The discovery of natalizumab, a potent therapeutic for multiple sclerosis

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Multiple sclerosis (MS) is the major inflammatory demyelinating disease of the central nervous system. There is strong evidence that an immune response in the brain is a critical component of the disease. In 1992, in a collaboration between academia and biotechnology, my colleagues and I showed that α4 integrin was the critical molecule involved in the homing of immune cells into the inflamed brain. Was it sheer luck that these results led to the development of a drug for MS?

Multiple sclerosis (MS) is the most prevalent inflammatory disease of the brain and spinal cord in Europe and North America. More than one million are affected worldwide, including 400,000 in the US. Symptoms often commence in young adulthood and include motor paralysis, visual disturbances and blindness, bowel and bladder incontinence, sensory loss, and incoordination and ataxia. Neurological deficits depend on the location of inflammation in the central nervous system. Moreover, the disease has the propensity to relapse and remit. Until the past 20 years, there was only one approved treatment for MS, the use of adrenocorticotropic hormone (ACTH) or steroids (Frohman et al., 2006). In the past 20 years, eight drugs have been approved for MS, with natalizumab (also known as Tysabri) regarded as the most potent (Rudick et al., 2012).

In MS, there is strong evidence supporting the idea that an immune response targets molecules in the central nervous system, including some of the proteins and lipids of the myelin sheath. We now know from genomic, transcriptomic, proteomic, and even lipidomic studies that there is a major adaptive immune response involving T cells and B cells targeting various molecules in the white matter and in the gray matter of the brain (Han et al., 2008; International Multiple Sclerosis Genetics Consortium, 2011; Lucchinetti et al., 2011; Ho et al., 2012; Srivastava et al., 2012). This inflammatory immune response, which may be "autoimmune," involves various key proinflammatory cytokines, including interferon-γ, IL-6, and IL-17, and related cytokines (Lock et al., 2002; International Multiple Sclerosis Genetics Consortium, 2011). Experiments in the early 1990's were aimed at elucidating how the key cellular components of the adaptive immune system, the T cells and B cells, breached the blood–brain barrier and migrated into the central nervous system.

At that time, while investigating the molecules involved in lymphocyte homing to the inflamed brain we found that α4 integrin was critical for the adhesion of lymphocytes to the inflamed endothelium in brain (Yednock et al., 1992). Within 12 years, rather fast for drug development, a humanized antibody to α4 integrin, natalizumab, was approved for the treatment of relapsing remitting MS. The successful translation of cell biology was based on an adaptation in an experimental system that was widely used to study the molecular interactions between lymphocytes and venules in lymphoid tissue. I focus here on the pivotal experiment, published in 1992, that enabled identification of α4 integrin as the key adhesion molecule in homing to the brain and to many other organs, including the intestines and pancreas (Yednock et al., 1992; Yang et al., 1993, 1994; Steinman, 2005).

The historical context

Gowans and Knight demonstrated in 1964 that lymphocytes, though not other leukocytes, enter lymph nodes through specialized blood vessels called high endothelial venules (Gowans and Knight, 1964). This experiment focused attention on the molecular interactions between the endothelium and lymphocytes (Rosen, 2006). A key experimental system for analyzing these interactions, the frozen-section binding assay, was developed in the mid-1970s by Stamper and Woodruff (1976). In this assay, tissue sections are prepared from suitably frozen histological sections. Lymphocytes are layered onto these sections and adhere to their ligands on the exposed tissue. The assay proved useful in the mapping of molecules involved in the adherence of lymphocytes within lymphoid follicles. It was applied in this case to the study of interactions between lymphocytes and endothelium in remote lymph nodes in the intestines called Peyer's Patches. Experiments by Butcher, Gallatin, and Weissman used the Stamper

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Abbreviations used in this paper: EAE, experimental autoimmune encephalomyelitis; MS, multiple sclerosis; PML, progressive multifocal leukoencephalopathy.

