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Leukocyte Traffic Blockade in Inflammatory Bowel Disease

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Abstract

Dysregulated recruitment of leukocytes into the intestine is a characteristic feature of IBD. Several families of molecules regulate the influx of these cells into sites of inflammation within the gastrointestinal tract. Interference with molecules that mediate the formation of stable bonds (integrins) with their endothelial ligands has already shown efficacy in the clinics. Antibodies that target participant molecules have been approved by the US Federal Drug Administration for use in Crohn's, multiple sclerosis (MS) (i.e. natalizumab) and psoriasis (i.e. efalizumab). A more recent additional family of drugs, which might also interfere with lymphocyte traffic (i.e. shingosine-1-phosphate receptor agonists: fingolimod) is in clinical use for MS and just recently entered the clinical trial stage for ulcerative colitis. In the present review we discuss basic aspects of clinically relevant molecules and compile the clinical studies that support the targeting of specific steps of the leukocyte adhesion cascade for therapeutic purposes in IBD.

Keywords

adhesion molecules; chemokines; Crohn's disease; integrins; sphingosine1-phosphate; ulcerative colitis

Introduction

Immune-mediated chronic inflammatory diseases (e.g. rheumatoid arthritis (RA), psoriasis, multiple sclerosis, and inflammatory bowel disease (IBD)) are characterized by dysregulated leukocyte recruitment [1–3]. Neutrophils, which are short-lived outside the circulation, migrate to sites of inflammation and undergo apoptosis while monocytes remain in inflamed tissues for several days, while other monocytes become permanent residents. By contrast, naïve T cells migrate to lymphoid organs where they encounter antigens, proliferate and acquire effector functions, along with a repertoire of adhesion molecules, cytokine and chemokine receptors that allow them to recognize specific vascular beds [4, 5]. Effector lymphocytes then are able recirculate from the blood into tissues, followed by migration to lymphoid organs before returning to blood. Their ability to recirculate is essential for the perpetuation of chronic inflammatory processes, such as IBD. The molecules that mediate leukocyte traffic have therefore been the focus of investigation due to their potential as therapeutic targets.

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The Leukocyte Adhesion Cascade

Leukocytes migrate into sites of inflammation across the walls of post-capillary venules by engaging specific molecules expressed on specialized endothelial cells. Endothelial molecules serve as mechanical anchors and confer tissue specificity to the recruitment process [6]. These molecules mediate a sequential series of steps (i.e. capture, rolling, activation and firm adhesion) that allow leukocytes to escape the circulation and migrate to sites of inflammation. The major families of molecules involved in leukocyte recruitment include: selectins and their glycoprotein ligands, chemokines and their receptors, integrins and immunoglobulin-superfamily molecules.

Although interference with any of these steps might conceivably be of therapeutic value, only targeting the integrins, which mediate arrest and firm addition, have crossed from the bench into the clinics. Both $\alpha_4\beta_1$ (VLA-4) and $\alpha_4\beta_7$ integrins have been shown to mediate firm adhesion to their respective ligands Vascular Cell Adhesion Molecule (VCAM)-1 and Mucosal Addressin Cell Adhesion Molecule (MAdCAM)-1 [7, 8].

Integrins

Integrins are cell-adhesion receptors expressed on leukocytes as heterodimeric transmembrane glycoproteins [9]. They interact with molecular components of the extracellular matrix but also with ligands displayed by endothelial (and other) cells [10]. Binding of integrins to their respective ligands is stable, results in arrest of leukocytes on the vessel wall and ensures migration of lymphocytes to sites of inflammation. Besides cell adhesion, integrins have been shown to play important roles in other cellular processes, such as antigen recognition, cell proliferation and survival [11]. Consequently, integrins constitute integral components of diverse biological phenomena, including cellular development, immunological reactions, hemostasis and neoplastic transformation [10].

Integrins are heterodimeric molecules formed by the noncovalent association of two subunits, namely the large (α, molecular weight: 120–170 kDa) and the small (β, molecular weight: 90–100 kDa) subunits. As of today, 18 α and 8 β-subunits have been described, which combine to generate at least 24 different integrin heterodimers in vertebrates, 14 of which are detected in cells of the immune system (Figure 1) [11–14]. The high number of possible combinations ensures the wide functional diversity of these molecules. Each subunit is a type I transmembrane glycoprotein consisting of a large extracellular domain, a single pass transmembrane domain which induces intracellular signaling via binding to cytoskeleton [15] and a short cytoplasmic tail (with the notable exception of β_4 integrin) [13]. Extracellular domains contain the site for ligand binding, which is dependent upon the presence of divalent cations (Ca^{2+}, Mg^{2+}, and Mn^{2+}).

The type of the β-subunit that is present in each heterodimer defines discrete subtypes of integrins, which display unique patterns of structure, tissue-specificity and function. According to this functional scheme, the β_2, α_4 and β_7 families of integrins play the most prominent roles during immunological and inflammatory conditions, as they are leukocyte-specific[10, 16]. Further diversity results from the fact that different types of leukocytes display unique patterns of integrin expression. Indeed, lymphocytes, macrophages, and polymorphonuclear cells all express distinct sets of β integrins which differ between subclasses and also between the resting state and activated states [17].

