Leukocyte integrins and their ligand interactions

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Abstract

Although critical for cell adhesion and migration during normal immune-mediated reactions, leukocyte integrins are also involved in the pathogenesis of diverse clinical conditions including autoimmune diseases and chronic inflammation. Leukocyte integrins therefore have been targets for anti-adhesive therapies to treat the inflammatory disorders. Recently, the therapeutic potential of integrin antagonists has been demonstrated in psoriasis and multiple sclerosis. However, current therapeutics broadly affect integrin functions and, thus, yield unfavorable side effects. This review discusses the major leukocyte integrins and the anti-adhesion strategies for treating immune diseases.

Keywords

Integrin; LFA-1; Mac-1; VLA-4

Introduction

Integrins are adhesive transmembrane receptors that mediate bidirectional signal transmission between the cell and its environment. “Inside-out” integrin signaling, often stimulated by chemokines or chemoattractive cytokines, regulates the affinity of the integrin for ligand binding. “Outside-in” signaling, which can involve ligand binding and receptor clustering, triggers intracellular signaling pathways that control diverse cell functions. Integrins have critical roles at each step during the process of leukocyte emigration from the blood to inflammatory sites and lymphoid tissues. Heterodimeric integrins expressed predominantly by leukocytes consist of a β_2 subunit coupled with one of several α subunit counterparts ($\alpha_L\beta_2$, $\alpha_M\beta_2$, $\alpha_X\beta_2$, and $\alpha_D\beta_2$), or an α_4 subunit with its β subunit counterparts ($\alpha_4\beta_1$ and $\alpha_4\beta_7$). These leukocyte integrins are dynamically up- or down-regulated depending on the stage of leukocyte activation and extravasation, and each integrin has specific functions contributing to leukocyte movements. Insufficient integrin activity contributes to recurrent infectious episodes and impaired wound healing, and excessive integrin activity leads to a sustained and exaggerated inflammatory response with associated tissue damage. Antagonistic monoclonal antibodies and small molecules targeted against leukocyte integrins have been developed for monotherapies to treat autoimmune and inflammatory diseases. These function-blocking reagents have generally been shown to be efficacious, although their mechanisms of action still need to be defined in detail. Hereby, we summarize the expression and ligand interactions of LFA-1, Mac-1 and VLA-4 in the immune response and discuss the current therapeutic strategies for each.

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LFA-1

1. Expression

Lymphocyte function-associated antigen-1 (integrin LFA-1, $\alpha_L\beta_2$, CD11a/CD18), is expressed exclusively by leukocytes, and plays a role in leukocyte recruitment to inflammatory sites and lymphoid tissues [1]. LFA-1 is involved in various cell-cell interactions, such as T cells-antigen presenting cells, B cells-T cells and natural killer (NK) cells-target cells [2], and in the formation of the immunological synapse [3]. The importance of LFA-1 is demonstrated in β_2 integrin-deficient patients with leukocyte adhesion deficiency (LAD). LAD patients exhibit a deficiency to clear pathogens, recurrent infections and death at an early age [1]. Animal studies using CD18-deficient mice demonstrated defects in a wide range of immune responses, including attenuated recruitment of myeloid cells [4]. In addition, in CD11a- and CD18-deficient mice, both subunits of LFA-1 were shown to be critical for host resistance against *Mycobacterium tuberculosis* infection [5].

2. Ligands

LFA-1 binds to intercellular adhesion molecules (ICAMs), including ICAM-1, ICAM-2, ICAM-3, ICAM-4, ICAM-5 [6–10], and the junctional adhesion molecule (JAM)-1 [11]. Among the ICAMs, ICAM-1 is the principal ligand for LFA-1. LFA-1 binds the D1 domain of the ICAM-1 molecule that consists of five tandem immunoglobulin superfamily (IgSF) domains [12]. It was shown that Glu³⁴ in the D1 domain of ICAM-1 binds to the Mg²⁺ within the metal ion-dependent adhesion site (MIDAS) of the α_L I domain and stimulates conformational change of the integrin [13]. The interaction of LFA-1 with ICAM-2 and ICAM-3 is involved in diapedesis and antigen recognition, respectively, and ICAM-1 is involved in both processes [6,14]. ICAM-4, a red cell membrane glycoprotein originally named Landsteiner-Wiener (LW) blood group antigen [15], is still being investigated for its physiological role in binding to LFA-1, but may be involved in erythropoiesis [10]. ICAM-5 (telencephalin), which is expressed by neurons, is suggested to act as an adhesion molecule for leukocyte trafficking in the central nervous system [9]. JAM-1 is a recently discovered ligand for LFA-1 and plays an important role in the sequential steps of adhesion and transmigration during the recruitment of memory T cells and neutrophils [11].

