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Natalizumab and Progressive Multifocal Leukoencephalopathy: What are the causal factors? Can it be avoided?

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Abstract

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Natalizumab (Tysabri®) was the first monoclonal antibody approved for the treatment of relapsing forms of multiple sclerosis (MS). After its initial approval, three patients undergoing natalizumab therapy in combination with other immunoregulatory and immunosuppressive agents were diagnosed with progressive multifocal leukoencephalopathy (PML). The agent was later re-approved, and its use restricted to monotherapy in patients with relapsing forms of MS. Over the past year, five additional cases of PML were reported in MS patients receiving natalizumab monotherapy. Thus, there is currently no convincing evidence that natalizumab-associated PML is restricted to combination therapy with other disease modifying or immunosuppressive agents.

The initial section of this review focuses on the scientific rationale for natalizumab in MS treatment. In the second part, our understanding of PML will be outlined. Thirdly, recent results on altered immune surveillance under natalizumab treatment are reviewed. In the forth section, the link of viral reactivation and very late activation antigen 4 (VLA-4) antagonism will be discussed. Finally, this review will address the potential impact of our current knowledge on the use of natalizumab in clinical practice.

The natalizumab experience

Multiple Sclerosis is an inflammatory demyelinating disorder of the central nervous system (CNS) and one of the most common causes of sustained neurological disability in young adults.[1] The presence of leukocytes in cerebral perivascular spaces (CPVS) in areas of disease activity is one of the pathological hallmarks.[2–4] An absolute requirement for the influx of leukocytes from the peripheral blood into the CNS is their expression of adhesion molecules, called integrins, and their interaction with counter-receptors from the immunoglobulin supergene family proteins on endothelial cells. Alpha(α)4-beta(β)1-Integrin (very late activation antigen 4 (VLA-4)) is one of the four main integrins required for the firm arrest of leukocytes following their rolling adhesion.[5]

Natalizumab (Tysabri®) is a recombinant humanized monoclonal IgG4-antibody that binds, amongst others, to the α-4-subunit of the α4β1 integrin, and interferes with the α-4 mediated binding to its natural ligands of the extra cellular matrix and endothelial lining, vascular cell adhesion molecule-1 (VCAM1) and fibronectin (FN).[6–7] Although inhibition of leukocyte migration and extravasation is believed to be the leading mode of action of natalizumab, additional mechanisms might modulate the therapeutic and adverse effects of this antibody. Lindberg et al. recently showed that natalizumab has a direct effect on gene expression relevant for function and differentiation of T-lymphocytes, B-lymphocytes, neutrophils and erythrocytes.[8]

In vivo, antibodies against VLA-4 interfere with the binding of leukocytes to cerebral blood vessels, and effectively prevent signs of experimental autoimmune encephalomyelitis (EAE), an animal model of MS.[9] Although natalizumab is highly immunogenic in mice, it reduces the influx of T cells and monocytes into the CNS and substantially ameliorates the clinical course of EAE.[10]

The efficacy of natalizumab in EAE led to clinical trials for the treatment of Multiple Sclerosis (MS). Following very promising results in phase II studies,[11–13] phase III clinical

trials were performed that compared natalizumab alone versus placebo (AFFIRM trial[14]), and natalizumab plus interferon beta-1a (IFNβ-1a) versus placebo plus IFNβ-1a (SENTINEL trial[15]). Both studies showed significant advantage for the natalizumab-treated groups with respect to the primary clinical endpoints. In the AFFIRM monotherapy trial, natalizumab reduced the rate of clinical relapses at one year by 68 percent and the risk of sustained progression of disability by 42 percent over two years. Post hoc analysis of the AFFIRM trial showed disease remission defined as no activity on clinical (no relapses and no sustained disability progression) and radiological measures in 37% of the natalizumab group compared to 7% of the placebo group.[16]

In November 24, 2004, the Food and Drug Administration (FDA) approved natalizumab for the treatment of relapsing forms of MS. On February 28, 2005, the manufacturers of natalizumab announced the voluntary withdrawal from the market after two MS patients in the SENTINEL trial (combination therapy with IFNβ-1a) and one patient with Crohn's disease were diagnosed with progressive multifocal leukoencephalopathy (PML).[17–19]