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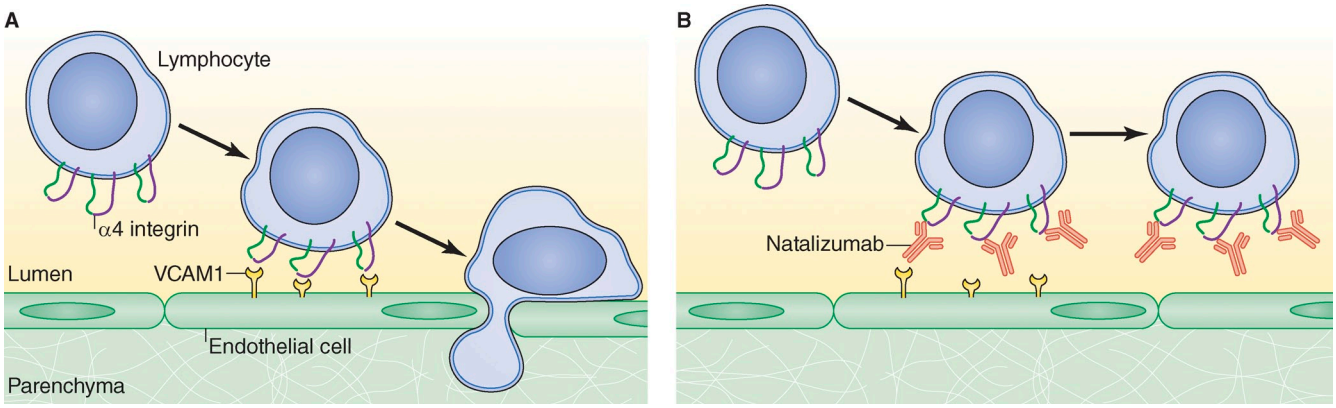

Figure 1. **Natalizumab blocks lymphocyte homing in MS.** (A) α4 integrin binds to vascular cell adhesion molecule 1 (VCAM1) and to osteopontin (not depicted) on inflamed brain endothelium. This interaction gives lymphocytes access to the central nervous system (CNS). The presence of immune cells in the brain is a prominent feature of MS. (B) Natalizumab, a humanized antibody to α4 integrin, blocks binding of lymphocytes to VCAM and osteopontin on inflamed brain endothelium, thereby preventing lymphocyte entry into the CNS.

Woodruff assay to study the binding of a B cell lymphoma to high endothelial venules (Gallatin et al., 1983). This lymphoma was used to produce MEL-14, an antibody that was shown by Yednock and Rosen to bind to a carbohydrate receptor on high endothelial venules (Yednock et al., 1987), which was cloned in 1989 (Lasky et al., 1989) and termed L-selectin (Lasky et al., 1989; Rosen, 2006). These experiments and techniques set the stage for the critical experiment that led to the development of natalizumab.

The team assembled for this experiment included Ted Yednock, a scientist at Athena Neuroscience, a biotechnology company in South San Francisco, who was familiar with the Stamper Woodruff assay and participated in the identification of L-selectin (Yednock et al., 1987; Lasky et al., 1989). The Stanford team included Nati Karin, a member of my group who was skilled in producing T cell lines and clones that homed to the central nervous system (Zamvil et al., 1985; Lohse et al., 1989; Zamvil and Steinman, 1990). We had studied radiolabeled T cell clones to assess their homing properties to brain and spinal cord (Steinman et al., 1983), and we were interested in studying the properties of these clones, especially which molecules they used in homing from the periphery through inflamed brain venules into the central nervous system (Steinman et al., 1983; Zamvil et al., 1985; Zamvil and Steinman, 1990). An attractive idea that was in vogue at the time was that specific molecules might guide lymphocytes to recognize regions of inflammation in a specific organ. The concept was likened to the delivery of mail to a specific postal address and came to be known as the "zip code hypothesis." The critical experiment might therefore be considered as a search for the "zip code" for immunological homing to the brain (Steinman, 2005).

