Therapeutic uses of anti-α4-integrin (anti-VLA-4) antibodies in multiple sclerosis

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Abstract

Multiple sclerosis (MS) is a disorder of putative autoimmune origin, where immune cells invade the central nervous system and cause damage by attacking the myelin sheath of nerve cells. The blockade of the integrin very late antigen-4 (VLA-4) with the monoclonal antibody natalizumab has become the most effective therapy against MS since its approval in 2004. It is assumed that the inhibition of VLA-4-mediated immune cell adhesion to the endothelium of the blood–brain barrier (BBB) alleviates pathogenic processes of MS and, therefore, reduces disease severity and burden. Not all approaches to treat additional immune-mediated disorders (e.g. Rasmussen encephalitis and neuromyelitis optica) with natalizumab have been successful, but allowed researchers to gain additional insight into mechanisms of specific immune cell subsets’ migration through the BBB in the human system. While the long-term efficacy and general tolerability of natalizumab in MS are clear, the over 400 cases of natalizumab-associated progressive multifocal leukoencephalopathy (PML) have been of great concern and methods of risk stratification in patients have become a major area of research. Modern risk stratification includes established factors such as treatment duration, previous immune-suppressive therapy, and anti-John Cunningham virus (JCV) antibody seropositivity, but also experimental factors such as anti-JCV antibody titers and levels of L-selectin. Today, anti-VLA-4 therapy is reserved for patients with highly active relapsing-remitting MS and patients are monitored closely for early signs of potential PML.

Keywords: natalizumab, progressive multifocal leukoencephalopathy, risk stratification

Introduction

Therapeutic monoclonal antibodies interfering with immune-cell trafficking have provided major advancements to the treatment of some inflammatory disorders. Such antibodies have been effective in a number of conditions, ranging from suppression of transplant rejection in the case of Muronomb (anti-CD3) (1) and Daclizumab (anti-CD25) (2), to using anti-integrin antibodies for amelioration of autoimmune disorders like psoriasis using anti-CD11a treatment (3) or multiple sclerosis (MS) using anti-CD49a (anti-α4-integrin) treatment (4, 5). Integrins are dimers with an α chain linked to a β chain: for example, CD11a plus CD18 form lymphocyte function-associated antigen-1; whereas CD49d (also called the α4-integrin chain) plus CD29 form very late antigen-4 (VLA-4) and CD49d plus CD103 form lymphocyte Peyer’s patch HEV adhesion molecule-1 (LPAM-1).

Concerns have, however, been raised that therapeutic monoclonal antibodies may inhibit beneficial immune responses as well as detrimental ones (6, 7). Anti-α4-integrin treatment with natalizumab has been approved for the therapy of MS, a chronic-relapsing inflammatory demyelinating disorder of the central nervous system (CNS), since 2006. In 2008, the Food and Drug Administration (FDA) approved natalizumab for the therapy of treatment-resistant Crohn’s disease (CD). In MS, the majority of patients react very favorably to the treatment, providing clear evidence for the relevance of leucocyte migration into the CNS. However, despite the undisputable benefits, success is clouded by the fact that anti-α4-integrin treatment is associated with an unfavorably severe adverse event, namely John Cunningham virus (JCV)-mediated progressive multifocal leukoencephalopathy (PML) (8–10).

Our review summarizes the state of knowledge on therapeutic use with the anti-α4-integrin antibody natalizumab, including its mechanism of action, its clinical efficacy and its safety profile. The differential effects of natalizumab on different aspects of clinical MS as well its differing effects in other autoimmune diseases studied, i.e. CD and rheumatoid arthritis (RA), provide important lessons for the relevance of the pathway mediated by VLA-4 and its ligand, vascular cell adhesion molecule-1 (VCAM-1), in different organ-specific autoimmune diseases. Furthermore, the selective association of VLA-4 neutralization with proposed loss of CNS immune...
surveillance in some patients (i.e. those with PML) teaches further important lessons on the relevance of VLA-4 in JCV homeostasis.

**Anti-α4-integrin treatment in MS and CD**

To understand the therapeutic use of anti-α4-integrin antibodies, it is important to understand the related disorders of interest, i.e. MS and CD. In both conditions, anti-α4-integrin treatment has been studied, with different success.

MS is broadly seen as a disorder of autoimmune origin, affecting the CNS and with a strong bias towards female patients (female:male ratio is 2–3:1). Patients today are usually diagnosed in their late-twenties (median 28 years) (11). The most common form of MS is relapsing-remitting MS (RRMS; 80–85% of MS patients), which is characterized by acute clinical episodes (relapses) following remissions.

