artery catheter and there were several disease processes that warranted study.[10,14] Concern was voiced, however, that it could be difficult to convince physicians to participate in the trials.[12] This concern was allayed somewhat by the successful enrollment of 201 patients in a randomized, controlled trial of pulmonary-artery catheterization in critically ill patients.[15] Although that trial was not large enough for its results to be conclusive, it served as a pilot study that demonstrated the feasibility of trials of pulmonary-artery catheterization in critically ill patients.[12]

Sandham and colleagues have substantially furthered the progress in research in critical care with their current study. They have clearly demonstrated that physicians will allow their patients to be randomly assigned to either treatment group in a clinical trial that involves not only the insertion of a pulmonary-artery catheter, but also the implementation of therapy directed by that catheter. Furthermore, their study demonstrates the feasibility of conducting large, adequately powered, multicenter, controlled trials of pulmonary-artery catheterization. These accomplishments represent milestones in research in clinical critical care that would not have been possible less than two decades ago.

Whether the results of this trial extend to groups of patients other than the high-risk surgical patients who were studied is not known. The use of the pulmonary-artery catheter is currently being studied in patients with other clinical syndromes, including acute lung injury and congestive heart failure.[14] The design and execution of these trials have enhanced our understanding of the complexity of studying a technology that is already so widely used in clinical practice. The determination of which clinical questions are important to ask and the designing of appropriate trials with which to answer those questions have led to debates that would not have been considered a decade ago. These debates represent the progress we have made in research related to critical care and the difficulty posed by the legacy of an over-enthusiastic embracing of technology without adequate assessment. I hope that we are learning from our experience.

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α_4 Integrins as Therapeutic Targets in Autoimmune Disease

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In this issue of the *Journal*, two groups of investigators report on clinical trials of natalizumab, a recombinant monoclonal antibody against α_4 integrins, for the treatment of multiple sclerosis[1] and Crohn's disease.[2] Miller et al. report that a group of patients with multiple sclerosis who received monthly injections of natalizumab had significantly fewer new inflammatory central nervous system lesions than the placebo group (a reduction of approximately 90 percent) and had approximately

half as many clinical relapses.[1] Ghosh et al. report that patients with Crohn's disease also had an improved response to natalizumab,[2] with response and remission rates that were approximately twice as high in the patients who received two injections of the antibody than in the placebo group. The rate of adverse events did not differ significantly between the natalizumab and placebo groups in either trial.

Integrins are adhesion molecules that confer mechanical stability on interactions between cells and their environment.[3] They also act as cellular sensors and signaling molecules. All integrins are composed of noncovalently linked α and β chains. The α_4 integrin chain dimerizes with either the β_1 chain or the β_7 chain. The $\alpha_4\beta_1$ integrin is also known as very late antigen 4 or CD49d–CD29, and the $\alpha_4\beta_7$ integrin is sometimes referred to as lamina propria–associated molecule 1. Natalizumab binds the α_4 chain irrespective of its associated β chain.

Natalizumab probably has therapeutic effects because it blocks the ability of $\alpha_4\beta_1$ and $\alpha_4\beta_7$ to bind to their respective endothelial counter-receptors, vascular-cell adhesion molecule 1 (VCAM-1) and mucosal addressin-cell adhesion molecule 1 (MAdCAM-1) (Fig. 1). These molecular interactions are required for lymphocytes to enter the central nervous system (mediated by $\alpha_4\beta_1$ and VCAM-1) and the intestine (mediated by $\alpha_4\beta_7$ and MAdCAM-1). It is now well established that the recruitment of leukocytes from the blood into virtually every tissue is regulated by sequential engagement of adhesion and signaling molecules on leukocytes and endothelial cells.[4-6] Leukocytes make an initial adhesive contact that allows them to slow down and roll along the vascular wall. This step, which is readily reversible, can be mediated by selectins, α_4 integrins, or both. In order for the rolling leukocyte to stop, it must receive an activating signal, such as a signal from a chemokine, that switches integrins to a high-affinity state and allows the cell to arrest itself.[4,6] Leukocytes are recruited into a tissue only if they are successful at undergoing each step. The two α_4 integrins and their endothelial counter-receptors have a unique role in this multistep cascade because they are the only molecules known to mediate both rolling (when the integrins are in a low-affinity state) and arrest (when they are in a high-affinity state).[7,8]