The two major components of the experiment involved the use of T cell clones in the animal model known as experimental autoimmune encephalomyelitis (EAE), which induces brain inflammation, and the use of the Stamper Woodruff assay. In rats, injection of a single T cell clone, made from a $CD4^+$ T cell reactive to myelin basic protein, caused EAE and paralysis. In the critical experiment, we examined the binding of lymphocytes to the inflamed endothelium in this animal model. The T cells infiltrate the central nervous system in 4–12 h, where they initiate inflammation and subsequent paralysis within 4 d. We showed that either human monocytes, or rat or mouse lymphocytes, but not human blood neutrophils, could bind to the inflamed venular endothelium in brains with EAE (Fig. 1). We then asked whether various monoclonal antibodies to adhesion receptors might interfere with the binding of human monocytes, or rat or mouse lymphocytes. Our "war chest" included various monoclonal antibodies to α3, α4, α5, and α6 integrin; to β1 and β2 integrin; to LFA-1 (CD18 and CD11a) and Mac-1 (CD18 and CD11b); to L-selectin; to CD2, CD4, and CD45; to OX44; and to Thy 1.1 (Yednock et al., 1992).

Remarkably, lymphocyte attachment to the lumen of inflamed vessels was almost entirely blocked by antibodies to β1 integrin. The integrin molecule's β chain binds to 1 of 6 unique α chains. Antibodies specific for the α chains were applied to the frozen section assay; an antibody to α4 integrin inhibited lymphocyte binding to the frozen brain sections. Thus, binding was inhibited with antibodies to α4 or to β1 integrin. Surprisingly, other integrins and L-selectin had no effect on binding to inflamed brain endothelium (Yednock et al., 1992).

Next, we asked whether an antibody to α4 integrin would inhibit the progression of paralysis and inflammation in EAE. We administered the α4 integrin antibody 2 d after injection of the highly pathogenic clones, which can home to the brain within 4–12 h. Remarkably, treatment with this antibody prevented paralysis in 75% of animals. Even in those animals with paralysis, the signs were weaker and appeared later than those who received a control antibody. Thus, importantly, we could have a clinical impact long after some cells had reached the brain. We found that the α4 integrin antibody blocked the appearance of inflammatory cells in the brain (Yednock et al., 1992). We stated audaciously in the final sentence of the paper "that therapy based on inhibiting α4 β1 integrin may prove effective in treating inflammatory diseases of the central nervous system" (Yednock et al., 1992).

Translation from experimental cell biology to pivotal clinical trials

In 2004, a humanized antibody to α4 integrin was approved by the Food and Drug Administration (FDA) for treatment of relapsing remitting MS after succeeding in phase 1, 2, and 3 clinical trials (Steinman, 2005; Rudick et al., 2012). On the basis of year 1 results from two phase 3 trials, the FDA conducted an accelerated review and approved natalizumab for the treatment of relapsing forms of MS in November 2004, 12 years after discovery of the target molecule and 7 years after the start of clinical testing. Approval based on accelerated review indicated that the FDA felt that natalizumab offered significant advantages over existing drugs in an area of high unmet medical need (Rudick et al., 2012).

Natalizumab, though powerful in reducing relapses and halting progression of disease, produced a major vulnerability: after blockade of this integrin for two years, more than 1 in 500 individuals developed a devastating opportunistic infection of the brain, progressive multifocal leukoencephalopathy (PML; U.S. Food and Drug Administration, 2005; Gorelik et al., 2010; U.S. Food and Drug Administration, 2012; Bloomgren et al., 2012). The drug was withdrawn from the commercial market in 2005, and was then reinstated by regulatory authorities with strict monitoring (Steinman, 2005; U.S. Food and Drug Administration, 2005; Gorelik et al., 2010; Bloomgren et al., 2012; U.S. Food and Drug Administration, 2012; Rudick et al., 2012). Over the past seven years, a biomarker, antibodies to John Cunningham (JC) Virus, the causative agent of PML, has emerged that enables clinicians to know who is at risk, and who is essentially risk-free. More than 90,000 patients have now taken natalizumab (Rudick et al., 2012).