Integrin ligands – adhesion molecules of the immunoglobulin superfamily

Integrins that are displayed on the surface of circulating leukocytes associate with adhesion molecules that are expressed on endothelial cells, directing the migration of leukocytes from the blood to sites with inflammation. The endothelial ligands for integrins are members of

the immunoglobulin superfamily. Proteins of this class share structural and genetic features with immunoglobulin molecules; they contain at least one immunoglobulin domain, comprising of two β-pleated sheets held together by a disulfide bond (Figure 2). These immunoglobulins play pivotal roles in IBD as they are major mediators of the aberrant trafficking of lymphocytes and other immune cells to the inflamed mucosal sites. In addition they may also participate in the pathogenesis of extraintestinal inflammatory reactions that are a frequent manifestation of IBD. Among the several members of the immunoglobulin superfamily, the following have established pathogenetic roles in IBD: intercellular adhesion molecule-1 (ICAM-1) or CD54, Vascular Cell Adhesion Molecule-1 (VCAM-1) or CD106, and Mucosal Addressin Cell Adhesion Molecule-1 (MAdCAM-1).

β_2 Integrins (CD18)

The β_2 integrins are also known as the "leukocyte" integrins because they are exclusively expressed on bone marrow-derived cells. Upon cellular activation, a process termed inside-out signaling or integrin activation is initiated whereby the extracellular region undergoes modulation that results in enhancement of ligand-binding capacity [13, 16]. Integrin activation is triggered by the engagement of various membrane receptors such as the binding of a chemokine or other chemoattractant to its heptahelical G-protein coupled receptor (GPCR) on the leukocyte surface [18, 19]. This triggering event is followed by inside-out signaling within milliseconds [13].

β_2 integrins/CD18 associate with different α chain ligands to generate four β_2-heterodimers (Figure 1). Their cellular distribution varies between the different heterodimers and some such as CD11c are now commonly seen as markers of monocyte lineage cells. Critical information for the functional importance of β_2 integrins has been gained from the study of the clinical and immunological phenotype of mice or humans bearing mutations in the respective genes. In particular, lymphocytes and neutrophils mainly express CD11a/CD18 (LFA-1). LFA-1 binds to ICAM-1 and ICAM-2 expressed on endothelial cells and mediates migration, antigen presentation and cell proliferation [20, 21]. The existence of a null mutation in the gene encoding the LFA-1 α chain results to defects in lymphocyte migration to secondary lymphoid organs [22]. CD11b/CD18 (Mac-1) is also expressed on lymphocytes; nevertheless its main cellular localization is on monocytes, granulocytes and some NK cells. In phagocytes, Mac-1 is necessary for respiratory burst and certain forms of phagocytosis [23, 24]. The other two β_2-heterodimers are not expressed on lymphocytes; instead CD11c/CD18 (p150, p95) is detected on monocytes and dendritic cells and CD11d/CD18 predominantly on macrophages and granulocytes in the red pulp of the spleen [25]. Recent genetic studies have clearly elucidated an important role of CD18 integrins in innate immunity. Indeed, humans [26] and mice [21], bearing null or hypomorphic mutations in the Itgb2 gene (which encodes for the β_2 (CD18) integrin subunit]) result in the genetic disorder leukocyte adhesion deficiency-1 (LAD-1) [26]. This condition is characterized by recurrent bacterial infections. The pathophysiological disturbance that underlies the clinical phenotype is ineffective recruitment of granulocytes in response to bacterial invasion [26].

As mentioned above, ICAM-1 is a very important ligand for integrins LFA-1 and Mac-1 [27]. LFA-1/ICAM-1 interaction is dominant for the trafficking of inflammatory cells, whereas Mac-1 may associate with various ligands [28]. ICAM-1 is constitutively expressed on endothelial cells. Nevertheless, its expression is highly upregulated when endothelial cells are stimulated by pro-inflammatory signals, including cytokines, such as IL-1α and IFN-γ [29]. These cytokines are integral components of the mucosal milieu in IBD and may be responsible for the increased adhesiveness for integrins that characterizes the inflamed gut [30]. In fact, mucosal upregulation of ICAM-1 has been reported in both CD and UC, whereas, in a recent study, response to treatment with anti-TNF agents correlated to decreases in mucosal ICAM-1 expression [31].

Therapeutic targeting of CD18 integrin/ICAM-1 pathway in IBD

Efalizumab in CD

Efalizumab (RAPTIVA) is a humanized monoclonal antibody (IgG1) that targets the integrin α_L subunit (CD11a) of LFA-1 [32]. As a result, LFA-1 expressing lymphocytes are prevented from interacting with ICAM-1-expressing endothelial cells. This, in turn, results in compromised trafficking and defective activation of T-cells. Efalizumab was approved in 2003 for the treatment of plaque psoriasis [33]. In 2011 the results of a clinical trial of efalizumab administration in treatment-refractory, moderate to severe CD (Crohn's Disease Activity Index [CDAI] score 220–450) CD were published [34]. Efalizumab was administered on a weekly schedule of subcutaneous 1 mg/kg injection for 8 weeks. The primary endpoint of clinical response (≥70 points decrease in the CDAI score) at 8 weeks was reached by 67% of patients. Clinical remission was achieved in 40% of cases and there was a significant increase in the mean Inflammatory Bowel Disease Questionnaire (IBDQ) score from 124 to 168 (P<0.001). Clinical response typically occurred during the first weeks of treatment (before week 4). There were no serious adverse events during the follow-up period (16 weeks). The most frequent adverse effect was headache that usually occurred after the first 3 doses and lasted for 1–2 days post-injection. In April 2009 efalizumab was voluntary withdrawn from the US market, due to the report of 3 cases of progressive multifocal leukoencephalopathy (PML) in patients with psoriasis.