The earliest evidence of α_4-integrin–mediated central nervous system disease came from studies of autoimmune encephalomyelitis, a T-cell–mediated autoimmune disease that resembles multiple sclerosis, in animals.[9] Cerebral microvessels normally express low levels of VCAM-1, but in autoimmune encephalomyelitis, inflammatory stimuli induce increased expression of VCAM-1, which precedes perivascular leukocyte infiltration. VCAM-1 is similarly induced in central nervous system endothelium in patients with multiple sclerosis.[10] Antibodies to α_4 integrins reduce cellular infiltration, inhibit the development of autoimmune encephalomyelitis, and halt the progression of disease or even reverse existing symptoms by preventing inflammatory cells from crossing the blood–brain barrier. Intravital microscopical studies have shown that α_4 integrins mediate lymphocyte rolling and arrest in pial venules during autoimmune encephalomyelitis.[11] Encephalitogenic T cells express high levels of activated $\alpha_4\beta_1$ and can even adhere to venules within the spinal cord in healthy mice, which express VCAM-1 sparsely.[12] These findings suggest that α_4-integrin inhibitors should block the egress of pathogenic T cells into unaffected regions of the central nervous system, where their stimulation by antigen would precipitate the inflammation cascade. Such blockade may explain the effectiveness of natalizumab in preventing the formation of new lesions in patients with multiple sclerosis, as reported by Miller et al.[1]

The α_4 integrins also interact with extracellular-matrix molecules such as fibronectin[3] and with VCAM-1 on nonendothelial cells, especially in the bone marrow and lymphoid tissues. Through these interactions, α_4 integrins exert additional effects on the immune system. Indeed, in autoimmune encephalomyelitis, the blockade of α_4 integrins is more effective than the blockade of VCAM-1, and an antibody that binds α_4 integrins but does not affect lymphocyte migration can nevertheless interfere with the development of autoimmune encephalomyelitis.[13] Thus, it is possible that natalizumab blocks additional pathologic events that are unrelated to T-cell migration. Such further therapeutic mechanisms notwithstanding, the results of the trial of natalizumab in patients with multiple sclerosis[1] lend strong support to the notion that the continuing assault on brain tissue in this disease depends on the recruitment of blood-borne leukocytes, which require α_4 integrins to exert their pathologic effects.

The effects of natalizumab in patients with Crohn's disease are likely to involve inhibition of the binding of $\alpha_4\beta_7$ to MAdCAM-1. MAdCAM-1 is selectively expressed in venules in the gut and gut-

associated lymphoid tissues.[5] Its expression is enhanced in inflammatory bowel disease.[5] Animal models of colitis have shown that antibodies to $\alpha_4\beta_7$ or MAdCAM-1 attenuate T-cell–mediated intestinal inflammation.[14,15] However, VCAM-1 is also induced in inflammatory bowel disease and predominates in some animal models of experimental colitis.[16] The extent to which natalizumab exerts its therapeutic effects by inhibiting $\alpha_4\beta_1$ in addition to its blockade of $\alpha_4\beta_7$ remains to be determined.

