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Natalizumab: Targeting α_4-Integrins in Multiple Sclerosis

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Abstract
In 1992, it was shown that monoclonal antibodies blocking α_4-integrins prevent the development of experimental autoimmune encephalomyelitis, an animal model for multiple sclerosis (MS). As $\alpha_4\beta_1$-integrin was demonstrated to mediate the attachment of immune-competent cells to inflamed brain endothelium in experimental autoimmune encephalomyelitis, the therapeutic effect was attributed to the inhibition of immune cell extravasation and inflammation in the central nervous system. This novel therapeutic approach was rapidly and successfully translated into the clinic. The humanized anti-α_4-integrin antibody natalizumab demonstrated an unequivocal therapeutic effect in preventing relapses and slowing down the pace of neurological deterioration in patients with relapsing-remitting MS in phase II and phase III clinical trials. The occurrence of 3 cases of progressive multifocal leukoencephalopathy in patients treated with natalizumab led to the voluntary withdrawal of the drug from the market. After a thorough safety evaluation of all patients receiving this drug in past and ongoing studies for MS and Crohn's disease, natalizumab again obtained approval in the US and the European Community. A treatment targeting leukocyte trafficking in MS has now re-entered the clinic. Further thorough evaluation is necessary for a better understanding of the risk-benefit balance of this new treatment option for relapsing MS. In this review, we discuss the basic mechanism of action, key clinical results of clinical trials and the emerging indication of natalizumab in MS.

Introduction

Under physiological conditions, entry of circulating immune-competent cells into the central nervous system (CNS) is very low and strictly controlled by the endothelial blood-brain barrier (BBB). In contrast, in multiple sclerosis (MS) and in its animal model experimental autoimmune encephalomyelitis (EAE), circulating leukocytes get access to the CNS, where they start the molecular events leading to inflammation, edema and demyelination, which provide the basis for the development of the disabling clinical picture of the disease. Interaction of circulating immune cells with the BBB is thus a critical step in the pathogenesis of EAE and MS.

It is now well established that leukocyte recruitment from blood into tissue is regulated by the sequential interaction of adhesion and signaling molecules on leukocytes and endothelial cells lining the vascular wall [1–3]. The multistep leukocyte-endothelial interaction starts

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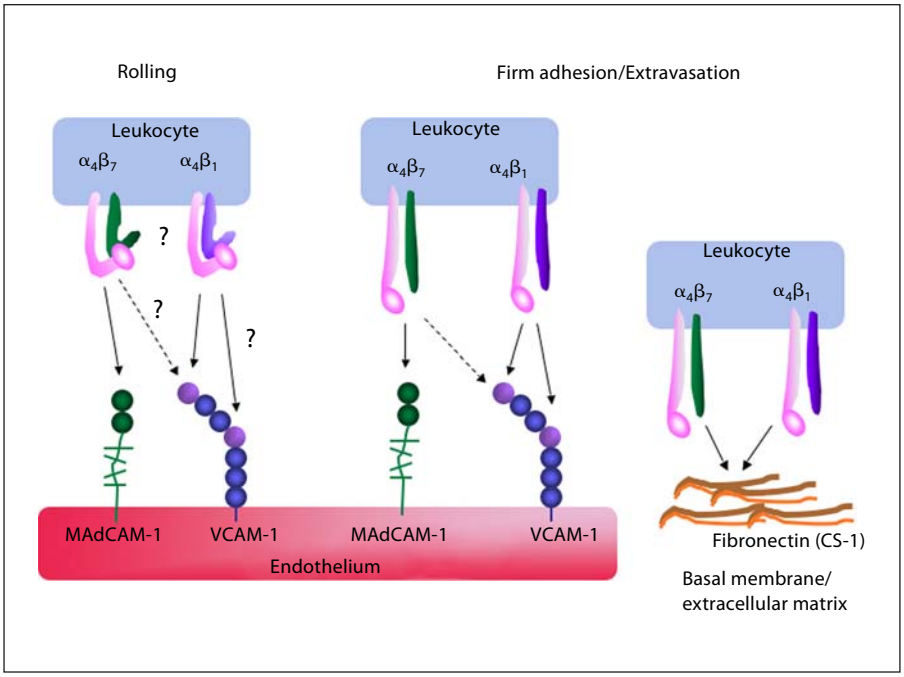

