic precursor cells to enter the CNS and to infect glial cells. Thus, it is conceivable that diminished immune surveillance plays a substantial role in the natalizumab-associated cases of PML (Lysandropoulos and Du Pasquier 2010) (Fig. 1).

Nearly all patients with MS, who develop PML following treatment with natalizumab, develop also immune reconstitution inflammatory syndrome (IRIS), which carries a high morbidity and mortality rate. The rapid restoration of T cells' capacity to cross the BBB, following the suspension of natalizumab treatment, can lead to a massive infiltration of PML lesions by JCV-specific cytotoxic $CD8^+$ T lymphocytes. Although the most important factor for a favorable outcome of PML is immune reconstitution, in the case of IRIS, there can be a transient worsening of the symptoms due to the massive inflammation. This syndrome seems to be more common and more severe in patients with natalizumab-associated PML than in patients with HIV-associated PML. Management of PML has routinely used plasma exchange or immunoabsorption to hasten clearance of natalizumab and shorten the period in which natalizumab remains active (usually several months) (Clifford et al. 2010; Tan et al. 2011).

Efalizumab

Efalizumab is a humanized IgG1 monoclonal antibody targeting CD11α, the alpha subunit of the LFA-1 on antigen-presenting cells. It was used for the treatment of moderate to severe plaque psoriasis, and was withdrawn from the market in April 2009 after three patients were diagnosed with PML. All three had received efalizumab as monotherapy for longer than 3 years (Kothary et al. 2011).

By binding to the domain I of the α chain of CD11α, it triggers a conformational change in LFA-1, the site that binds to intercellular adhesion molecule (ICAM) and can affect apoptosis, cytotoxicity, cell proliferation, cytokine production, antigen presentation, and gene activation. This set of events affects psoriasis pathogenesis at multiple levels, perhaps most importantly by inhibiting the initial T cell activation in lymph nodes, preventing binding of T cells to endothelial cells and blocking trafficking of T cells from the circulation into the psoriatic skin, preventing their reactivation in the dermal and epidermal layers (Lebwohl et al. 2003). Blockade of costimulatory molecules on T cells, particularly CD11α, as occurs with efalizumab, also results in a sustained unresponsiveness to viral and other pathogens in animal models. It has been also demonstrated to reduce T cell activation produced by polyclonal stimuli; this T cell hyporesponsiveness is fully reversible following efalizumab washout. Similarly, during active therapy but not following its elimination, it has been demonstrated to reduce the cellular immune response to intracutaneous recall antigens (Krueger et al. 2008). Although as natalizumab and efalizumab result in an increase in peripheral blood leukocytes during treatment, these cells are predominantly circulating $CD3^+$ cells with the largest increase in memory $CD8^+$ T cells. Moreover, efalizumab reduces cutaneous dendritic cells, but its effect on cerebral perivascular dendritic cells is unknown (Berger 2010).

On 8 April 2009, Genentech (South San Francisco, CA, USA) announced a phased voluntary withdrawal of efalizumab from the market based on its association with PML. Interestingly, as natalizumab, efalizumab is directed against members of the integrin family, which raises the question whether there may be a relationship between anti-integrin agents and JCV/PML (Lysandropoulos and Du Pasquier 2010).

Rituximab

Rituximab is a chimeric anti-CD20 mAb that depletes mature circulating B lymphocytes in the blood, and apparently also in the CNS. It is approved for CD20-positive B cell non-Hodgkin's lymphoma, untreated chronic lymphocytic leukemia (approved by EMEA in 2009), and as a second-line treatment for RA. To date, 114 PML cases have been associated to treatment with rituximab (Carson et al. 2009a; Keene et al. 2011). Its mode of action may be based on the decrease of both the humoral and cellular immune responses, due to dimished help provided by B cells to T cells (Cross et al. 2006). In a recent work, Carson and colleagues (Carson et al. 2009b) reported 57 cases of PML in patients treated with rituximab either for lymphoma/leukemia or for autoimmune

diseases, the latter group including two patients with systemic lupus erythematosous (SLE). However, rituximab was always associated with other immunosuppressive treatments. Rituximab is not recommended for SLE; nevertheless, at least 8,000 SLE patients have been treated with this drug, and two of them developed PML (Carson et al. 2009a). Conversely, there have been so far 30 cases of PML reported in SLE patients who had never received rituximab. Thus, on the basis of these data, it seems that SLE rather than rituximab may be a trigger of PML. Of note, no case of PML has been reported so far in MS patients treated with rituximab (Hauser et al. 2008).