The patients experiencing such a ‘relapse’ suffer from clinical symptoms ranging from visual and sensory deficiencies, pain, and loss of motor function depending on where the damage in the CNS is being inflicted. The periods between relapses are called ‘remission’, where the patient recovers from a relapse and regains most of his former abilities (7). However, remissions can be complete or partial, which leaves patients with some form of residual symptoms and contributes to accumulating disability over time (12). Neurodegeneration starts to play a more important role in the later stage of the disease, when relapses are less frequent, but the patient’s disability still progresses; this part of the disease is then called secondary-progressing MS (SPMS) (13).

Available therapies for disease modification in MS are largely successful for relapsing forms of MS, where inflammatory mechanisms are most important. The pathogenic processes leading to the array of symptoms summarized as ‘MS’ supposedly stem from an attack of the immune system against proteins of the myelin sheath in the CNS (14). Data suggest that a primary attack of T cells against oligodendrocytes in the brain leads to subsequent tissue inflammation consisting of T cells and macrophages, which then mediate damage of the brain tissue (15).

It stands to reason that the first T-cell mediated attack is initiated by few activated, auto-specific T cells, migrating over the blood-brain barrier (BBB) into the perivascular space between the endothelium of the capillary and the glia limitans, which marks the beginning of the CNS parenchyma (16). There, these T cells are confronted with special dendritic cells, which have been collecting (auto) antigens and present these to the T cells (17), which then start proliferating and migrate to the sites of primary inflammation (18) to mediate damage via direct cytotoxicity and secretion of cytokines (19). Antigens released by cytotoxicity damage then lead to secondary inflammation and the spread of the inflammatory target to other, secondary antigens (epitope spreading) (20). The release of cytokines activates the surrounding tissue cells and attracts myeloid cells such as microglial cells, dendritic cells, and ultimately other T cells.

CD is a type of inflammatory bowel disease, which has long been thought to be caused by an overactive adaptive immune system and infiltration of T cells into the gastro-intestinal tract (21), similarly to MS albeit in a different compartment. However, recent research suggests that CD might be caused by a reduced activity of the innate immune system, resulting in a shift in T-cell cytokines and, therefore, might only be secondarily mediated by the adaptive immune system (22). In any case, the infiltration of lymphocytes into the CNS or the intestine is one key pathogenic hallmark of CD and MS and it is therefore important to understand how immune cells migrate over cellular barriers.

**Principle mechanism of action of VLA-4 therapies**

Several key molecules and sequences of events are relevant for immune cell migration. The most important molecules in this process are integrins (23). Depending on the composition of the α and β chains, the integrin heterodimer fulfills different functions and reacts with different ligands. VLA-4, which is the main focus of this review, consists of the specific α4-integrin chain (CD49d) and the more common β1-integrin chain (CD29). The α4 chain can, however, also be part of LPAM-1 (α4–β7), which binds to mucosal vascular addressin cell adhesion molecule-1 (MAdCAM-1) on endothelium. Therefore blockade of the α4 chain with anti-CD49d antibodies blocks both α4–β1 (VLA-4) and α4–β7 (LPAM-1) integrins, the latter molecule LPAM-1 playing a role in the treatment of CD with natalizumab (24).

For the purpose of this review we will focus on the treatment of MS patients and, therefore, the blockade of VLA-4 by natalizumab. If VLA-4 is activated by a chemokine trigger and changes its conformation to the active form (25), it is able to bind to VCAM-1 expressed on activated endothelial cells of the BBB and to mediate firm adhesion of the immune cells, which is a pre-requisite of transmigration (26). VLA-4 is expressed on most leukocytes, but not on granulocytes and was therefore a promising candidate to inhibit the infiltration of immune cells of the adaptive immune system into the CNS, but leaving the innate functions of granulocytes largely intact.

**Development of the drug natalizumab**

As the expression of VLA-4 on immune cells seemed to be an ideal target to inhibit immune-cell infiltration, the development of inhibitory mechanisms was the next logical step. In 1992 Ted Yednock presented his work on an anti-α4-integrin antibody, which was tested experimentally in vitro on brain slices and in vivo in the model of adoptive-transfer experimental autoimmune encephalomyelitis in Lewis rats and prevented the induction of disease in treated animals (4). After promising results in the animal models, the antibody, which as a substance was now called ‘natalizumab’ (composed of a human IgG4 framework grafted to CDRs from a mouse anti-CD49d mAb), was tested in clinical studies with MS patients, progressing to a first phase I trial in 1999 (27), a phase II trial in 2003 (28), and finally resulting in two phase III trials in 2006 (AFFIRM, SENTINEL) (29, 30) with unprecedented, highly impressive clinical efficacy in reducing annualized relapse rates (68% relative reduction compared with placebo) and reducing lesion load assessed using magnetic resonance imaging (MRI).