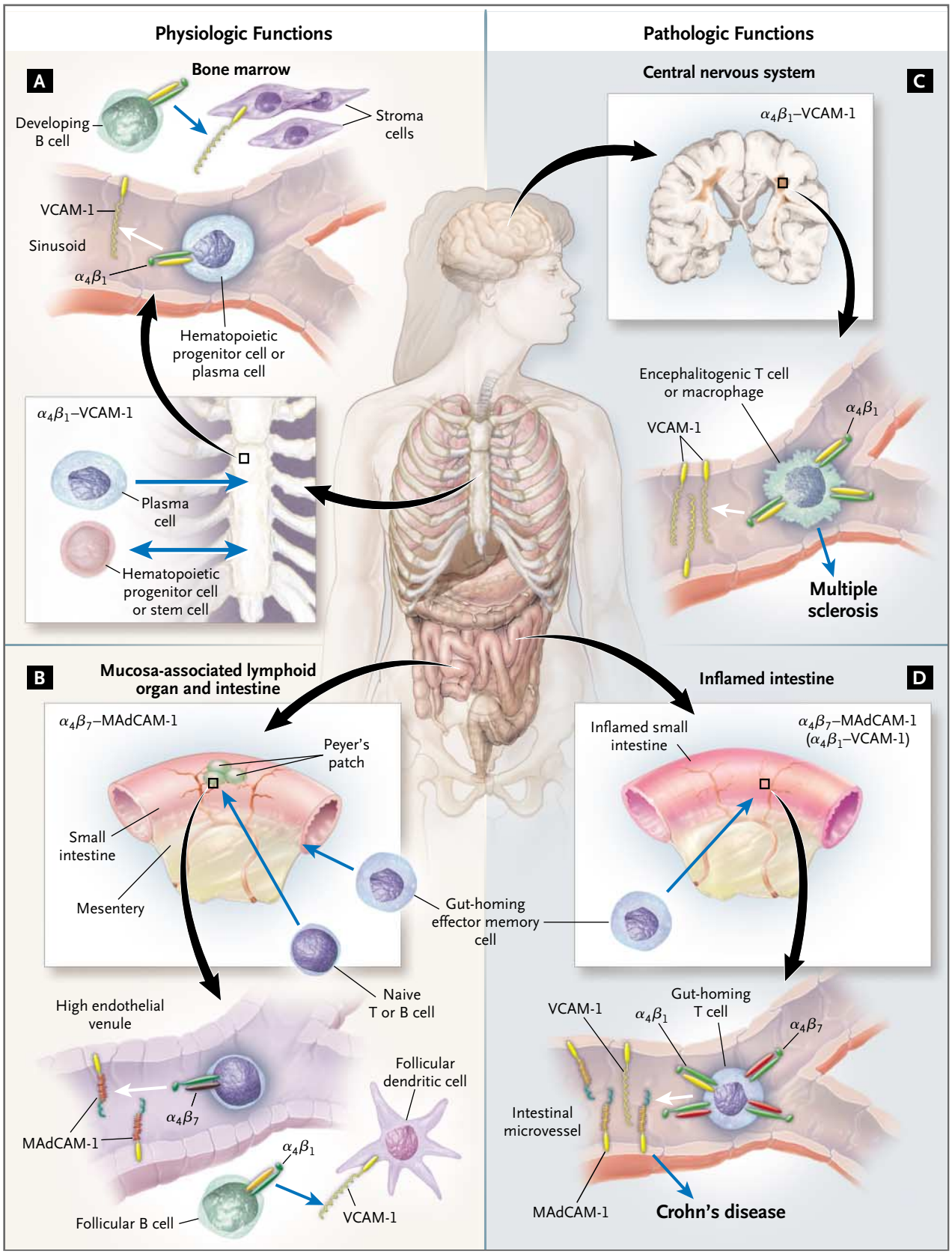
Whatever the precise mechanism of natalizumab's efficacy in patients with Crohn's disease, the drug had only partial antiinflammatory effects during the relatively short treatment period,[2] suggesting that intestinal inflammation involves other pathways of leukocyte recruitment. Indeed, the inflammatory infiltrate in Crohn's disease is dominated by neutrophils, which do not normally express α_4 integrins, but instead rely on β_2 integrins to travel to sites of inflammation.[4] Neutrophils can express $\alpha_4\beta_1$ once they have emigrated out of blood vessels,[17] but it is questionable whether the inhibition of α_4 integrins at this stage can interfere with leukocyte-mediated disease. Why, then, did natalizumab work in patients with Crohn's disease, even though it probably inhibited only intestinal homing of a relatively small population of T cells? One plausible explanation is that the T cells are essential for inducing the secondary signaling molecules in the gut, such as cytokines and chemokines, that are needed to sustain neutrophil recruitment.[14]

Orally available small molecules may eventually replace natalizumab as the α_4-integrin inhibitors of choice. It has already been shown that small molecules can specifically inhibit α_4 integrins, but clinical data are not yet available. Their use should have several advantages over monthly injections of recombinant protein. For example, binding antibodies against natalizumab developed in 11 percent of patients with multiple sclerosis[1] and in 7 percent of those with Crohn's disease.[2] Small molecules are much less likely to elicit such antibody responses.

Chronic inhibition of α_4 integrins could also have undesirable effects that are independent of the immunogenicity of the pharmacologic inhibitor. For example, in mice, an embryonic deficiency in the α_4 or β_1 integrin chain or in VCAM-1 is lethal before birth,[3] suggesting that the use of agents that inhibit these pathways should be avoided during pregnancy. On the other hand, in the clinical trials reported by Miller et al. and Ghosh et al., the rate of adverse events did not differ significantly between the natalizumab and placebo groups.[1,2] Considering the physiologic role of α_4 integrins in hematopoiesis and in mucosal and humoral immunity in animals, this finding is somewhat surprising. The recipients of natalizumab had elevated levels of lymphocytes, monocytes, and eosinophils in the circulation, which is consistent with the expression of α_4 integrins on these leukocytes, whereas neutrophil levels did not change.[1,2] This suggests that the antibody did not interfere with neutrophil functions that are independent of α_4 integrins and are necessary to combat bacterial and fungal infections. The natalizumab-treated patients with multiple sclerosis did have a higher incidence of infections, especially pharyngitis, than the patients who received placebo, but that trend was not statistically signifi-

Figure 1. Physiologic and Pathologic Functions of α_4 Integrins.