In the summer 2006, natalizumab was re-approved in the United States (US), and approved in the European Union (EU) as monotherapy for the treatment of relapsing forms of MS. In the US, recommendations were made to limit the use of natalizumab to highly active (more than 2 severe relapses per year) relapsing remitting (RR)-MS and for patients not responding to or tolerating first line treatment (IFNβ-1a, IFNβ-1b, glatiramer acetate). This restricted approval was the result of a risk-benefit analysis. The initial diagnosis of PML in two patients from the combination therapy trial[17–18] led to the restricted approval and to risk minimization plans (TYSABRI Outreach: Unified Commitment to Health (TOUCH), TYSABRI Global Observation Program In Safety (TYGRIS), Crohn's Disease - Investigating Natalizumab through Further Observational Research and Monitoring (CD INFORM)). Systematic review of all patients treated in those studies (more than 3700 patients) showed no additional cases, therefore, calculated risk of PML was 1:1000 (95% CI) after an average treatment time of about 18 months.[20] As there is no increased risk of JC-polyomavirus-replication and spread throughout the CNS in patients with MS per se,[21] there is little doubt that treatment with natalizumab is linked to these cases of PML.

According to information provided by Biogen Idec, about 53,000 patients had been treated with natalizumab by December 2008 (including patients enrolled in clinical trials). Of those patients, 20,000 have received at least one year of natalizumab therapy, approximately 10,700 patients have been on therapy for 18 months and 4,300 patients have received natalizumab for at least 24 months.[22]

In the past 12 months, five new cases of PML have been reported, bringing up the total number of PML under natalizumab therapy to eight thus far.[22–23] Because of the restricted use of natalizumab, all of those new patients had been treated with monotherapy. PML was observed after 8, 12, 14, 14, 17, 26, 28 and 37 infusions. These new cases remind us of the necessity to further investigate mechanisms that predispose certain patients to an increased risk of developing PML under natalizumab therapy.

PML – Primary infection and places of latency

PML is an opportunistic demyelinating disease of the brain. It is caused by reactivation of JC-Virus, a dsDNA-virus belonging, together with SV-40- and BK-virus, to the family of polyomaviridae.[24] PML has almost exclusively been reported in immunocompromised patients, in particular in patients with reduced cellular immunity, including patients with HIV, patients with haematological diseases, or in patients receiving immunosuppressive medication.[25–26] Therefore, detection of JCV-specific cytotoxic CD8+ T lymphocytes in PML patients is associated with a favourable outcome and early disease control[27] and assays measuring CD4 cell immune functions are currently being investigated as monitoring tools for immunocompromised populations to estimate their risk of polyomavirus infections.[28]

Primary infection by JC-virus takes place in childhood and is asymptomatic. JC-virus-antibodies are seen in 50 – 85% of all adults.[29–31] JCV persists in the tonsillar tissue, bone marrow, the kidney and the spleen.[32–34] The presence of JCV-DNA has been detected in urine, different blood compartments, and in CSF.[35–37] JCV viruria was found as frequently in HIV-positive individuals as in control subjects, suggesting that its detection has no clinical value.[38] In a recent study, viruria was seen in 25.5 % of healthy volunteers.[39]

JCV-DNA has been detected in peripheral blood mononuclear cells (PBMC) in HIV-positive patients with and without PML but not in HIV-negative control subjects. There appears to be no specificity for any leukocyte subtype. The presence of JCV-DNA detection correlates with low CD4+ lymphocyte counts.[38] Furthermore, JCV-DNA was recently found in peripheral blood of immunosuppressed patients with Crohn's Disease (CD),[39] but not in controls. In contrast, only one of the first three confirmed cases of PML (the patient with CD) on natalizumab had consistently elevated JCV-DNA plasma levels before onset of PML symptoms. Consequently, JCV-load might correlate with immunosuppression but may have limited predictive value as a screening tool for PML.[40]

Currently, our knowledge about transmission of JCV infection and its replication cycle in the healthy human population is limited. The transmittable form of JCV is commonly referred to as the JCV-archetype, as it is thought that all other genotypes originate from it. The JCV-archetype is detectable in the urine.[41] In contrast, the JCV-PML-type, referred to as MAD-1 (named after the University if Wisconsin at Madison, where it was characterized) appears more neurotropic, and can be isolated from brains of PML patients. This pathological variant is characterized by deletions, duplications and point mutations in a specific JCV regulatory region.