Despite of these cases of PML emerged after treatment with rituximab, always used in combination with other immunosuppressive treatments, the pathophysiology of PML associated with this mAb remains uncertain. Rituximab treatment does not result in T cell depletion, as seen in AIDS. However, it is possible that after rituximab B cell depletion, subsequent repletion of the mature B cell population results in expansion of pre-B cell harboring latent JC virus that can use these lymphocytes to reach the CNS and to infect permissive cells, such as oligodendrocytes (Fig. 1). However, additional studies are warranted to confirm this hypothesis. For the time being, in Europe and in the USA, the manufacturer in collaboration with the respective health agencies proposes a patient alert card mentioning the risk of PML for patients treated with rituximab (Carson et al. 2009a; Lysandropoulos and Du Pasquier 2010).

Anti-TNF-α mAbs

The anti-TNF-α biological agents, such as adalimumab and infliximab, are widely used in several autoimmune diseases, mainly in CD and severe forms of plaque psoriasis. Several cases of demyelinating events of the nervous system in association with the anti-TNF-α therapy have been reported, prompting a heightened surveillance of treated patients, althought the incidence of such events is relatively low (Keene et al. 2011).

There are contradictory data in literature as to whether anti-TNF-α treatment could worsen demyelination or even cause demyelination. TNF-α is thought to play a significant role in the pathophysiological mechanism of several inflammatory diseases such as RA, ankylosing spondylitis, inflammatory bowel diseases (IBD), such as CD and MS. High levels of TNF-α have been found in plaques and in CSF of MS patients (Andreakos 2003). In chronic progressive MS, CSF levels of TNF-α correlate with disability and the rate of neurological deterioration. In animal model of MS, administration of anti-TNF-α therapy improves the outcome of the disease (Selmaj and Raine 1995). Since then, additional anti-TNF-α drugs have been developed, including the mAbs adalimumab and infliximab. These are recognized treatments for RA, psoriatic arthritis, ankylosing spondylitis, and CD, but not for MS, since patients treated with anti-TNF-α drugs develop different forms of CNS and peripheral nervous system demyelination. Up to July 2009, 175 cases of CNS demyelination after starting anti-TNF-α therapies have been reported, including optic neuritis, MS or MS-like, and cognitive dysfunction. The majority of cases occurred between 1 month and 1 year after initiation of the biological agent. Moreover, 44 patients on anti-TNF-α also presented with demyelination of the peripheral nervous system, including Guillain–Barrè syndrome, multifocal motor neuropathy, chronic inflammatory demyelinating polyradiculoneuropathy, axonal polyneuropathy, and Lewis–Summer syndrome (Ramos-Casals et al. 2010). The mechanism by which anti-TNF-α medications would trigger demyelination remains unexplained. It has been hypothesized that exposure to anti-TNF-α might, between other effects, increase survival of autoreactive peripheral T cells penetrating the CNS, produce proinflammatory cytokines such as interferon gamma (IFN-γ), and cause demyelination (Mitoma et al. 2005). However, recently, the first case of PML in a man with erosive RA after 3 years of treatment with infliximab (Kumar et al. 2010) has been reported. The TNF-α blocking could also result in decreased expression of interleukin (IL)-6 and IL-1, IFN-γ, and other proinflammatory cytokines, with a subsequent reduction in inflammation at the macrophage site of TNF-α production. A reduction in cell recruitment occurs also through reduced expression of $CD3^+$, $CD68^+$, VCAM-1, ICAM, and E-selectin. A reduction in T cell activity has been theorized to occur through a reduction in IFN-γ, STAT-1, granzyme B, and T cell inflammatory gene expression as well as a reduction in dendritic cell-mediated T cell activation (Gottlieb et al. 2005). Therefore, although infliximab reduced clinical symptoms, it also could unbalance the local immune surveillance, particularly the affect on T cell activation and the consequential reduction of IFN-γ expression, involved in the anti-viral state control, and enhance the latent JCV reactivation, with direct implications on the risk of PML (Bellizzi et al. 2010) (Fig. 1).

However, tempering these alarming data, members of the BIOGEAS project conducted a meta-analysis of randomized control trials and postmarketing studies and found that these demyelinating events were rare, ranging between 0.05% and 0.20% of anti-TNF-α-treated patients. Nevertheless, because of the concern that anti-TNF-α treatment may trigger or worsen demyelination in some patients, a baseline brain MRI is recommended prior to the initiation of anti-TNF-α treatment (Ramos-Casals et al. 2010).