Alicaforsen in IBD

Alicaforsen (ISIS2302) is a 20 base pair antisense oligonucleotide that inhibits the translation of ICAM-1 mRNA, leading to reduced expression of ICAM-1. The efficacy of this compound was originally studied in CD in various therapeutic protocols, including intravenous and subcutaneous administration. Analysis of these studies led to the conclusion that ICAM-1 is ineffective for the treatment of CD [35].

The efficacy of local alicaforsen delivery by enema was subsequently studied in patients with active, left-sided UC. Bioavailability studies did show that following rectal enema the concentration of alicaforsen at the colonic mucosa was more than 100-fold higher when compared to the maximum concentrations obtained in plasma [36]. The efficacy of the compound was studied in a randomized, double-blind, both placebo and active-controlled multicenter studies, with mesalazine enema as the active comparator [37, 38]. Patients with mild to moderate active, left-sided colitis were administered daily enemas of different doses of alicaforsen, mesalazine or placebo for 6 weeks. The major conclusions from these findings were the favorable safety profile and the durability of response to alicaforsen, which exceeded that of mesalazine.

α_4 Integrins (CD49d) and their ligands: $\alpha_4\beta1$/VCAM-1 and $\alpha_4\beta7$/MAdCAM-1

There are two integrins that contain an α_4 subunit, namely the $\alpha_4\beta_1$ and the $\alpha_4\beta_7$. The $\alpha_4\beta_1$ homodimer is also known as very late antigen-4 (VLA-4, CD49d/CD29) [39, 40]. VLA-4 binds to its ligand VCAM-1 (CD106), and is chiefly responsible for lymphocyte and monocyte adhesion to vascular endothelium. The steady state-expression of VCAM-1 on the endothelium is very low/undetectable. Nevertheless, under inflammatory or other stimulatory conditions VCAM-1 expression is upregulated. VCAM-1 is not exclusively expressed on endothelial cells. Studies have reported detection of this molecule on epithelial cells, dendritic cells (DCs), Kupffer cells and on smooth muscle cells within atherosclerotic lesions [41–43]. Of particular importance is the fact that VLA-4/VCAM-1 system regulates the trafficking of lymphocytes to the central nervous system. VCAM-1 is expressed at very low levels in CNS microvessels at the healthy state; nevertheless its expression is significantly induced under inflammatory conditions. This was shown in the murine model of

experimental autoimmune encephalitis and further confirmed in patients with multiple sclerosis [44]. These findings may have important implications for the pathogenesis of PML, a devastating adverse effect of anti-integrin-based therapies.

The other α_4 integrin, $\alpha_4\beta_7$ is critically involved in gut-homing. Indeed, $\alpha_4\beta_7$ expression is upregulated on plasma cells that are positive for the mucosa-associated immunoglobulin IgA and in memory/activated $CD4^+$ subsets that re-circulate to the gut (defined as, $\alpha4\beta7^{high}$ memory T cells) [45]. This is in contrast to the immunophenotype displayed by non-mucosa associated memory T-lymphocytes. In this population, the expression of β_7 integrins is lacking and replaced by upregulation of $\alpha_4\beta_7$, which binds to VCAM-1 and directs T-cells to non-mucosal sites [46, 47]. It has been recognized in recent years that the $\alpha_4\beta_7$-mediated gut tropism of memory/activated gut-homing T cells (but also of and circulating B cells) is induced by retinoic acid, which originates from intestinal DCs [48, 49].

The ligand for $\alpha_4\beta_7$ is mucosal addressin cell adhesion molecule-1 (MAdCAM-1) [50–52] which is selectively expressed by high endothelial venules of Peyer's patches and gut associated lymphoid tissue [53]. The $\alpha_4\beta_7$/MAdCAM-1 interaction is pivotal for the homing of lymphocytes to Peyer's patches. MAdCAM-1 may also be involved in L-selectin-mediated rolling of inflammatory cells[54]. Therefore, MAdCAM-1 may be the common critical factor through which L-selectin and $\alpha_4\beta_7$ integrin synergize for lymphocyte homing to the intestine [7, 55].

Recent evidence shows increased numbers of intestinal mucosal vessels that stain positive for MAdCAM-1 patients with CD or UC [51]. In addition, animal models of intestinal inflammation, such as the colitic IL-10 KO mice overexpress mucosal MAdCAM-1 [56]. This upregulation may be the result of the proinflammatory milieu that predominates at the inflamed mucosa. TNF-α and IL-1 are both abundant in areas of active CD or UC and have been shown to induce upregulation of MAdCAM-1 expression in the intestine, colon and MLN [56, 57]. Very interestingly, during active IBD, MAdCAM-1 expression is detected in extra-intestinal sites, such as the joints, eyes, skin and liver[58]. As these organs are frequently affected in patients with IBD, the aberrant expression of a gut-homing molecule in these tissues may attract pathogenic cells and induce extra-intestinal inflammation. In addition to IBD, MAdCAM-1 expression is upregulated on inflamed venules in several other chronic inflammatory conditions as those occurring in diabetes, primary sclerosing cholangitis, and cirrhosis [59].

Therapeutic targeting of α_4 integrins/ligands in IBD

Natalizumab in CD

Natalizumab is a recombinant humanized IgG4κ monoclonal antibody that binds to α4-integrin. It was produced in murine myeloma cells and humanized by engrafting the complementarity-determining regions of a murine anti-α_4 antibody (AN100226m) to a human immunoglobulin IgG4 framework. This resulted in a 95/5 % human /murine protein composition of natalizumab, with a molecular weight of 149 kD. Natalizumab is marketed under the brand name of Tysabri (formerly Antegren).