**Anti-α4-integrin treatment-associated PML and immune reconstitution inflammatory syndrome (IRIS)**

There were two cases of PML in the phase III trials of 2006 (31, 32) in MS patients and one additional case in a CD trial.
IRIS given a corresponding clinical setting. Contrast-enhancing lesions, may be interpreted as indicative of IRIS is not primarily based on radiological criteria, newly occurring inflammatory processes seen on MRI, such as contrast-enhancing lesions, may be interpreted as indicative of IRIS given a corresponding clinical setting.

All PML survivors are left with residual neurological damage and recovery is very limited and difficult (38). PML occurs as mentioned above in cases of HIV infection (39), but also in rare cases of lymphopenia (40), leukemia (41), and more recently, treatment with monoclonal antibodies (42). There is currently no method for prevention or treatment of PML, which only increases the severity of this problem in cases where it occurs as an adverse event during treatment.

Current strategy is to detect PML as soon as possible to ensure a maximum of survivability and to limit residual neural damage (43). In the case of antibody-associated PML, elimination of therapeutic antibody from the patient using plasma exchange is considered a treatment option to accelerate immune reconstitution (10, 44). Because of its long biological half-life time, natalizumab requires rapid elimination from the circulation. This can be achieved by five or more exchanges of 1.2-fold blood plasma volumes over 5–8 days. This lowers the concentration of natalizumab in peripheral blood and desaturates α4-integrin molecules. Alternatively, immunoadsorption of natalizumab using a tryptophan or protein A column treating a plasma volume of at least 2000 ml can be performed. The comparative efficacy of plasmapheresis versus immunoadsorption with a tryptophan column or protein A columns is not established.

Elimination of natalizumab can be accompanied by antiviral therapy, albeit none has yet proven to improve clinical outcome. However, this recovery usually leads to IRIS in these patients (45), where the strong influx of immune cells into the virus-affected brain tissue has unwanted (and sometimes lethal) consequences (46). Therefore, the immune system recovery has to be carefully controlled to allow for a virus clearance with as little neurological damage as possible (9). IRIS is defined as a paradoxical deterioration of the clinical condition of a patient with PML due to reconstitution of the immune system following efficient elimination of natalizumab. This is to be distinguished from a rebound of the inflammatory MS disease activity following cessation of natalizumab treatment, which can be also very severe.

IRIS can be observed in the initial phase of intensive highly active anti-retroviral therapy (HAART) in HIV patients and regularly after cessation of natalizumab therapy in MS in the context of PML. The clinical and radiological findings as well as the time interval between cessation of natalizumab treatment and plasma exchange therapy and the occurrence of IRIS is variable (3 weeks up to several months). Although diagnosis of IRIS is not primarily based on radiological criteria, newly occurring inflammatory processes seen on MRI, such as contrast-enhancing lesions, may be interpreted as indicative of IRIS given a corresponding clinical setting.

No data from controlled trials are available for the treatment of IRIS. As IRIS may cause space-demanding effects together with the increase of intracranial pressure, an aggressive anti-inflammatory treatment regimen using high-dose glucocorticoids is required. This can be performed via methylprednisolone pulse therapy using 1g per day for five consecutive days with an oral taper for 2 weeks. If symptoms deteriorate during taper, the same scheme has to be applied again. Alternatively, methylprednisolone pulse therapy using 2g per day for five consecutive days with subsequent oral taper may be considered. Brain edema may require extensive osmotherapy.

Recent studies show that although JCV is responsible for PML, the virus needs to undergo specific mutations in the regulatory region of its DNA to become pathogenic (47). Although most humans are infected with JCV and many shed virus copies in the kidneys and urine, virus genomes in non-PML subjects only show the ‘archetype’ of the virus genome (48). The mutated virus forms could only be sequenced in PML cases, and then only in PML-affected tissues like brain or cerebrospinal fluid (CSF) (49). This led to the hypothesis that pathogenic mutations can only occur in patients in whom the virus is able to replicate (50). Consistent with this hypothesis is the fact that, so far, only subjects detectable antibody response against JCV have developed PML (51), meaning that PML can only occur in patients where there is viral activity leading to antibody production. Up to now, there have only been two cases of ‘anti-JCV antibody sero-negative’ PML, which might or might not have been false-negatives of the antibody assay (52).