There is growing evidence on persistency of JCV in the CNS.[42–44] A more recent study investigated viral protein and DNA load in brains of immunocompetent non-PML patients. No viral proteins were expressed in any of these cases. Nevertheless, fragments of the viral DNA were present in various regions of normal brain. JCV DNA was found in oligodendrocytes and astrocytes, but not in neurons.[42] These findings suggest that JCV has access to the brain in immunocompetent individuals. In the setting of immunosuppression, it is conceivable that either the passing of virus during JC-viremia, or resident virus persisting in normal brain may express its genome and initiate its lytic cycle in oligodendrocytes.[42]

The prognosis of PML is poor. Notwithstanding that mortality has substantially dropped since the introduction of highly active antiretroviral therapy (HAART) for treatment of the acquired immune deficiency syndrome (AIDS), PML still is fatal in about 50% of HIV-associated PML cases within the first three months after diagnosis.[25] No pathognomonic initial symptoms of PML have been defined, which often makes an early clinical diagnosis of this disorder very challenging. Some of the classic clinical signs and symptoms of PML include rapidly progressive dementia, motor dysfunction and vision loss, which can be difficult to differentiate from MS relapses.[33, 45–46]

Critical for the diagnosis of PML is the demonstration of virus by JCV-PCR in the CSF and surrogate disease markers by magnetic resonance imaging (MRI). Before the introduction of HAART, JCV-PCR had a sensitivity of 72–93% and a specificity of 92–100%.[25] Since HAART therapy, sensitivity is reported to be lower (about 58%) most likely due to an improved immune response under antiretroviral treatment.[47–49] A recent study showed positive JCV-PCR results in some MS patients without any clinical or radiological evidence for PML. Thus, low numbers of JCV-copies within the CSF might also be part of the physiological replication process.[50] As there are only 8 cases in natalizumab treated patients so far, sensitivity and specificity of CSF-JCV-PCR for this subpopulation is unknown, although one case of false negative PCR in one of the recently reported PML cases proved limited sensitivity and gives reason for concern.[23]

MRI is a sensitive detection method, but many surrogate disease markers are not specific for PML. Typically, there are hyperintense, multifocal asymmetric signal abnormalities throughout the supratentorial subcortical white matter on T2-weighted and fluid attenuated inversion recovery (FLAIR) sequences.[20,51] While there is a relative absence of gadolinium uptake in PML, in cases of immune reconstitution inflammatory syndrome (IRIS), contrast enhancement is frequently observed. This syndrome is defined as a combination of clinical worsening in a PML patient despite recovery of the immune system, explained by an overwhelming inflammatory immune response.[52] This syndrome was first seen in AIDS patients after initiation of HAART, but has also been reported in natalizumab treated patients.[23]

Restoring the immune system is the only proven intervention for PML.[26,53] The downside of restoring an immune response against JCV is the risk of IRIS. There is some evidence that IRIS can be attenuated through the administration of corticosteroids.[54] While immune reconstitution is realized by HAART therapy in AIDS patients, early cessation of immunosuppressive medication was associated with favourable clinical outcomes in transplant recipients.[55–56] Plasma exchange has been proposed as a therapeutic tool that may allow a faster restoration of immune effector function in natalizumab-treated patients.[53,57] Unfortunately, it appears that the pharmacological half-life of natalizumab far exceeds its biological half-life.[58] Therefore, even accelerated clearance of treatment may not have a beneficial effect in all affected individuals. Currently a randomized multicenter trial is underway for the treatment of PML with mefloquine. The primary outcome measure is the JCV-PCR product in the CSF over up to 24 weeks.[59] This trial is based on positive in vitro data on efficacy of mefloquine on JCV-replication.[60]