JCV and cancer in the era of biological therapies

The phenotypic complexity of cancer makes it difficult to determine the specific roles for infectious agents, such as viruses and bacteria, that might be considered carcinogenic,

and even more difficult to determine their implication at a particular stage in disease progression (Hanahan and Weinberg 2011). More recently, the association between the development of lower gastrointestinal tract neoplasias and infectious agents, such as between CRC and JCV infection (Burnett-Hartman et al. 2008), has been reported. JCV is a virus very well adapted to humans; thus, its widespread infection and adaptation to humans complicate the determination of its etiologic contribution to cancer development. The mechanistic role of infectious agents is even more complex because they might contribute in several different ways to oncogenesis. Thus, causality of infectious agents in cancer is somewhat more difficult to demonstrate (Jiang et al. 2009). CRC is the third most common tumor in women and the fourth in man, and more than 500,000 deaths are caused by this malignant disease. Most colorectal cancers are sporadic in their origin and associated to different risk factors, such as a diet rich in fat and animal protein intake, but we cannot forget that patients with ulcerative colitis and Crohn's disease (CD) are at increased risk for developing CRC (Parkin et al. 2005). Chronic inflammation is believed to promote carcinogenesis. The risk for CRC increases with the duration and anatomic extent of colitis and presence of other inflammatory disorders (Ullman and Itzkowitz 2011). In particular, CD is a chronic IBD, with increasing prevalence that reflects the interaction of at least three components: a genetic predisposition, an environmental trigger, and an unregulated or deregulated immune response (Mayer 2010). Recent introduction of infliximab and adalimumab in the management of CD has been immediately associated with the development of serious life-threatening infections, such as JCV reactivated infection. As already explained above, the TNF-α blocking results in decreased expression of IL-1, IL-6, INF-γ, and other proinflammatory cytokines that could unbalance the local immune surveillance and enhance JCV reactivation from its several sites of latency (Bellizzi et al. 2010). Moreover, the association between JCV infection and chronic idiopathic intestinal pseudo-obstruction has led to speculate that this neurotropic virus may undergo activation from a latent state in the enteric glial cells to a productive lytic infection, with serious clinical consequences in affected patients (Selgrad et al. 2009). Since the use of infliximab in CD patients blocks the TNF-α and interferes with the recruitment of the activated T lymphocytes causing a decreasing of IFN-γ levels, involved in the anti-viral state control, JCV could leave its latent state within the enteric glial cells and perhaps within the intestinal epithelium, where the virus might establish latency (Ricciardiello et al. 2000).

Generally, there are two possible outcomes to JCV infection: permissive cells, as oligodendrocytes, are able to support viral DNA replication resulting in a lytic infection; in nonpermissive cells, as those of the colorectal epithelium, resulting in silent or abortive infection, or probably in cell transformation and cancer (White and Khalili 2004). Despite these evidences, the role of JCV in human malignancies, and of its oncoproteins in promoting transformation of cells in vitro and in vivo, is still far from clear. JCV does not infect experimental animals, and its roles have been implied by analogy to SV40. However, JCV can also infect and transform different cell types in culture and is highly oncogenic in several laboratory animal models (Maginnis and Atwood 2009). Furthermore, JCV DNA sequences have been detected in a broad range of human tumors of glial and non-glial origin, including gliomas, ependymomas, and medulloblastomas, as well as in several non-neural clinical specimens of upper and lower gastrointestinal tumors, such as CRC (Burnett-Hartman et al. 2008), suggesting that the virus can infect a wide range of cell types, but the role of JCV in human malignancies is still unclear. A very important issue is to determine whether the genomic sequence of JCV presents any difference between tumor of different grades and its normal surrounding mucosa, but very few studies have been performed in this context and in none of them there is any indication about the JCV strain implicated. The variability in detected JCV genome suggests that in some infected colon cells, there might be integration with partial loss of JCV DNA, which may have a pathogenic role in cancer development, probably allowing additional events that will lead to cancer progression with a selection of a cell subpopulation. When human CRC samples were grown as xenographs in nude mice that permit expansion of the cancer cell population, all of them resulted positive for JCV, suggesting that the cell subpopulation containing JCV might be selected for its growth and adaptation characteristics (Laghi et al. 1999). JCV might participate in different ways in the pathogenesis of CRC as a consequence of the complexity of the mechanisms contributing to cancer phenotype, which have many different phases, ranging from initiation, promotion, morphological progression with different biological characteristics to tumor maintenance and dissemination. Thus, to pinpoint a unique mechanism of action for a virus represents a very simplistic approach to the problem (Coelho et al. 2010).