Recent studies have shown that a previously proposed treatment with mefloquine (an anti-malarial) does not result in a more positive outcome during PML (53), whereas the treatment of one patient with IL-7 showed promise in the treatment of PML (54) and a case report of a patient treated with maraviroc (a CCR5 antagonist) might suggest that perhaps the detrimental effects of IRIS could be mitigated pharmacologically (55).

Long-term safety and efficacy with anti-α4-integrin treatment

After the two cases of PML in the clinical MS trials of natalizumab were discovered, the pharmaceutical company voluntarily withdrew the drug from the market in February 2005 for further analysis, after it had been approved several months earlier in 2004. The fear at the time was that there might be even more cases of PML developing in the post-treatment period of the phase III patients. After thorough analysis, the FDA approved the continued treatment of RRMS patients with natalizumab in 2006, but it was suggested that the drug should be reserved for severely affected patients, where the benefit of natalizumab would justify the potential PML risk. Additionally, patients should enter a monitoring program to ensure maximum safety. Natalizumab is currently not prescribed to CD patients in the EU, as the risk of PML outweighs the benefits in this disorder, for which other effective therapies are available.

Natalizumab has been on the market continuously since 2006 and some MS patients have been treated with the drug for over 10 years after having taken part in a clinical study in
2002/2003 with continued efficacy. In fact, these long-term data from the STRATA study (56) suggest that continued treatment with natalizumab reduces the annualized relapse rate to a potential minimum of 0.09, which would mean that on average the treated MS patient would suffer from one relapse every 11 years and many patients are left relapse-free. Additionally, the data suggest that the drug effectively stops disease progression as reflected in the Expanded Disability Status Scale (EDSS; a method to quantify disability in MS) score with a mean reduction from 2.90 to 2.79 after nearly 5 years of treatment. Further real-world data (e.g. from the ‘tysabri observational program’ TOP) (57) confirm the efficacy but also the safety profile of natalizumab.

Treatment of relapsing MS patients with natalizumab: guidelines in 2014

Natalizumab is approved for disease-modifying monotherapy of patients with highly active RRMS in Europe and the USA (escalation therapy) in two subgroups of patients:

(i) Patients with high disease activity despite treatment with either IFN-β or glatiramer acetate. These patients should have had at least one relapse in the past 12 months and have lesions visualized using different techniques for cerebral MRI: at least nine T2-hyperintense lesions or at least one gadolinium-enhanced lesion.

(ii) Patients with high disease activity showing at least two relapses with confirmed disability progression in the past 12 months and at least one gadolinium-enhanced lesion or a significant increase in the number of T2-hyperintense lesions on cerebral MRI within the past 6–12 months.

Natalizumab is administered intravenously at a dose of 300 mg every 4 weeks. Some adverse effects occur frequently (hypersensitivity reactions, elevations of liver enzymes) whereas PML occurs infrequently. Therefore, careful clinical surveillance including cognitive and neuropsychological assessments is mandatory. Contraindications are active bacterial infections (urinary tract, lung, hepatitis), systemic mycosis in the past 6 months, viral infections (herpes zoster or herpes simplex infections with acute reactivations) in the past 3 months, HIV-infection and subsequent opportunistic infections in the past 3 months, other chronic or recurrent viral or bacterial infections, malignant tumors, organ transplantation with on-going immunosuppression, pregnancy and lactation.

Current approach for natalizumab-associated PML risk stratification

Risk stratification concerning PML is an important point in today’s clinical practice when natalizumab or other monoclonal antibodies are concerned. Patients treated with natalizumab are currently stratified using three validated parameters: previous treatment with immunosuppressive drugs; duration of natalizumab treatment; and presence of antibodies against the PML-inducing virus (JCV) in the serum above a certain threshold (58). However, statistical risk stratification of groups instead of individuals is problematic: patients with all risk parameters have a combined risk of 1 in 91 of developing PML, which leads to 90 patients potentially stopping an efficient therapy to avoid one case of PML (8).

There is, therefore, an apparent disconnect between theoretical risk stratification and practicability. Recent research suggests that the stratification efforts have—so far—not been successful in reducing the rate of PML (59) and the mean treatment duration does not necessarily correlate with JCV sero-status (60). These data suggest that patients undergo risk stratification for the potential of reducing the psychological burden that comes with PML risk. However, if this fails, meaning if there is indeed a high risk in their respective group, the patients do not draw consequences from these results, but continue to receive treatment with natalizumab, albeit with a higher level of fear. It stands to reason that this is due to the aforementioned statistical nature of previous risk-stratification parameters, as the prognosis of ‘risk’ is always allocated to a patient group and not an individual patient.