Impaired immune surveillance in Natalizumab-treated MS patients

Reactivation of a latent infection in natalizumab treated patients is likely linked to its therapeutic principal of action. Natalizumab was specifically designed to reduce trafficking of lymphocytes into peripheral tissues; therefore, it was postulated that treatment with natalizumab results in reduced immune surveillance of the CNS. Indeed, cerebrospinal fluid (CSF) from natalizumab-treated patients with MS contained significantly fewer white blood cells (WBC), CD4+ T cells, CD8+ T cells, CD19+ B cells and CD 138+ plasma cells compared to MS patients not treated with natalizumab.[58] Furthermore, the CD4/CD8-ratio in the CSF of natalizumab-treated patients was significantly reduced to levels comparable to those of HIV-patients.[61] In contrast to HIV-patients, there was no reversed CD4/CD8-ratio in peripheral blood. It was also shown, that CD4+ cells express significantly less unbound α-4-integrin pre and post natalizumab therapy compared to CD8+ cells.[61] Therefore, an absolute threshold of unbound VLA-4 may be required for migration across the endothelial barrier, perhaps partially explaining reduced migration of CD4+ T cells into the CNS. Surprisingly, reduced cell counts were still detectable six months after cessation of natalizumab treatment.[58] This was unexpected, as natalizumab has a biological half-life of eleven days, and its biological activity therefore can not be expected to continue beyond six weeks after cessation.[62]

The preferential and prolonged effect of natalizumab on CD4+ T cell number in the CNS could possibly be explained by a decrease in the number of antigen presenting cells (APC), and the expression of major histocompatibility complex (MHC) class II antigens in cerebral perivascular spaces (CPVS). Autopsy material from a patient who died of PML while on natalizumab treatment[17] was investigated for MHC-expression and the number of APCs within the cerebrovascular spaces,[63] and compared to anatomically-matched healthy brain tissue from MS patients not treated with natalizumab, and tissue from patients with PML not associated with natalizumab treatment. MHC II expression and the number of APCs in CPVS were significantly reduced compared to control brains. In addition, not a single CD4+ T cell was detectable in the CPVS of natalizumab treated PML patients, whereas CD8+ T cell numbers were not reduced in the CPVS as compared to controls. The latter might not be surprising, as MHC I was shown to be significantly upregulated in PML patients treated with natalizumab compared to all other controls. While it is difficult to detect JCV-responsive $CD4^+$ T cells in patients with PML,[64] JCV-specific $CD8^+$ T cells are detectable in the peripheral blood of PML patients infected with the HIV, and their presence has been associated with a more favorable outcome.[27,65,66] $CD8^+$ T cell responses are directed toward an HLA-A*0201-restricted JCV epitope, $VP1_{p36}$.[67] However, Initiation and perpetuation of antigen-specific $CD8^+$ T cell responses are likely to require the help of $CD4^+$ T cells in the form of cytokines and other inflammatory mediators.[68]

VLA-4 Antagonism, viral reactivation and malignancy

There are two possible mechanisms of JCV reactivation discussed in the literature. Either the persisting virus within the CNS or passing virus during JC-viremia is responsible for JCV reactivation in the setting of immunosuppression or impaired immunosurveillance. Interestingly, CD34+ cells are susceptible to JCV infection,[69] and JCV-DNA is detectable in

peripheral lymphocytes from patients with and without PML.[35,38,70] In addition, CD34+ haematopoietic precursor cells express high levels of VLA-4 on their surface[71–73] and CD34+ bone marrow cells have, in comparison to circulating cells in the peripheral blood, a higher VLA-4 expression rate and VLA-4-avidity.[74] Bonig and co-workers and our group recently showed that natalizumab mobilizes CD34+ haematopoietic progenitor cells.[75–76] Binding of natalizumab to α4-integrin may block the VLA-4 mediated interaction of CD34+ cells of the bone marrow with its ligands in the extra cellular matrix, e.g. VCAM1, and may lead to mobilization of CD34+ cells to the peripheral blood. Antibody mediated blockage of CD34+ cell-homing into the bone marrow could play an additional role.[77–78] Mobilization of haematopoietic progenitor cells by monoclonal antibodies against VLA-4 in primates and mice was also demonstrated.[79–81]

As outlined above, natalizumab upregulates transcription factors important for differentiation of B lymphocytes.[8] Thus, during natalizumab induced B cell differentiation, JCV-infected bone marrow cells might be activated leading to JC-viremia and PML as a consequence of natalizumab therapy.[82–84] In this context, rearrangement of archetype-JCV to PML-type to the MAD-1 genotype could occur.[41,85] This hypothesis could also help to explain PML cases under treatment with monoclonal antibodies against CD20 and CD52. After initial depletion, reconstitution of the B cell line might cause JC-viremia and PML.[83] However, so far there is no conclusive evidence for an increased incidence of JC-viremia in natalizumab treatment. Furthermore, given the fact that natalizumab significantly reduces the extravasation of cells that express VLA-4, the presence of CD34+ cells in peripheral tissues under natalizumab therapy needs to be verified.