By targeting the α_4 subunit of integrins, natalizumab blocks both $\alpha_4\beta_1$/VCAM-1 and $\alpha_4\beta_7$/MAdCAM-1 interactions. These properties were demonstrated in *in vitro* studies, as natalizumab effectively prevented adhesion of human Jurkat cells that expressed $\alpha_4\beta_1$ to purified recombinant VCAM-1 and of RPMI-8866 cells that expressed $\alpha_4\beta_7$ to recombinant MAdCAM-1. These data were complemented by *in vivo* studies in guinea pigs with experimental allergic encephalomyelitis (EAE). This model is mediated by T-lymphocytes that infiltrate regions of the central nervous system via $\alpha_4\beta_1$/VCAM-1-mediated migration.

Natalizumab administration did not allow leukocytes from crossing the blood-brain barrier and both prevented the development of neurological manifestations as well as reversed established disease [60]. In all, these results provided a direct proof for the efficacy of natalizumab as an anti-adhesion drug. Pre-clinical studies were also performed in tamarins with IBD and provided evidence for an anti-inflammatory effect of α4 blockade in experimental intestinal inflammation[61, 62]. These pre-clinical studies were followed by a multicenter study of natalizumab in patients with active multiple sclerosis and a small phase I study in 26 healthy male volunteers, which showed that a single 3-mg/kg intravenous dose was safe and well tolerated. These data set the background for clinical studies of natalizumab in patients with IBD.

In a first study by Gordon et al., 30 patients with mild to moderate, active CD (CDAI >151 and <450) were blindly randomized to receive a single, 3-mg/kg infusion of natalizumab (n=18) or placebo (n=12) (n=12)[63]. The primary outcome was the change in CDAI at week 2 after infusion and the presence of clinical remission as defined by a CDAI<150. Among secondary outcomes were Inflammatory Bowel Disease Questionnaire (IBDQ) score, concentration of C-reactive protein (CRP) in the serum, and peripheral blood T-cells and B-cells counts. At week 2, the CDAI decreased significantly from baseline after infusion of natalizumab (mean 45 points) but not placebo (mean 11 points). The number of patients achieving clinical remission at week 2 was 7/18 (39%) and 1/12 (8%) for the natalizumab and control groups, respectively. Nevertheless, comparisons between natalizumab and placebo-treated patients regarding clinical response and remission did not reach statistical significance. At 4 weeks, patients treated with natalizumab experienced significant improvement of IBDQ scores, and decreases in the levels of inflammatory markers (CRP, ESR). Rescue therapies were needed by 4/12 (33%) of the placebo-treated patients in contrast to only 2/18 (11%) patients in the natalizumab group. Significant increases in circulating B and T lymphocytes were detected 1, 2, and 4 weeks after drug administration indicating that natalizumab interrupted lymphocyte trafficking. There were no significant differences in the frequency of adverse events between natalizumab and placebo-treated patients, the most common being headache, CD exacerbation, and abdominal pain. The overall modest results achieved in the study by Gordon may be attributed to the study protocol that included a single infusion of natalizumab. It was later found that this administration resulted in a satisfactory mean serum concentration of natalizumab by week 2, whereas at week 4, drug levels were lower, and potentially suboptimal according to the results of leukocyte saturation studies.

A second, double-blinded randomized study on natalizumab in CD was performed by Gosh et al.[64] Patients (n=248) with moderate to severe CD (CDAI >220 and <450) were recruited from 35 centers. Patients were randomized to receive two infusions 4 weeks apart (week 0 and week 4) according to four different treatment regimens: wk-0: placebo, wk-4: placebo; wk-0: 3 mg/kg natalizumab, wk-4: placebo; wk-0: 3 mg/kg natalizumab, wk-4: 3 mg/kg natalizumab; or wk-0: 6 mg/kg* natalizumab, wk-4: 6 mg/kg* natalizumab. The primary outcome was the proportion of patients in remission (CDAI<150) by week 6 which was not reached as there were no significant differences between the rates of remission between the group that received 2 infusions of 6 mg/kg* natalizumab and the placebo group. In contrast, there were significantly more patients in remission in the 6 mg/kg* group both at 4 weeks (29% vs. 14% in the placebo group, P=0.028) and at 8 weeks (43% vs 16%, P=<0.001). In addition, patients receiving 3 mg/kg* of natalizumab had a significant higher chance of being in remission by week 4 (29% vs. 14%, P=0.027, week 6 (44% vs. 27%, P=0.03), week 8 (41% vs. 16%, P<0.001) and week 12 (42% vs. 27% in the placebo group, P=0.042). In addition, administration of 3 or 6 mg/kg* of natalizumab resulted in significant differences on several secondary outcomes of the study, including higher percentages of patients achieving clinical response by week 4, 6, 8, and 12, significant improvements in mean

IBDQ scores at week 6, as well as significant decline of the baseline serum CRP levels at week 6 ($P<0.05$ vs. placebo treated). No significant differences in study outcomes were seen between the groups receiving 3 or 6 mg/kg* of natalizumab. Similar to the previous study, there were increased numbers of lymphocytes in the systemic circulation, a finding compatible with a natalizumab-mediated inhibition of lymphocytic extravasation due to inhibition of α_4 integrin blockade.