Fig. 1. α_4-Integrins are unique. Circulating immune-competent cells can slow down in the blood stream by engaging the endothelial α_4-integrin ligand MAdCAM-1 or VCAM-1 on the vascular endothelium under the influence of physiological flow, leading to leukocyte rolling at reduced velocity. Hereby, $\alpha_4\beta_7$ interacts with the first Ig domain of MAdCAM-1, and $\alpha_4\beta_1$ binds to the first Ig loop of VCAM-1. In addition, α_4-integrins mediate G-protein-dependent adhesion strengthening of leukocytes to the vascular endothelium. G-protein-mediated inside-out signals lead to a conformational change of integrins and probably to integrin clustering. On activated lymphocytes, $\alpha_4\beta_7$ can mediate adhesion strengthening by binding to the first Ig loop of MAdCAM-1 and VCAM-1. $\alpha_4\beta_1$ can bind to the first and the fourth Ig domain of VCAM-1. Additionally, both $\alpha_4\beta_1$ and $\alpha_4\beta_7$ bind to the CS-1 domain of fibronectin, and therefore, can mediate leukocyte binding to the extracellular matrix.

with an initial transient contact of the circulating leukocyte with the vascular endothelium, generally mediated by adhesion molecules of the selectin family and their respective carbohydrate ligands, or alternatively by α_4-integrins and their endothelial ligands, which slows down the leukocyte in the blood stream. After the initial tether, the leukocyte rolls along the vascular wall with greatly reduced velocity and is then exposed to chemotactic factors of the family of chemokines presented on the endothelial surface. Chemokines bind to G-protein-coupled receptors expressed on the leukocyte surface. G-protein-coupled receptors deliver a pertussis toxin-sensitive 'inside-out signal', activating heterodimeric adhesion molecules of the integrin family, which are constitutively expressed on the leukocyte surface in an inactive stage. Activated integrins display an increased avidity and mediate the firm adhesion of the leukocytes to the vascular endothelium by binding to their endothelial ligands of the immunoglobulin (Ig) superfamily. Firm adhesion is the prerequisite for subsequent leukocyte diapedesis across the endothelium into the tissue. Concerning the multistep extravasation of circulating leukocytes, α_4-integrins and their endothelial counterreceptors are unique, as they can exert two functions, namely initial rolling and firm arrest, depending on whether the integrins are in a low or high affinity state [4, 5].

α_4-Integrins

α_4-Integrins belong to the large family of adhesion receptors of the integrin family, which mediate both cell-extracellular matrix and cell-cell interactions [6]. In addition to their adhesive function, these receptors act as cellular sensors and signaling molecules [6]. All integrins are composed of noncovalently linked α- and β-chains. The α_4-integrin chain dimerizes with either β_1 or β_7. $\alpha_4\beta_1$ is also known as very late antigen 4 [7] or CD49d/CD29, while $\alpha_4\beta_7$ was originally described as lamina propria-associated molecule [8]. The major endothelial ligand for $\alpha_4\beta_1$-integrin is vascular cell adhesion molecule (VCAM)-1 (fig. 1). VCAM-1 is upregulated on endothelial cells during inflammation. $\alpha_4\beta_7$ binds to mucosal addressin cell adhesion molecule (MAdCAM)-1, which is constitutively expressed on high endothelial venules in mucosa-associated lymphatic tissue, such as in the mesenteric lymph nodes and Peyer's patches. Both α_4-integrins can additionally bind the extracellular matrix protein fibronectin (fig. 1). In contrast to the fibronectin receptor $\alpha_5\beta_1$-integrin, which binds the RGD amino acid sequence of fibronectin, α_4-integrins interact with the LDV sequence on the connecting segment 1 (CS-1) of fibronectin. Last but not least, it is important to stress that on activated lymphocytes, $\alpha_4\beta_7$ can also bind to VCAM-1 (fig. 1) [9]. Additional reported ligands for $\alpha_4\beta_1$-integrins

include the secreted highly acidic glycoprotein osteopontin [10] and the tight junction protein junctional adhesion molecule B [11].