To improve these measures, two numerical parameters for individual risk stratification have recently been suggested: anti-JCV antibody titers represented as a JCV index value (61) and L-selectin (CD62L) as a possible biomarker to indicate individual risk to develop PML under therapy with natalizumab (62) or during HIV infection (63). Future prospective and retrospective studies will have to show whether these additional markers can be validated, are useful in daily clinical practice, and can help to reduce the rate of PML among patients.

Treatment failures, unexpected outcomes, and predicted future usage in MS and other disorders: implications for the relevance of the VLA-4–VCAM pathway

Natalizumab has proven to be an effective treatment against RRMS. However, treatment of patients presenting with other neuroinflammatory disorders such as neuromyelitis optica (NMO) was less successful (64, 65), even though NMO was once considered to be a separate, but closely related, clinical presentation of MS. Additionally, there are rare cases of MS patients who do not respond well to treatment with natalizumab without developing anti-natalizumab antibodies and it still remains to be seen if SPMS and primary progressive MS can be treated successfully with the drug (66).

All these cases imply that VLA-4 might play a more differentiated role in the migration of immune cell subtypes than previously thought. Consistent with this hypothesis, recent studies in animal models showed that the putatively pathogenic cells in MS and CD, T17 cells, which were genetically modified not to express VLA-4, were not inhibited in their ability to cross the BBB (67, 68). This prompted us to investigate this phenomenon in the human system and in natalizumab-treated MS patients. Interestingly, not only did we observe similar effects of the natalizumab blockade in the human system, but one important molecule in the migration of these ‘VLA-4-independent’ T17 cells might be P-selectin glycoprotein ligand 1 (PSGL-1), which binds selectins and is upregulated on leukocytes during natalizumab therapy, leading to enhanced rolling (behavior that occurs before cell adhesion). The key molecule, however, could be the melanoma cell adhesion molecule (MCAM) on T cells (69, 70), as MCAM-expressing (putatively T17) cells could not adhere to endothelium expressing when both VLA-4 and MCAM were blocked (71) (Fig. 1).
Experimental and clinical data are therefore consistent with a profound shift in the immune reaction during treatment with natalizumab, which can also be observed in the change of CSF cell composition (71, 72). This might have implications not only for the understanding of immune homeostasis, immune surveillance, and immune-cell infiltration, but also for potential additional future uses of natalizumab, when used in combination with other drugs (e.g., MCAM-blocking antibodies) or even in completely different disease entities such as Rasmussen encephalitis (73).

Conclusions

Treatment with the anti-α4-integrin antibody natalizumab has introduced a new era of therapy for MS. Blocking lymphocyte entry with monthly intravenous infusions has robust effects on measures of MS disease activity. Despite this undisputed benefit, the success of this paradigm is clouded by its association with PML, a rare disease with high morbidity and mortality that is caused by an opportunistic affliction. This created a new area of research, entitled ‘drug-associated’ PML (74). Furthermore, the differences in treatment effects in different phases of MS have brought a new level of understanding of inflammatory versus degenerative mechanism contributing to the pathomechanisms, including major differences between MS and other neuroinflammatory disorders (75).

Research on trafficking requirements revealed differences between CNS routes and different immune cell subsets. It further explains why anti-α4-integrin treatment shows major benefits in MS, but failed in RA and NMO. The size of its effect in CD is considered rather moderate. Anti-VLA-4 strategies have, therefore, further deepened our understanding of CNS immune surveillance and MS immunopathogenesis.

Fig. 1. Schematic overview of the blood-CSF migration mechanisms and molecular requirements of central and effector memory T cells in MS or under long-term natalizumab therapy. MS patient (naive): CD4+ T memory cells use PSGL-1 for rolling by binding to P-selectin on the endothelial barrier of the choroid plexus. Binding of VLA-4 to VCAM-1 on the endothelium leads to adhesion and allows for further transmigration. This is reflected in the cellular composition of the CSF with a high percentage of central memory (CM) cells, few effector-memory (EM) cells, and few Tn17 cells (MCAM+). MS patient (long-term treatment with natalizumab): under long-term treatment with natalizumab, VLA-4 expression is strongly downregulated and the remaining VLA-4 molecules on CD4+ T cells are saturated with the therapeutic antibody. The immune cells are still able to roll over the endothelium using their strongly upregulated expression of PSGL-1, which results in enhanced rolling capacities. However, as almost all T cells need VLA-4 to adhere to the endothelium and natalizumab is bound to the few remaining VLA-4 molecules, adhesion is efficiently blocked with the exception of Tn17 cells. They use MCAM to mediate adhesion to the endothelial barrier. The ligand for this interaction is currently not known. This is reflected in the cellular composition of the CSF with only VLA-4− EM cells remaining, a high percentage of which are MCAM+.

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