3. Signaling and regulation

Many signaling molecules have emerged as players in inside-out and outside-in signaling pathways for regulating LFA-1 function. Rap-1, a member of the Ras family of small GTPases, is an upstream regulator that modulates the affinity and avidity of LFA-1 [16]. The Ras proteins, including Rap-1, reversibly cycle between GTP-bound active and GDP-bound forms, regulated by guanine exchange factors (GEFs) and GTPase-activating proteins (GAPs). Calcium and diacylglycerol-regulated guanine nucleotide exchange factor I (CalDAG-GEFI) is a key GEF that activates Rap-1 by releasing GDP and loading GTP in response to calcium and DAG [17]. Therefore, CalDAG-GEFI plays a critical role in Rap-1-mediated integrin-dependent immune responses, such as platelet aggregation and leukocyte motility. It has been reported that CalDAG-GEFI knockout mice are deficient in integrin-dependent platelet aggregation [18]. Although Rap-1 is an important inside-out activator of LFA-1 and VLA-4, the regulation of Rap-1 by CalDAG-GEFI is exclusively involved in LFA-1 activation induced by chemokines and phorbol 12-myristate 13-acetate (PMA) [19]. A recent study reported that one of the LAD syndromes, LAD-III, involves the deficient expression of CalDAG-GEFI in lymphocytes, neutrophils, and platelets [20].

Regulator of adhesion and polarization enriched in lymphocytes (RAPL), an effector molecule that associates with Rap-1, is crucially involved in Rap-1-mediated integrin activation during T cell receptor (TCR) and chemokine triggered LFA-1 adhesion to

ICAM-1 [21]. In Rap-1/RAPL-mediated LFA-1 activation, the Ser/Thr kinase Mst1 is a downstream effector molecule of RAPL that binds to RAPL in association with the cytoplasmic domain of the α_L subunit of LFA-1 [22]. RAPL switches the integrin from the low affinity to the high affinity state and stimulates the extension of the extracellular headpiece of the integrin, whereas Mst1 plays an apparent role in the transport of active LFA-1 to the leading edge of motile cells [22,23]. Studies of RAPL knockout mice have shown that RAPL-deficient lymphocytes exhibit impaired ability in homing to lymphoid tissues, are much less adherent to ICAM-1 and fibronectin, and are deficient in migration [24]. Thus, RAPL and Mst1 are critical regulators of leukocyte trafficking via LFA-1. In addition to RAPL, Rap1-GTP interacting adapter molecule (RIAM) is also a crucial effector molecule for Rap-1-mediated integrin activation [24,25], though RIAM and RAPL act through independent signaling pathways [26]. RIAM associated with Rap-1 stimulates the binding of talin to the β subunit and subsequent opening of the extracellular headpiece of the integrin for high affinity ligand binding.

RhoH, a member of the Rho family of small GTPases, is deficient in GTPase activity and exists constitutively in a GTP-bound state [27]. RhoH has been shown to be a leukocyte-specific inhibitory molecule that negatively regulates LFA-1 activation [28]. Thus, in the absence of positive regulatory signals, such as Rap-1, RhoH maintains LFA-1 in the basal state and leukocytes remain in their resting non-adhesive state.

Jun activating binding protein-1 (JAB-1) is a transcriptional co-activator that interacts with LFA-1 [29]. JAB-1 is expressed in the nucleus and the cytoplasm of cells, and a fraction of JAB-1 is colocalized with LFA-1 at the membrane. JAB-1 is implicated as an outside-in signaling modulator, as LFA-1 cross-linking triggers an increase of JAB-1 in the nucleus and activation of activator protein-1 (AP-1) [29].

Cytohesin-1 is a signaling molecule that interacts with the cytoplasmic domain of the β_2 subunit of LFA-1 and thus regulates LFA-1 activation [30]. Cytohesin-1 has a pleckstrin homology (PH) domain and a domain homologous to the yeast SEC7, a GEF for ADF ribosylation factor (ARF)-GTPases [30,31]. The molecule is suggested to be involved in inside-out and outside-in signaling [32,33].