Preclinical Studies on α_4-Integrin-Mediated CNS Pathology

The earliest evidence for α_4-integrin-mediated CNS pathology has come from studies in the animal model EAE in the rat [12]. Yednock et al. [12] searched for adhesion molecules involved in the extravasation of circulating immune cells into the CNS during EAE. Using the Stamper-Woodruff frozen section adhesion assay [13], they could demonstrate that antibodies which blocked the α_4- or the β_1-integrin chain, but not other antibodies, interfered with T cell and monocyte binding to inflamed vessels in frozen sections of EAE brains and concluded that $\alpha_4\beta_1$ mediates the binding of leukocytes to the inflamed BBB. This was further supported by their observation that anti-α_4-integrin antibodies inhibited the development of EAE by preventing the accumulation of inflammatory cells in the CNS of the treated animals. Numerous subsequent studies confirmed and extended these original findings. Frozen section adhesion assays on EAE brains in mice demonstrated the binding of lymphocytes via both α_4-integrins, namely $\alpha_4\beta_1$ and $\alpha_4\beta_7$, to the inflamed cerebral vessels via their respective endothelial ligand VCAM-1 in vitro [14]. Furthermore, adhesion but not the transendothelial migration of encephalitogenic T cells to brain endothelial cells in vitro was shown to be mediated via α_4/VCAM-1 interactions [15–18]. Different antibodies blocking α_4-integrins were shown to inhibit the development of EAE and even reverse the ongoing disease by preventing inflammatory cells from crossing the BBB in EAE models in rats, mice and guinea pigs [19–21]. However, discordant effects of anti-α_4-integrin treatment were also reported depending on the application before or after the onset of relapsing EAE [22]; no therapeutic effect was seen if certain mouse strains were used in the EAE studies [23]. On the other hand, antibodies blocking the endothelial α_4-integrin ligand VCAM-1 were again demonstrated to interfere with the development of EAE in a comparable fashion with blocking α_4-integrin [24, 25]. Furthermore, it was recognized that encephalitogenic T cells require expression of α_4-integrin to enter the CNS and cause EAE [24]. Interestingly, although encephalitogenic T cells were shown to express both $\alpha_4\beta_1$ and $\alpha_4\beta_7$, neutralizing $\alpha_4\beta_7$ antibodies did not inhibit EAE in SJL mice [25]. In contrast, β_7-integrin-deficient mice exhibited mild EAE [26], suggesting that the precise role of $\alpha_4\beta_7$-integrin in EAE pathogenesis and probably MS remains to be clarified. Finally, although the CS-1-containing fibronectin isoform binds α_4-integrins, its potential involvement in EAE or MS has not been addressed in detail [27, 28]. This is important in the context of MS, where the expression of VCAM-1 by the cerebral vasculature remains controversial. One study reported VCAM-1 expression on CNS venules during MS [29], whereas others did not confirm these findings [30, 31].

More recent intravital microscopy studies were able to provide direct evidence for α_4-integrin-mediated leukocyte interaction with the endothelial BBB in vivo. During EAE, α_4-integrins were shown to mediate leukocyte rolling and G-protein-dependent arrest in pial venules [32]. Interestingly, encephalitogenic T cells already use α_4-integrins to interact with the noninflamed BBB. Performing intravital microscopy of the spinal cord white matter in mice, it was shown that the interaction of encephalitogenic T lymphoblasts with the noninflamed spinal cord microvasculature is unique as T cells do not roll and due to the predominant involvement of α_4-integrins in initial T cell capture and subsequent G-protein-dependent adhesion strengthening [33]. The endothelial ligand was identified as VCAM-1, which is constitutively expressed at low levels in CNS microvessels, at least in mice [34].