The two α_4 integrins, $\alpha_4\beta_1$ and $\alpha_4\beta_7$, and their respective ligands, vascular-cell adhesion molecule 1 (VCAM-1) and mucosal addressin-cell adhesion molecule 1 (MAdCAM-1), have been implicated in a number of physiologic events. The $\alpha_4\beta_1$–VCAM-1 pathway mediates the homing and retention of hematopoietic progenitor cells and IgG-producing plasma cells in the bone marrow (Panel A). This pathway also mediates interactions between bone marrow stroma cells and developing B cells, which are necessary for B-cell maturation. In addition, interactions of mature B cells with follicular dendritic cells in the B-cell follicles of secondary lymphoid organs, such as the spleen, lymph nodes, and Peyer's patches, are mediated by $\alpha_4\beta_1$–VCAM-1 (Panel B). B-cell communication with follicular dendritic cells is necessary for the generation of high-affinity antibodies to microbial agents. The $\alpha_4\beta_7$–MAdCAM-1 pathway is important for maintaining intestinal mucosal immunity, especially the homing of naive lymphocytes, which must adhere to MAdCAM-1 in high endothelial venules within gut-associated lymphoid tissues (Peyer's patches, appendix, and mesentric lymph nodes) and the migration of gut-homing memory cells to the intestinal lamina propria. A minor role of $\alpha_4\beta_1$ and VCAM-1 during the homing of naive lymphocytes to peripheral lymph nodes has also been observed. One of the pathologic functions of α_4 integrins in humans is the recruitment of circulating activated T cells, monocytes, and macrophages to the central nervous system (Panel C). Activated T cells can enter the central nervous system even in the absence of inflammation, because low levels of VCAM-1 are constitutively expressed in central nervous system microvessels. Microvascular VCAM-1 expression is markedly increased in the vicinity of multiple sclerosis lesions and contributes to enhanced recruitment of $\alpha_4\beta_1$-expressing cells at these sites. In Crohn's disease (Panel D), pathogenic effector T cells are recruited to the intestine predominantly through the $\alpha_4\beta_7$–MAdCAM-1 pathway, but the $\alpha_4\beta_1$–VCAM-1 pathway may contribute to their recruitment.


Physiologic Functions
Pathologic Functions
A
Bone marrow
Developing B cell
Stroma cells
VCAM-1
Sinusoid
$\alpha_4\beta_1$
Hematopoietic progenitor cell or plasma cell
$\alpha_4\beta_1$–VCAM-1
Plasma cell
Hematopoietic progenitor cell or stem cell
C
Central nervous system
$\alpha_4\beta_1$–VCAM-1
Encephalitogenic T cell or macrophage
VCAM-1
$\alpha_4\beta_1$
Multiple sclerosis
B
Mucosa-associated lymphoid organ and intestine
$\alpha_4\beta_7$–MAdCAM-1
Peyer's patch
Small intestine
Mesentery
Gut-homing effector memory cell
Naive T or B cell
High endothelial venule
Follicular dendritic cell
$\alpha_4\beta_7$
MAdCAM-1
$\alpha_4\beta_1$
Follicular B cell
VCAM-1
D
Inflamed intestine
Inflamed small intestine
$\alpha_4\beta_7$–MAdCAM-1 ($\alpha_4\beta_1$–VCAM-1)
VCAM-1
$\alpha_4\beta_1$
Gut-homing T cell
$\alpha_4\beta_7$
Intestinal microvessel
MAdCAM-1
Crohn's disease

cant. Given the small number of patients and the relatively short duration of natalizumab treatment in the current phase 2 trials, firm conclusions about the safety of α_4-integrin inhibition must await the results of much larger, phase 3 studies.

Larger numbers of patients and a longer duration of treatment will also be needed to determine whether resistance to natalizumab can develop. In addition, it will be important to determine whether natalizumab can reverse existing defects, especially in patients with multiple sclerosis. The α_4 integrins have also been implicated in other inflammatory conditions, such as ulcerative colitis, rheumatoid arthritis, insulitis, vasculitis, atherosclerosis, and asthma.[6] Treatment with α_4-integrin antagonists may lead to improvement in at least some of these diseases.

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