Myosin II in nonmuscle cells is involved in cytokinesis and cell migration [34]. There are three isoforms of human nonmuscle myosin, termed MyH9, MyH10, and MyH14 [35]. Among them, MyH9 is expressed in T cells and recruited to LFA-1 positioned at the uropod during migration [36]. When the association of MyH9 and LFA-1 was inhibited, T cells migrating on ICAM-1 exhibit extremely elongated uropods and are deficient in tail detachment. Consequently, T cell migration mediated by LFA-1 on ICAM-1 was inhibited. Thus, MyH9 is a crucial regulator of the mechanical link between LFA-1 and the cytoskeleton, and is important for LFA-1 de-adhesion during T cell migration.

4. Clinical implications

LFA-1 has been targeted with specific antagonists to treat lymphocyte-based diseases including psoriasis, rheumatoid arthritis and organ transplant rejection [2]. Efalizumab, a monoclonal antibody to the α_L subunit of LFA-1, selectively and reversibly inhibits trafficking of T cells and successfully treats plaque psoriasis with moderate-to-severe symptoms by blocking the interaction of T cells with Langerhans cells, endothelial cells and keratinocytes [37,38]. Efalizumab was also shown to inhibit the development of allergen-induced cellular inflammatory responses and attenuate the late asthmatic response [39]. The antagonist-driven therapies for LFA-1 function may potentially be used for the treatment of bone marrow graft failure, kidney allograft rejection, asthma and graft-versus-host disease [40–43]. As described above, Rap-1 and Mst1 are involved in α_L subunit binding with

RAPL and subsequent LFA-1 activation. Thus, instead of global blockade of Rap-1/RAPL, which is involved in regulating multiple integrins, the interaction of Rap-1/RAPL with α_L or Mst1 has been proposed as an alternative therapeutic target for the selective inhibition of LFA-1 in the treatment of inflammatory diseases with minimal side effects [44]. However, it is still unclear how these molecules interact with each other in detail and additional molecular studies are required before therapies can be developed.

Mac-1

1. Expression

Macrophage antigen-1 (integrin Mac-1, $\alpha_M\beta_2$, CD11b/CD18) is expressed by cells of the myeloid lineage [45,46], including neutrophils, monocytes, macrophages and dendritic cells. Mac-1 is also expressed, to a limited extent, by certain subsets of T lymphocytes [47]. In response to inflammatory stimuli, the cell surface expression of Mac-1 on neutrophils [48] and monocytes [49] is upregulated by an order of magnitude by the rapid delivery of stores within secondary granules [50]. However, studies indicate that this increase in Mac-1 expression is neither sufficient nor necessary for the adhesive function of Mac-1 during leukocyte recruitment and extravasation. Inhibition of degranulation or depletion of granule stores did not affect the adherence of neutrophils to cultured endothelium in the presence of inflammatory stimuli [51]. In addition, blocking only a small portion of Mac-1 on stimulated neutrophils with antibodies specific to the high affinity I domain resulted in nearly complete inhibition of adhesion to ICAM-1 and fibrinogen [52], indicating that the increase in integrin affinity, rather than density, regulates neutrophil recruitment. The increased cell surface expression of Mac-1 after degranulation does not persist when neutrophils are exposed to stimulatory agents for an extended time [53]. In response to the bacterial product N-formyl-Met-Leu-Phe peptide (fMLP) in the presence of other pro-inflammatory factors, or to PMA alone, the amino-terminal portion of the α_M subunit is cleaved by a Ser proteinase resulting in a decrease in Mac-1-dependent neutrophil adhesion [53]. The precise role of Mac-1 in the sequential steps of the inflammatory cascade remains a topic of active investigation. Recent studies have surprisingly found that Mac-1 deficiency does not significantly impair the adhesion of neutrophils to the endothelium during inflammation, but that Mac-1 alone mediates intraluminal migration after neutrophil arrest [54].