At this point, we would like to stress that natalizumab binds the α_4-integrin chain irrespective of its associated β-chain and therefore blocks both $\alpha_4\beta_1$- and $\alpha_4\beta_7$-integrins. In most publications, natalizumab is referred to as blocking $\alpha_4\beta_1$-integrin [35, 36]. Although this statement is absolutely correct, it might still be misleading as natalizumab equally well binds $\alpha_4\beta_1$- and $\alpha_4\beta_7$-integrins and therefore probably exerts its therapeutic effects because it blocks α_4-integrins binding to their ligands including both VCAM-1 and MAdCAM-1, which is required for leukocyte recruitment from blood into inflamed tissues.

Therapeutic Effects of Natalizumab in MS

Based on the dominant role of α_4-integrins in leukocyte migration across the BBB observed in EAE, this approach was translated into the clinic. A mouse monoclonal antibody directed against the α_4-integrin chain was humanized and placed on the IgG4 framework and named natalizumab.

Three trials were looking at magnetic resonance imaging (MRI) outcomes only, and all demonstrated that na-

talizumab was able to significantly lessen MRI activity compared with placebo alone [37–40]. One of these phase II clinical trials also showed a promising reduction in the relapse rate by approximately 50% over 6 months in a group of MS patients with active relapsing and secondary progressive disease [40].

Four randomized placebo-controlled trials studied both clinical and MRI outcomes. Two trials compared natalizumab with placebo [40, 41], one studied the combination of natalizumab and IFN-β_{1a} (Avonex) 30 μg per week intramuscularly compared with IFN-β_{1a} alone [42], and one phase II study analyzed natalizumab as an addition to ongoing glatiramer acetate treatment [43]. In the large phase III trial against placebo, AFFIRM [41], patients had a mild disability and were entered quite early in their disease course [mean Expanded Disability Status Scale (EDSS) 2.3, median duration 5 years], whereas in the large phase III trial studying combination therapy, SENTINEL [42], patients were at a somewhat later stage, having already had breakthrough activity while on IFN-β_{1a} therapy (mean EDSS 2.4–2.5, median duration 7 years). By contrast, in the phase II trial, which included both relapsing-remitting MS and secondary progressive MS patients [39, 40], the disability at baseline was considerably greater (mean EDSS 4.2–4.4, mean duration 11.7 years). All three of these natalizumab trials [39–42] showed a significant benefit of treatment on both clinical and MRI measures of disease activity, with MRI activity being suppressed by 80–90% and relapse rates being reduced by 50–70%. In addition, in both of the two larger (and longer) trials [41, 42], there was a significant reduction in progression of impairment/disability (as measured by the EDSS or MS Functional Composite, as well as by quality of life scales). The effect size and the statistical significance for natalizumab with each of these outcomes were generally larger than those reported using any of the other currently available therapies [44–55], especially with respect to the clinical outcomes.

However, the real magnitude of any such improved efficacy is impossible to define accurately because there are no direct head-to-head trials. The cohorts studied in the respective pivotal trials are probably different, although many baseline characteristics were similar. The patient cohorts for the IFN-β and glatiramer pivotal trials were drawn a decade or more in the past (when disease-modifying treatments were unavailable) and probably differ in their implicit disease characteristics from cohorts drawn at a time when it is standard practice to initiate disease-modifying therapy at the time of confirmed relapsing-remitting MS diagnosis. In all of these trials, the therapeutic benefits of natalizumab seemed to be accompanied by very few notable side effects over the 2 years of therapy. Although many patients (in both the treatment and comparative arms in both of the phase III trials) experienced adverse events, there were few statistically meaningful differences between groups and very little consistency of any reported differences between trials [41, 42]. Nevertheless, approximately 2–4% of patients had anaphylactic or other hypersensitivity reactions to natalizumab, and in approximately 1%, these were considered serious by the investigators [41, 42]. Approximately 6% of patients developed persistent binding antibodies to natalizumab and, in these patients, the therapeutic effect of natalizumab seemed to be completely neutralized [41, 42].