The ENACT study was the first to include both induction and maintenance arms for the administration of natalizumab to patients with CD[65]. In addition, a fixed dose of 300mg of natalizumab was given to patients instead of body weight based adjustments. The study was conducted in 142 centers around the world and patients with moderate to severe CD (CDAI >220 and <450) were recruited. In the induction arm, patients received 3 infusions of 300 mg natalizumab (n=724) or placebo (n=181) at weeks 0, 4, and 8. The primary outcome of the study was clinical response at week 10, which was defined as a decrease in CDAI score of at least 70 points, whereas clinical remission (CDAI <150) was a secondary endpoint. At week 10 the response and remission rates were 56% and 37% for the natalizumab group and 49% and 30% for placebo-treated patients, respectively, the differences not being statistically significant ($P=0.05$ for response and $P=0.12$ for remission, respectively). However, when patients with elevated CRP at baseline were analyzed separately, significant differences were found.

In the maintenance phase of the study, primary responders of the induction arm (n=399) were randomly assigned to receive natalizumab or placebo every 4 weeks through week 56. Analysis of the data showed that significantly more patients on continuous natalizumab treatment remained in clinical response as compared with the placebo group (61% vs. 28%, $P<0.001$). In addition the time to loss of response was longer in the natalizumab group.

A final study (ENCORE trial) was published in 2005 by Targan et al.[66] Only patients with objective evidence of active inflammation, as indicated by elevated CRP were included. Patients with moderate to severe CD were enrolled in 112 centers from around the world. Participants were randomized 1:1 to receive natalizumab 300mg on week 0-4-8 or placebo infusions. The primary outcome was clinical response (reduction of CDAI by 70 points from baseline) at week 8 that was maintained through week 12. The primary endpoint was met by 124/259 (48%) of patients receiving natalizumab and 81/250 (32%) of placebo-treated patients. The difference was significant ($P<0.001$). The secondary endpoint (clinical remission) was also significantly higher in the natalizumab group (26% vs. 16%, $P=0.002$). At week 12, the rates for clinical response and remission were 60% and 38% for the natalizumab group and 44% and 25% for the placebo group (both statistically significant). In addition, the median time to response was shorter for the natalizumab group (31 days) as compared with the placebo-treated group (51 days). Other secondary endpoints included significant improvements in IBDQ scores and consistent decreases in CRP concentrations in natalizumab treated patients. The rates of adverse events (including serious) did not differ between active drug and placebo groups.

Taken together, the aforementioned studies provided important evidence for the efficacy of natalizumab as a remission-inductive and maintenance therapy for CD.

Natalizumab in UC

In addition to the reviewed studies in CD, the efficacy of natalizumab was also tested in a small group of patients with UC[63]. Ten patients with active disease (Powell-Tuck activity score >4, median baseline score=10) received a single 3 mg/kg natalizumab infusion. There were significant decreases in the median Powell-Tuck score at 2 weeks (primary endpoint, decrease of 7.5 points) but also at 4 weeks post-infusion (decrease of 6). Patients also

achieved significant improvements in quality of life scores by week 4. Further evaluation of the efficacy of natalizumab in UC in a randomized, placebo-control trial has not been reported yet and under the TOUCH program implemented after the reports of PML, patients with UC may not receive this drug in the United States.

AJM300 in CD

AJM300 is an oral compound that acts as an antagonist of α_4 integrins. Several studies have reported the efficacy of this small molecule in animal models of IBD (i.e. TNBS, DSS, and adoptive transfer); nevertheless all have been presented in abstract form and associated full manuscripts are yet to be published. Similarly the results of a randomized, double-blind, placebo controlled trial in Japanese patients with active CD was presented during DDW 2009 by Takazoe et al. (DDW2009, A-181, presentation #S1066). In this trial seventy-one patients with active CD (CDAI>150 and elevated CRP) received placebo or one of 4 doses of AJM300: AJM300 40 mg TID, 120 mg TID, or 240 mg TID orally for 8 weeks. AJM300 was safe and well tolerated. The study did not meet the primary (decrease in CDAI at week 4) or secondary (decrease in CDAI by 70 points at week 4) endpoints as the differences between AJM300- and placebo-treated patients were not significantly different. However, patients with a CDAI≥200 at week 0, had a significant decrease of CDAI (41.5 ± 57.5 in the 120 mg group, P=0.0485, 41.6 ± 94.1 in the 240 mg group); in addition, there was a significant decrease in CRP level but only in the 240 mg group (1.87 mg/dL at week 0 to 0.96 mg/dL at week 8, P=0.022). Further evaluation of the efficacy of AJM300 in IBD is required.

Vedolizumab in IBD

An antibody that binds to a combinatorial epitope on $\alpha_4\beta_7$ integrin (i.e. vedolizumab, MLN002) has been tested in patients with IBD. The topic is discussed elsewhere in this issue (see Parikh and Danese).