Among the viral proteins, the main role in the tumor is played by the JCV early protein TAg, involved in the cell cycle progression to the S phase (Khalili et al. 2008). Binding of TAg to pRb promotes the activation of the E2F family of transcription factors,which induce the expression of cellular genes required for S phase. In the context of lytic infection, this cell cycle progression is necessary for viral replication because polyomaviruses rely on S phase-specific host factors for their DNA synthesis. In the context of cellular transformation and tumorigenesis, TAg/pRb interaction is an indispensable event (White and Khalili 2006). Moreover, JCV TAg has the ability to interact with p53, allowing the occurrence of additional genetic damage that was considered a step forward in carcinogenesis (Nosho et al. 2009). JCV also by a hit and run mechanism, that is a transient effect, is able to trigger genetic instability by interacting with p53 and β-catenin

(Ricciardiello et al. 2003). In addition, there are several additional mechanisms by which TAg can also interfere with cellular functions. JCV TAg can interact with proteins involved in cellular regulation such as insulin receptor substrate-1, a major protein of the insulin-like growth factor I receptor (IGFIR) signaling pathway, which is activated and translocated to the nucleus in the presence of TAg. Activated IRS1 induces the PI3K signaling cascade, implicated in cell survival, and proliferation signals (Khalili et al. 2003). Thus, at the same time, it will permit survival and expansion of a JCV-containing subpopulation. These signaling effects associated to TAg can participate at any given stage of cancer progression, facilitating the expansion of a specific subpopulation, perhaps already pretumoral. TAg can also inhibit homologous recombination DNA repair causing DNA damage, mechanistically by its interaction with IRS1, which also interacts with Rad51 at locations of damaged DNA, and thus may contribute to generate some genetic instability in cells containing JCV (Reiss et al. 2006). TAg also contributes to the stabilization of β-catenin by a novel mechanism mediated by the small GTPase Rac1 (Bhattacharyya et al. 2007). β-catenin is an integral component of the Wnt signaling pathway whose stabilization is associated with increased transcription of genes that regulate cellular proliferation, e.g., c-myc and cyclin D1, despite the fact that the functional consequence of this JCV interaction in cancer development remains to be elucidated. TAg interacts with β-catenin, and β-catenin implication in colorectal cancer is well known (Enam et al. 2002). If the effect persists for some time in infected cells harboring JCV, they might contribute to expand a cell subpopulation that later might give rise to cancer (Fig. 1).

Another important component of tumorigenesis is represented by the tumor microenvironment, where local cytokines can play a stimulatory role. These cytokines may originate either in the tumor itself or in the local inflammatory infiltrate, and can activate JCV gene expression by a cis-acting transcription factor, Egr1; a factor which mostly activates the late promoter and affects VP1 expression and viral replication. The expression of this capsid protein can also trigger an inflammatory and immune reaction, with the corresponding local availability of several additional cytokines. This viral reactivation might also result in viral production, dissemination, and reinfection of neighboring cells. This mechanism could be important for maintenance or reactivation of a local subclinical infection (Coelho et al. 2010).

Conclusions

The link between the effects of the biological therapy on the immune system and the occurrence of PML has prompted investigations on JCV sites of latency in the bone marrow, the migration of bone marrow-derived cells (such as $CD34^+$ hematopoietic precursors) into the circulation, and intracellular virus entry into the brain. A hypothesis has gained general acceptance that JC virus causes a primary infection in childhood and enters a latent state, after which immunosuppression allows viral reactivation and genome rearrangement leading to PML. Nonetheless, many important aspects of PML pathogenesis remain unclear, including the molecular bases of latency and reactivation, the site(s) of latency and viral genome rearrangement, and the mode of virus transmission within the body and between individuals (White and Khalili 2011). Currently, there are also no drugs approved for the treatment of PML that have been demonstrated in the patient to effectively and reproducibly alter the course of disease progression. Therefore, since the number of patients undergoing immunomodulatory therapies that develop PML continues to rise (Major 2009), the understanding of JCV reactivation is increasingly important. Furthermore postmarketing surveillance is necessary to gather information on long-term efficacy of these mAbs and on identification and management of their side effects, although improved methods for the detection of low levels of viral DNA may help stratify PML risk and guide therapeutic strategies. At the same time, neurologists must follow the indication of these mAbs and be aware of their potential demyelinating side effects.

Moreover, considering JCV reactivation in nonpermissive cells after treatment with mAbs, such as intestinal epithelial cells in CD patients, we might take in account the emerging investigation field on JCV association and cancer, such as CRC. The role of any virus as a causal agent in cancer should not be considered simply as a direct cause–effect. The virus should be considered as a complex agent with potentially multiple and varying effects. In order to establish if JCV is an important risk factor for CRC, we should improve detection of viral DNA, as well as strain identification. This should also contribute to establish the potential and irreversible integration of remnant JCV DNA in cancer cells and permit identification of significant differences among malignant, benign, or normal surrounding tissue. Then, we should correlate JCV DNA presence with other known risk factors of cancer, as well as to identify the eventual viral protein expression at different stages of CRC progression. For this aim, development of better specific antibodies for JCV proteins is necessary. Finally, the host immune response to JCV characterization could be considered a very important achievement as it would allow to develop strategies to eradicate the virus from the human worldwide population or at least to manage the JCV infection in patients at risk of PML.

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Conflict of interest The authors declare that they have no conflict of interest.

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