Therapeutic Risks of Targeting α_4-Integrins in MS

Nevertheless, despite the overall encouraging results, there are reasons for caution. After completion of the clinical trial, 2 patients in the SENTINEL trial (both in the arm receiving combined natalizumab and IFN-β_{1a} therapy) developed progressive multifocal leukoencephalopathy (PML) and one of them died [56, 57]. In reviewing the previous experience with natalizumab in Crohn's disease, a third case of PML was identified in a patient receiving natalizumab alone [58]. However, this patient had previously been immunosuppressed with several agents (including natalizumab) and was still lymphopenic at the time natalizumab was restarted, prior to the development of PML. Consequently, the basis for this complication is not entirely clear. However, the possibility that concurrent immunosuppression (either from IFN-β or otherwise) contributes to the development of PML in patients on natalizumab cannot be excluded. Nor can we exclude the possibility that the risk is due to natalizumab alone and that this risk may increase with greater time on therapy. Although not definitely proven, it may be assumed that abrogated or at least significantly reduced immune surveillance in the CNS caused by inhibition of entry of activated immune cells leads to reduced suppression of the opportunistic JC virus. Other effects of natalizumab, as for example the increased release of hematopoietic progenitor cells from bone marrow, may have contributed to this complication [59].

As extensive studies of stored serum samples failed to reveal viremia in 2 of the 3 patients prior to the onset of clinical symptoms of PML, and because imaging features of MS and PML overlap to some degree, it seems that currently, there are no laboratory tests or imaging proce-

dures to reliably monitor patients for this potential complication. However, it is possible to monitor a patient's specific cellular immunity to the JC virus and, perhaps, such a strategy could be developed into a valid commercial test in the future.

In clinical practice, neurologists face the difficult challenge of accurately assessing both the risks and the benefits of therapy. Yousry et al. [60] have estimated the risk of PML as 1 per 1,000 patients treated for an average of 17.9 months (95% confidence interval 0.2–2.8 per 1,000), but this estimate is based on assumptions that may not be true. For example, if concomitant IFN-β therapy predisposes to PML, the risk for patients on natalizumab monotherapy will probably be much lower and possibly nonexistent. By contrast, if this complication can occur with natalizumab alone, the risk will possibly increase with higher exposure time to therapy so that the ultimate risk to patients (expected to be on treatment for many years) could be substantially greater.

In view of the lacking evidence for an additional beneficial effect of the use of a combined therapy of natalizumab with IFN – or with any other immune suppressive agent – such a combination is not recommended at present.

α_4-Integrins exert additional functions in hematopoiesis and mucosal immunity. Thus, natalizumab might block other relevant biological events unrelated to T cell migration. In this context, it is interesting to note that in EAE, blocking α_4-integrins is more effective than blocking VCAM-1, and that an antibody that binds α_4-integrins but does not block α_4-integrin-mediated lymphocyte homing [61] can nevertheless interfere with EAE development in the mouse [25].

According to current Food and Drug Administration and European Medicines Agency recommendations, it is anticipated that the majority of patients treated with natalizumab in the near future will be those who failed to tolerate or more likely failed to respond adequately to IFN-β or glatiramer acetate. Naive patients will only be considered for treatment with natalizumab if they present with unusually high disease activity as defined by relapses and gadolinium-enhancing lesions in MRI.

Chronic inhibition of α_4-integrins has shown a great therapeutic potential in relapsing forms of MS but could have undesired effects which at present are not predictable. Only further thorough evaluation of MS patients under long-term treatment with natalizumab will allow for a better assessment of its benefit-risk ratio.

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