PF-00547659 (anti-MAdCAM-1) in UC

PF-00547659 is a fully human IgG2 antibody against MAdCAM-1. Its functional characteristics and pharmacological properties have been extensively characterized[67]. In particular, its ability to bind to human MAdCAM-1 and block adhesion of $\alpha_4\beta_7$-expressing leukocytes was demonstrated both in *in vitro* systems and in animal models. In 2011, the results of the first human study utilizing PF-00547659 were reported in a population of patients with UC[68]. Eighty patients with active UC (defined as a total Mayo score ≥6, endoscopic subscore ≥2) were randomized in a double-blind way to receive either placebo or one of multiple PF-00547659 regimens (single intravenous infusions: 0.03, 0.1, 0.3, 1.0 or 10 mg/kg or a single subcutaneous injection of 3.0 mg/kg or multiple intravenous infusions: 0.1, 0.3 or 3.0 mg/kg 4 weeks apart or multiple subcutaneous infusions: 4 weeks apart 0.3 or 1.0 mg/kg). Clinical response was defined as the proportion of patients with ≥3-point reduction and 30% improvement in total Mayo score, and ≥1-point decrease in rectal bleeding subscore or absolute rectal bleeding score of 0 or1 and was achieved by 52% in PF-00547659-treated patients (vs. 32% in the placebo group) and 42% (vs. 21%) at week 4 and 12, respectively. Remission was defined as the proportion of patients with total Mayo score ≤2 points with no individual subscore exceeding 1 point, and was reached by 13% of PF-00547659-treated patients (vs. 11%) and 22% (vs. 0%) at week 4 and 12, respectively. Endoscopic response was defined as ≥1-point improvement in the Mayo endoscopic subscore and was achieved by 50% of PF-00547659-treated patients (vs. 26%) and 42% (vs. 29%) at week 4 and 12, respectively. These results were corroborated by objective measures of response such as the decrease in fecal calprotectin levels at week 4 (PF-00547659: 63%; placebo: 8%). There were no drug-related side effects in the study during the follow-up

period. Further studies will be required to elucidate the clinical applicability of this novel anti-MAdCAM-1 antibody in patients with IBD.

$\alpha_E\beta_7$ Integrin (CD103)

Integrin $\alpha_E\beta_7$ (CD103) is a heterodimer composed by the alpha E chain (molecular weight: 175kDa) which only pairs with the β_7 chain. The ligand for $\alpha_E\beta_7$ has been identified as E-cadherin that is expressed by epithelial cells [69]. The adhesion of $\alpha_E\beta_7$ T cells to epithelial E-cadherin is promoted by the interaction between epithelial CCL25 and T-cell CCR9. The gut selectivity of this molecule is exemplified by the fact that $\alpha_E\beta_7$ was originally identified by a monoclonal antibody that recognizes lymphocytes in intestinal tissue (i.e. human mucosal lymphocyte antigen-1, HML-1). A minority (2%) of peripheral blood lymphocytes express$\alpha_E\beta_7$. In sharp contrast, more than 90% of intraepithelial lymphocytes (IEL) express this marker, which is also present on fractions of DCs and lymphocytes at effector sites in the intestinal lamina propria[70]. In fact, the subpopulation of DCs that express $\alpha_E\beta_7$ may be responsible for imprinting gut tropism to T cells [71, 72]. Furthermore, it has been recently demonstrated that the expression of $\alpha_E\beta_7$ defines also a unique population of regulatory cells [72, 73]. The expression of $\alpha_E\beta_7$ by different leukocyte subsets might have implications for the targeting of this molecule in IBD.

Therapeutic targeting of β_7 integrins in IBD

Etrolizumab in UC

Etrolizumab is a humanized IgG1 monoclonal antibody that is directed against the β_7 integrin. Therefore, etrolizumab targets both the $\alpha_E\beta_7$ and the $\alpha_4\beta_7$ integrins and blocks interactions to their respective ligands, MAdCAM-1 and E-cadherin. Pre-clinical studies showed that etrolizumab effectively inhibits migration of T-cells to mucosal sites, without affecting their homing to non-mucosal tissue [74]. The results from a randomized, phase I study on the use of etrolizumab (PRO145223) in moderate to severe UC were recently reported [75]. The study had two components. In the single ascending dose protocol, patients received once either placebo or one of 4 ascending doses of etrolizumab (0.3, 1.0, 3.0, 10 mg/kg intravenous, or 3.0 mg/kg subcutaneous). In the multipledose (MD) stage, patients received etrolizumab every for weeks for 3 cycles in 4 different regimens (0.5 mg/kg SC, 1.5 mg/kg SC,3.0 mg/kg SC or 4.0 mg/kg IV.) or placebo. The safety, pharmacokinetics, and clinical response (as estimated by Mayo Clinic Score calculations) were examined at various time-points. This study showed that etrolizumab was well tolerated and safe. Serious adverse effects included exacerbation of UC and impaired wound healing in two patients who underwent colectomy. The most common side effect was headache. There was a decrease in 'availability" of β_7 receptors on target $CD4_+$ lymphocytes, providing evidence that etrolizumab administration decreases the number of gut homing lymphocytes and compromises lymphocytic migration to the gut. The duration of β_7 receptors was dose-dependent. Clinical response (≥3 points and 30% reduction from baseline in Mayo Clinic Score, and ≥1 point decrease in rectal bleeding subscore or absolute rectal bleeding score of 0 or 1) was achieved by 12/18 etrolizumab-treated patients. Clinical remission (Mayo Clinic Score ≤2 with no individual subscore>1) was achieved in 3/18. Nevertheless, similar trends were seen in the placebo group and no conclusion on efficacy could be drawn.

PML: a major complication of anti-integrin antibodies

The widespread use of anti-integrin monoclonal antibodies (natalizumab, efalizumab) for the treatment of IBD, multiple sclerosis or psoriasis has been hampered by the occurrence of a rare but potentially fatal complication, progressive multifocal leukoencephalopathy (PML)[76]. This condition is the result of reactivation of a polyoma virus, which is designated JC virus. The risk for developing PML after treatment with natalizumab has been estimated

to approximately 1:1000 for patients treated for more than 2 years. Efalizumab has also been associated with cases of PML in patients with psoriasis. Up to 2009, 4 cases were described within a cohort of 6000 patients that had received efalizumab for psoriasis. Development of PML has result in the voluntary withdrawal of natalizumab from the market in February 2005 (it returned in July 2006) and of efalizumab in 2009. PML appears to be a true drug-effect as neither MS, CD nor psoriasis have been associated with PML *per se*. Currently, natalizubab (TYSABRI) is available in the US only through a restricted distribution program, called the TOUCH Prescribing Program.