Thus far, there are only few reports on the reactivation of CNS latent virus other than JC virus in natalizumab-treated patients. Human herpes virus (HHV)-6 is a pleiotropic β-herpes virus commonly reactivated in the setting of acute and prolonged immunosuppression.[86,87] HHV-6 has also been suggested to be involved in pathogenesis of MS,[88,89] and it has also been associated with PML pathogenesis.[90] Elevated serum HHV6 IgG levels and HHV6A DNA in the CSF of a subset of patients treated with natalizumab were recently reported. Also, in vitro superinfection of JC-virus infected glial cells with HHV-6 increased JCV-expression.[91] Interestingly, HHV-6 has also been detected in CD34+ haematopoietic progenitor cells.[92,93]

JCV is probably not the only latent virus reactivated in natalizumab-treated patients. Mobilization of virus infected bone marrow cells might be a natalizumab-associated, but not a virus specific side effect. Altered immune surveillance combined with potential latent viral reactivation could also enhance the risk of malignancy. Thus far, there have only been three reported cases of melanoma in natalizumab-treated patients, which may present association by chance.[15, 94]

Alternative Treatment Paradigms

Existing data indicate that natalizumab is immunosuppressive, and that these properties may be a contributing factor in the susceptibility to CNS infections. Also, published reports suggest that there is a dose-duration effect on the risk of developing an infectious

complication in some patients. In addition, it has not been shown that prolonged continuous therapy with natalizumab is required to ensure its efficacy. Therefore, alternative treatment paradigms appear feasible: (1) Based on published observations we know that natalizumab has an immediate effect on the number and composition of leukocytes in the CSF.[58] (2) It is also known that the effect of natalizumab on leukocyte numbers in the CSF after cessation of treatment persists for at least 6 months,[58] and (3) that cell numbers normalize 14 months following the discontinuation of therapy.[95] In addition, it was demonstrated that the patients are clinically stable on first-line disease modifying therapies (DMTs) during the 14 months period after cessation of natalizumab.[95] While an increase in T2 lesion load on MRI 15 months after cessation of treatment has been shown in one study,[96] there was no change of surrogate disease markers on magnetic resonance imaging (MRI) in another.[95] Thus, it may be necessary to limit the use of natalizumab for a certain period of time, followed by a treatment holiday during which patients are treated with one of the conventional disease modifying therapies. In patients with very aggressive disease natalizumab may only be used as an induction therapy. Such treatment algorithms remain speculative at present and controlled clinical trials must be performed to shed further light on these clinically relevant questions.

Conclusions

Currently, natalizumab is only recommended as monotherapy in patients with MS not responding to first line treatment or treatment naïve patients with high clinical disease activity, or in those not capable of tolerating conventional therapy. This restricted approval originated from the observation of patients who developed progressive multifocal leukoencephalopathy (PML) under natalizumab therapy in combination therapy with IFNβ-1a in the context of clinical studies.[15] Recently, five more cases of PML in patients with MS who had received natalizumab in monotherapy were reported.[22–23] Thus, there is currently no convincing evidence that monotherapy is safer in this regard than combination therapy with disease modifying agents. PML is not a natalizumab-specific side effect, it has been diagnosed in the context of many other immunoactive drugs as well. Clearly, further studies are warranted to understand the immunological effects of natalizumab besides blocking cell migration across the BBB.

As there is currently no proven treatment for patients suffering from PML under natalizumab treatment other than accelerated clearance of therapy,[57] the early establishment of a diagnosis is crucial. Upon the re-approval of natalizumab, each country initiated a risk management program to closely monitor patients at risk. Kappos et al. developed a three step diagnostic algorithm for natalizumab-treated patients with new or worsening neurological signs and symptoms. Early suspension of natalizumab treatment and strategies for clinical, MRI, and laboratory assessments were proposed.[40]

As outlined above, impaired immune surveillance and viral reactivation caused by treatment with natalizumab are yet not fully understood. However, there is growing suspicion that long-term treatment with natalizumab may put patients at risk of PML and presumably other infections, as well as neoplastic growth.

These key findings suggest long-term effects on CNS intrinsic immune system and viral reactivation. These observations may provide a scientific rationale for alternative treatment algorithms for natalizumab, which need to be evaluated in controlled clinical trials. The major challenge in the near future will be to identify biomarkers associated with an elevated risk of viral reactivation.

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