The keys to the success of natalizumab

In my opinion, there were four important lessons to be learned from the pivotal experiment that led to the development of natalizumab:

"Off the shelf" technologies and reagents. We used techniques that were already tried and well tested, "off the shelf" if you will, in our experiments in the early 1990s. These included the Stamper Woodruff assay, this time applied to the inflamed brain, rather than being used to study homing to lymph nodes. The repertoire of techniques applied here also included the use of T cell clones that trigger EAE.

A collaborative team with highly complementary skill sets. T. Yednock was an authority on the Stamper Woodruff assay and was a key player in the biochemical characterization and subsequent cloning of L-selectin (Yednock et al., 1987; Lasky et al., 1989). Karin and I were experts on the use of T cell clones that cause EAE (Steinman et al., 1983; Zamvil et al., 1985; Lohse et al., 1989; Zamvil and Steinman, 1990). We used the T cell clones both to induce brain inflammation and then to test the therapeutic efficacy of anti–α4 integrin antibodies after we saw that they were critical in the homing process (Yednock et al., 1992).

Connection of animal models to human cell biology. We used a human cell line in the very first experiment, which connected the work from a study in an animal model all the way to an important insight on human monocytes (Yednock et al., 1992).

Test of a direct question with a potentially answerable outcome. Probably most importantly, we asked which molecules were involved in lymphocyte homing to inflamed brain. We did have luck with a small set of reagents available at the time, and got the surprisingly clear answer of α4 integrin.

Broader biological implications of the findings

Subsequently, we learned that that α4 integrin is critical in homing to other organs, and that the biology of lymphocyte homing to inflamed brain and to other organs has many biological features that are shared with other migratory processes across blood vessels, including the extravasation of tumor metastases to distant organs.

The identification of α4 integrin as the critical molecule in lymphocyte adhesion to the blood–brain barrier in brain inflammation exemplified its wider biological role in other contexts: α4 integrin is critical for homing to the intestines, to the β cells in the islets of Langerhans in the pancreas (Yang et al., 1993, 1994; Steinman, 2005). These findings were taken forward and led to approval for MS; natalizumab was also approved for Crohn's Disease (Steinman, 2005; Rudick et al., 2012). We now know that the interactions between integrins and small integrin binding proteins (SIBLING proteins) are critical in a variety of processes ranging from tumor metastasis to the triggering of relapses in MS and inflammatory bowel disease (Steinman, 2005; Bellahcène et al., 2008).

The physiology of lymphocyte homing to inflamed organs in MS and Crohn's disease is connected to another story involving a family of small integrin-binding proteins. In 2007, we showed that osteopontin was critical for inducing relapses in EAE, that osteopontin promotes proliferation and survival of autoreactive T cells, and that this SIBLING protein binds to α4 integrin (Fig. 1; Hur et al., 2007). Earlier work had described elevated levels of osteopontin around the time of relapse in MS (Vogt et al., 2004; Comabella et al., 2005; Hur et al., 2007; Steinman, 2009; Börnsen et al., 2011; Wen et al., 2012). Osteopontin promotes proliferation and survival of tumor cells, which then can circulate and bind to integrins on endothelium and thus enter tissues as metastases (Bellahcène et al., 2008). The parallels between how autoimmune monocytes home to inflamed brain and how tumor cells metastasize to specific anatomical locations are rather striking (Bellahcène et al., 2008).

Concluding remarks

In many ways, this work on lymphocyte homing used state-of-the-art cell biology, and the translation of the work from 1992 to 2004 led to a new drug. This epitomizes what is desired in so-called "bench-to-bedside" translation. The tale is filled with high points as well as a devastating low point, when clinicians first learned that the approach could lead to fatalities with PML (Steinman, 2005; Rudick et al., 2012). For now, it appears that "All's well that ends well," thanks to the biomarker test to largely mitigate risk (Gorelik et al., 2010; Bloomgren et al., 2012).

Early on, a very successful application of the Stamper Woodruff technique was used, melded with T cell cloning technology and enabling the discovery of α4 integrin as the key homing molecule to inflamed brain. This breakthrough led to the development of a powerful drug, natalizumab, for the treatment of MS.

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