The pathogenesis of PML in patients receiving natalizumab is largely unknown. Nevertheless, it may be primarily associated with the binding of α4β1 integrin by natalizumab. This may result in the blockade of migration of JCV-specific lymphocytes to the central nervous system, including cytolytic lymphocytes, as the latter have been related to increased survival from PML [77]. Alternative pathogenetic mechanisms may also participate such as mobilization of JC-infected pre-B-cells from the bone marrow due to $\alpha_4\beta_1$ blockade [78]. In any case, if $\alpha_4\beta_1$ blockade is mainly responsible for PML development it should be expected that selective blockade of $\alpha_4\beta_7$ will avoid this complication. Indeed, there have been no cases of PML in patients treated with the specific anti-$\alpha_4\beta_7$ antibody vedolizumab.

It is not clear whether PML is a ‘class” adverse effect. Cases of PML have been reported in patients receiving rituximab also [79]. Rituximab is an anti-CD20 monoclonal antibody that primarily targets B-cells. Nevertheless, a causal association between this drug and PML cannot be directly established since the conditions for which rituximab was administered (lymphoproliferative disorders, systemic lupus erythematosous and rheumatoid arthritis) may inherently increase the risk for developing PML. The frequency of PML in patients who are negative for JC virus (around 50% of patients) is near zero, thus it is possible that anti-integrin antibodies might be much more safely used in seronegative patients.

Chemokines as therapeutic targets

Chemokines are small proteins originally named for their role in leukocyte chemotaxis: **chemo**/tactic cyto/**kine**. They are classified based on the relative position of cysteine residues (i.e. C, CC, CXC, CX3C) and bind to G-protein coupled chemokine receptors (CCR). CCL25 is produced by small intestinal epithelial cells, and serves as a homing beacon for the homeostatic recruitment of lymphocyte subpopulations (e.g., IgA antibody secreting cells, CD8αα and T cells) to the small intestine [80–85]. The description of the restricted small intestinal expression of the chemokine CCL25/TECK [86] was exciting, as this expression pattern provided molecular evidence for the potential dichotomization of intestinal trafficking into distinct small- and large-intestinal compartments [87, 88]. Dichotomization of homing to small and large intestine for the first time allowed us to understand a subset of patient with Crohn’s develop disease strictly localized to small bowel. Patients with small intestinal CD have an increased number of $CCR9^+$ T cells increased in peripheral blood [89]. It has also been shown that CCL25 is induced aberrantly in the chronically-inflamed hepatic microvasculature of patients with primary sclerosing cholangitis (PSC), a chronic immune-mediated disease of the biliary tree frequently associated with IBD [90]. These findings imply that the role of CCL25/CCR9 may not be limited to homeostatic recruitment, but rather that this chemokine/receptor pair also participates in chronic inflammatory trafficking.

Traficet EN in CD

GSK-1605786 (Traficet-EN) is a small molecule that was developed as a selective antagonist of CCR9. Its mechanism of action relates to blockade of CCL25/CCR9

interaction, which is said to result in prevention of B- and T-cell trafficking to the intestine. An obvious theoretical advantage of this compound, besides its oral administration, is that migration of lymphocytes to non-intestinal tissues is theoretically unaffected, ameliorating therefore the risk for infectious complications, including PML. There has been one clinical trial of GSK-1605786 in CD (PROTECT-1 trial), which included an induction and a maintenance arm. So far, the results of this study have been presented only in abstract form. In the Protect-1 induction trial, 436 patients with active CD (CDAI>250 and <450) were randomized to receive one of three doses of Traficet-EN or placebo for 12 weeks. Clinical response (defined as the number of patients with decrease of ≥70 points on the CDAI) was 61% with Traficet-EN vs. 40% with placebo. In the maintenance trial, 241 responders from the induction phase were re-randomized to either Traficet-EN 250 mg twice daily (n=146) or placebo (n=95) for 36 weeks. At the end of the follow-up there was a significant difference in remission rates between patients on Traficet-EN (47%) in comparison with those who received placebo (31%) (P=0.01). This was associated with significant increases in the rates of corticosteroid-free remission and normal CRP levels. Traficet-EN was safe and well-tolerated with no increase in serious adverse events. Several phase III clinical trials are currently underway. Of interest is that we cannot find published evidence that this chemokine axis plays a role in traffic to the colon, yet patients with CD regardless of their disease localization (e.g. ileitis, colitis, ileocolitis) are being included. Results from these studies will define the applicability and efficacy of this attractive novel treatment for patients with CD.

BMS-936557 in UC

BMS-936557 is a fully human antibody that binds to the chemokine IP-10 (CXCL10), therefore blocking its interaction with its receptor CXCR3. Recently, the efficacy of BMS-936557 in moderate to severe UC was reported. This was a randomized, double-blind, placebo-control, phase II study which was carried out in 54 centers in 8 countries. Patients with active UC were administered BMS-936557 (10 mg/kg) or placebo at weeks 0, 2, 4, and 6. The primary endpoint was rate of clinical response at day 57 (≥3 points and 30% decrease in Mayo score, with ≥1 point decrease in rectal bleeding score or absolute rectal bleeding score ≤1). The primary endpoint was not met as the clinical response rate at day 57 was 52.7% (BMS-936557) vs. 35.2% (placebo) (P=0.083). The secondary endpoints of clinical remission and mucosal healing were also not met. Nevertheless, when patients with higher steady-state through concentration of BMS-936557 were analyzed, this population had a significantly increased clinical response (87.5% vs. 37%, P<0.001) and histological improvement (73% vs. 41%, P=0.004). More studies will be required to evaluate the efficacy of this compound in UC as well as the importance of achieving optimal drug levels.