2. Ligands

Mac-1 is the most promiscuous integrin with more than 30 reported ligands. The primary Mac-1 ligand binding activity resides within the I domain at the amino terminal portion of the α_M subunit [55]. The broad range of Mac-1 ligands includes members of the ICAM family [56,57], extracellular matrix components such as fibrinogen [58], complement C3 fragment C3bi [59], and protein and nonprotein microbial ligands [60]. Of particular significance in leukocyte recruitment and emigration are the ICAMs. While LFA-1 binds to the D1 domain of ICAM-1, Mac-1 recognizes the ICAM-1 D3 domain [61]. *In vitro* assays suggest that when both integrins are present, the LFA-1/ICAM-1 interaction takes precedence, indicating that LFA-1 and Mac-1 may compete for binding [62]. However, the binding characteristics of the integrin-ICAM-1 interaction appears to be much more complex *in vivo* where LFA-1 has been shown to play a dominant role over Mac-1 in mediating the firm adhesion of leukocytes to the endothelium [63], but Mac-1 interacts with ICAM-1 to mediate intraluminal crawling after leukocyte arrest [54].

Several studies have investigated the molecular basis for Mac-1 ligand recognition. In one of these studies, an important sequence in the α_M I domain was identified. A chimeric integrin was constructed in which the β_D - α_5 loop- α_5 helix segment of the α_M I domain was inserted into and replaced the complementary sequence within the α_L I domain of LFA-1

[64]. This integrin chimera was able to bind to several Mac-1 ligands, including fibrinogen, fibronectin, ovalbumin, and tissue culture plastic, to a similar extent as wild type Mac-1 [64]. Three amino acids (Phe²⁴⁶, Asp²⁵⁴, Pro²⁵⁷) within this region of the α_M I domain were identified as critical for Mac-1-dependent ligand binding [64]. However, it is likely that this segment of the α_M I domain does not solely mediate Mac-1 binding, as cells expressing the chimeric integrin in the study described above did not spread on Mac-1 ligand substrates to the same extent as cells expressing wild type Mac-1 [64]. In a separate study, ligand recognition sequences in three distinct regions of the α_M I domain, including the segment described above, were found to comprise the C3bi binding pocket [65]. Given the broad and diverse range of ligands that have been described for Mac-1, it is apparent that multiple mechanisms exist by which Mac-1 recognizes its binding partners.

3. Signaling and regulation

While the proximal events that regulate LFA-1 activation are relatively well defined, the molecular details of inside-out Mac-1 activation are poorly understood and remain a subject of intense investigation. There is an abundance of experimental evidence demonstrating that LFA-1 and Mac-1 are distinctly regulated. Several studies have shown that different chemokines and chemoattractants stimulate inside-out activation of LFA-1 or Mac-1 [66,67]. In a neutrophil chemotaxis assay, fMLP stimulated Mac-1-mediated migration in a p38 mitogen-activated protein kinase (MAPK)-dependent manner, whereas IL-8 stimulated migration mediated by LFA-1 that relied on phosphoinositide 3-kinase (PI3K) signaling [67]. That LFA-1 and Mac-1 are differentially regulated suggests an important role for the integrin α subunit in regulating their activation, and recent data support this hypothesis. Constitutive phosphorylation of a Ser residue near the integrin cytosolic C-terminus is shared by the α_L subunit of LFA-1 [68] and α_M subunit of Mac-1 [69], and has been shown to be important for activation and affinity upregulation. Mutating this Ser in the α_M subunit blocked the conformational rearrangements involved in Mac-1 activation, including extension of the extracellular domain and opening of the I domain, as indicated by activation-specific antibody recognition [69]. However, a similar mutation in LFA-1 did not affect the exposure of these activation epitopes, indicating that the α subunits of Mac-1 and LFA-1 differentially regulate their activation [69]. Further research is needed to determine the mechanism by which the α_M cytoplasmic tail regulates Mac-1 activation and the upstream signaling molecules that are involved.

4. Clinical implications

Few anti-adhesion therapeutics targeting Mac-1 have been pursued clinically and developed commercially. Recombinant neutrophil inhibitory factor (rNIF), whose target is the Mac-1 I domain [70], was shown in animal models to inhibit neutrophil recruitment to sites of cerebral ischemia and reduce inflammation, a contributor to ischemic injury after stroke [71]. Phase II clinical trials of rNIF, however, were abandoned after the drug failed to improve the recovery of stroke patients [72].

Although there have been few clinical trials of anti-Mac-1 agents, alteration of Mac-1 expression or activation state has been implicated in the efficacy of drugs not specifically targeting Mac-1. Abciximab was designed as a Fab fragment against the integrin $\alpha_{IIb}\beta_3$ to inhibit platelet aggregation, and used to prevent ischemic complications and restenosis in patients undergoing angioplasty [73]. Significant improvement in procedural complications [73,74], the short-term need for revascularization [74], and long-term mortality [75] was observed in patients treated with abciximab. However, the molecular mechanisms for the clinical benefits of abciximab are not clear. Activated leukocytes and enhanced Mac-1 expression, but not LFA-1, have been identified as markers of restenosis risk [76–78]. Subsequent studies suggested that regulation of Mac-1 by abciximab could contribute to the

inhibition of intimal thickening that occurs after angioplasty, as abciximab was shown to bind to monocytes and inhibit Mac-1-mediated adhesion to fibrinogen, C3bi, and the coagulation factor X [79]. Moreover, an anti-Mac-1 antibody reduced leukocyte recruitment and neointimal thickening after stent-induced injury in rabbits [80]. These results suggest that Mac-1 may be an effective target for therapies administered during vascular intervention.

Mac-1-binding agents are also being studied for use in cancer therapies. Anti-tumor antibodies can effectively stimulate the deposition of the opsonin C3bi on tumor cell surfaces. However, in the absence of Mac-1-associated polysaccharides, neutrophil binding to C3bi via Mac-1 does not result in degranulation or phagocytosis [81]. β -glucans are long glucose polymers that have been shown to bind to a lectin domain in the carboxy-terminal portion of the α_M subunit [82] and prime Mac-1 for cytotoxic activity [83]. In conjunction with antibody therapies, β -glucan has been shown to induce tumor regression and increase survival rate in animal models [84,85]. In these studies, neutrophils were found to be the primary effector cells in the therapeutic response [84]. Cancer immunotherapies in which β -glucan is employed as an immunoadjuvant have been used clinically since the 1980s.

Antibodies [86] and antibody-derived cyclic peptides [87] that exclusively recognize the active form of the Mac-1 I domain have been described. These reagents specifically block the binding of certain ligands to activated Mac-1, including ICAM-1 and fibrinogen, but not C3bi [87]. The antibody-derived blocking peptides inhibited the adhesion of stimulated monocytes to immobilized fibrinogen, while unstimulated cells were still able to bind fibrinogen under both static and flow conditions [87]. These studies suggest that therapeutics specifically targeting the active form of Mac-1 may be effective in treating inflammatory diseases, while preserving normal host defense functions such as phagocytosis of bacteria by recognition of C3bi [87].

VLA-4

1. Expression

Very late antigen-4 (integrin VLA-4, $\alpha_4\beta_1$, CD49d/CD29) is expressed by most resting lymphocytes, eosinophils, and monocytes [88,89]. In contrast to other integrins, the expression level of VLA-4 is not considerably increased in response to lymphocyte activation [41]. However, it was shown that VLA-4 expression is upregulated during the maturation of monocyte-derived dendritic cells [90]. VLA-4 also plays a role in the development and differentiation of several tissues and cell types [41]. VLA-4 is expressed by muscle cells and, in association with VCAM-1, is involved in myotube formation and alignment of the secondary myoblasts [91]. VLA-4 expression is also found in thymic epithelial cells and the integrin is suggested to play a role in thymus epithelial cell-thymocyte interactions [92]. VLA-4 is expressed by bone marrow CD34⁺ haematopoietic stem cells and thus related to mobilization of these cells and inhibition of homing of peripheral blood progenitors [93]. Consistent with these data, homozygous null mutation of VLA-4 (α_4 subunit deficient) in mice resulted in embryonic lethality with defects in placentation and the development of the epicardic vessels [94].

2. Ligands

The α_4 subunit of VLA-4 lacks the I domain that is a critical structural component of other integrins for which the α subunit I domain is the ligand binding site. Instead, the I-like domain in the β_1 subunit mediates ligand binding to VLA-4 [89]. VLA-4 mediates cell-extracellular matrix adhesion by binding to an LDV motif in an alternatively spliced region of fibronectin, the connecting segment 1 (CS-1). VLA-4 also mediates cell-cell adhesion by binding to an IDS motif in VCAM-1, a molecule containing six tandem IgSF domains.

VLA-4 also interacts with JAM-2 expressed by endothelial cells following prior engagement of JAM-2 with JAM-3 expressed by T cells [95]. JAMs are also members of the IgSF expressed at cell-cell contacts in leukocytes, platelets, epithelial and endothelial cells, and play a role in leukocyte-endothelial cell interactions and cell polarity regulation [96].