Future Directions

Targeting sphingosine-1-phosphate receptors in IBD

Shingosine-1-phosphate (S1P) is a bioactive sphingolipid that regulates an array of physiological processes, including lymphocyte traffic. T cells express G-protein coupled receptors (GPCRs) that bind S1P and lymphocytes rely on S1P gradients to recirculate. Fingolimod (FTY720, a prototype S1P receptor agonist) is derived from myriocin, a fungal derivative used in Chinese medicine. Like natalizumab, fingolimod is effective in MS and already approved by the FDA for that indication under the brand name Gylenia. Fingolimod binds to four of the five S1P receptors (S1P1, 3,4,5). Due to this lack of specificity its bradycardic effect on heart rate has already resulted in 1 death [91]. Refinement of the drug, to minimize their effect on heart rate is desirable. A more specific S1P1 agonist (RPC 1063) has been successfully tested in animal models of IBD[92] and is currently being tested in patients with UC (http://www.clinicaltrials.gov/ct2/show/NCT01647516).

Conclusions and implications for future anti-adhesion strategies

During the past decade, the success of anti-TNF-α strategies has revolutionized the treatment of IBD [93]. Yet, only about 70% of patients respond to this therapy, and another 20% lose response after a year, driving the continued search for other therapeutic strategies [93, 94]. Interference with leukocyte recirculation to the intestine by targeting specific molecules involved with leukocyte traffic has resulted in the development of agents that have advanced into clinical use (e.g. Natalizumab (Biogen/Elan Pharmaceuticals, www.tysabri.com), vedolizumab (Millenium Pharmaceuticals), RPC 1063 (Receptos)) have been either approved or are being evaluated in CD and UC [95]. Several other molecules that target these pathways are currently being evaluated. Despite their progression from the bench to bedside [64, 96], much remains to be learned regarding their fundamental mechanism of action. This enhanced understanding may allow us to optimize these therapies[64], minimize risks and potentially expand their use to other chronic inflammatory conditions which share self-perpetuating and dysregulated lymphocyte recruitment as a basic pathogenic mechanism.

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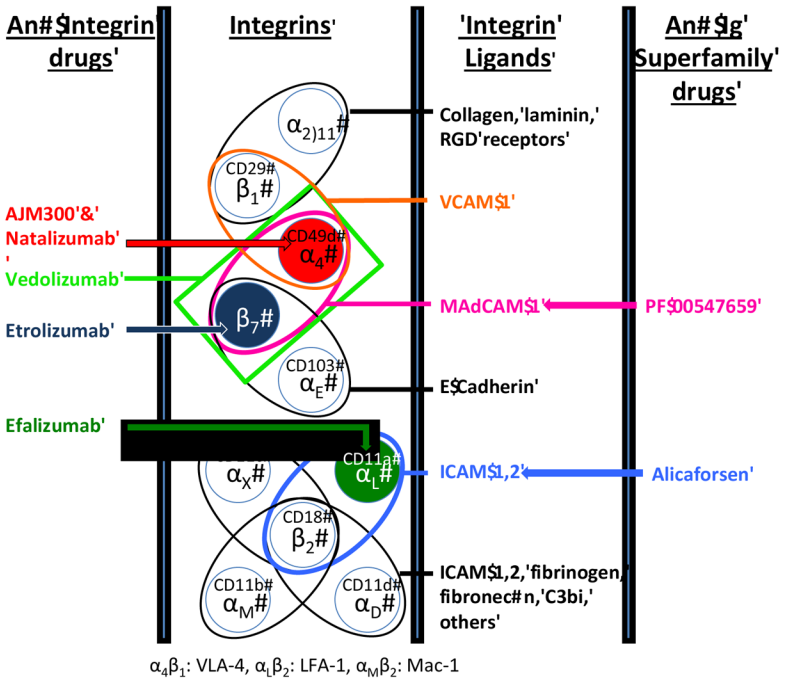

Figure 1.

The integrins are αβ heterodimers that share common subunits that bind distinct ligands. Illustrated in color are the integrins that have been targeted for the treatment of IBD. RGD: arginine-glycine-aspartic acid sequence found in some integrin ligands. C3bi: Complement 3b inactivated

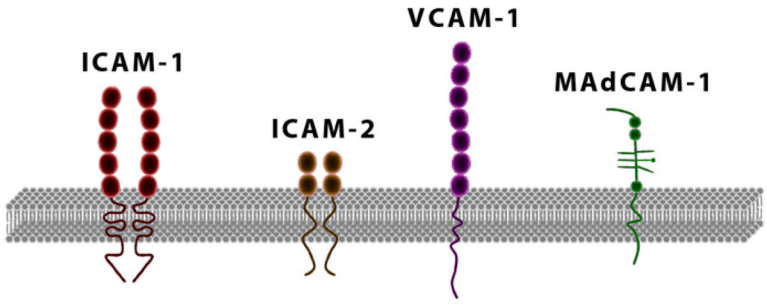

Figure 2.
Immunoglobulin superfamily. Members of this family contain two to seven immunoglobulin domains and serve as ligands for leukocyte integrins. ICAM-1 and ICAM-2 exist as homodimers on the cell surface.