3. Signaling and regulation

VLA-4 is an important component in immune function, playing a role in lymphocyte differentiation and homing, as well as in tissue-specific migration during inflammation [97]. α_4 integrins, including VLA-4 and $\alpha_4\beta_7$, have tightly regulated multi-step functions during rolling and arrest of leukocytes on the endothelium [98,99]. VLA-4 is also involved in cell-cell adhesion of leukocytes, such as at the immunological synapse [100]. Although the LFA-1-ICAM-1 interaction is crucial in the structure of the immunological synapse, the VLA-4-VCAM-1 interaction also contributes to immunological synapse formation [101]. Cytoskeletal adaptor molecules, such as Rap-1, paxillin, and talin, which regulate integrin adhesiveness, are involved in VLA-4 function during immune responses.

Inside-out signaling triggered by the small GTPase Rap-1 allows integrins on circulating leukocytes to become activated, resulting in ligand binding and cell adhesion. Rap-1 is an important regulator of β_1 integrins, including VLA-4, by mediating inside-out signaling [102] and is activated by various external stimuli, including TCR- and CD31-induced adhesion [103]. Rap-1 induces the binding of the cytoskeletal protein talin to the integrin cytoplasmic tail, which promotes integrin activation.

Paxillin is a signaling molecule that interacts with the cytoplasmic domain of the α_4 integrin subunit in a phosphorylation-dependent manner and subsequently triggers lymphocyte migration by downstream regulation of effector molecules [104,105]. When the α_4 integrin is phosphorylated at Ser⁹⁸⁸ by protein kinase A, its binding to paxillin is inhibited at the trailing edge of the cell and paxillin-free α_4 integrin is localized at the leading edge of the cell [104]. The localization of phosphorylated α_4 complexed with paxillin at the leading edge of migrating cells augments effective leukocyte migration [105]. Thus, the spatially-regulated interaction of α_4 integrin with paxillin is a critical signaling step for cell motility.

4. Clinical implications

VLA-4 is implicated in the pathogenesis of autoimmune diseases and chronic inflammation such as multiple sclerosis [106], Crohn's disease [107], asthma [108], stroke [109], rheumatoid arthritis [110], and inflammatory bowel disease [111]. Therefore, VLA-4 has been therapeutically targeted by antagonists such as blocking antibodies and ligand mimetic small molecules [112,113]. Multiple sclerosis is a neurological disease caused by autoimmune T cells. Immune reactions during early steps in the development of multiple sclerosis lesions include transmigration of CD4⁺ T cells (T_H1 and T_H17) from the blood to the central nervous system (CNS) across the blood brain barrier, where VLA-4 plays critical roles in the migration process [114,115]. Natalizumab, a monoclonal antibody to the α_4 subunit, blocks binding of VLA-4 and $\alpha_4\beta_7$ to VCAM-1 and MadCAM-1, respectively. Natalizumab showed efficacy by inhibiting inflammatory lesion in multiple sclerosis [116]. The number of CD4⁺ and CD8⁺ T cells, CD19⁺ B cells, and CD138⁺ plasma cells in the cerebrospinal fluid (CSF) was significantly reduced in Natalizumab-treated patients.

Although the current therapeutics to block α_4 integrin function have shown positive effects in the treatment of autoimmune diseases, they may also have unfavorable side effects on VLA-4-dependent processes, such as development, hematopoiesis and immune surveillance. Natalizumab treatment for multiple sclerosis patients resulted in the development of progressive multifocal encephalopathy (PML) and led to patient death in a few cases [117–

119]. PML is a demyelinating disease of the CNS that occurs in immune-suppressed patients. It results from lytic infection of oligodendrocytes in the CNS by the John Cunningham (JC) polyomavirus [120]. It has been reported that 1 in 1000 cases of Natalizumab treatments could lead to PML [121]. Thus, it is important to develop more specific antagonistic agents for targeting α_4 integrin function that affect leukocyte trafficking in an inflammatory-site specific manner rather than global blockade of integrin function.

Therefore, an alternative approach to regulate α_4 integrin signaling has been suggested for disease treatment [122]. As described above, paxillin binds to the α_4 integrin in a phosphorylation-dependent manner and regulates lymphocyte migration [104,105]. Therefore, the α_4 -paxillin signaling pathway could be a therapeutic target for site-specific blockade of integrin function [122]. It may be advantageous to treat diseases involving α_4 integrins by selectively blocking cell migration to the inflammatory site, while minimizing the obstruction of other functions of α_4 integrins.

Conclusion

In general, anti-adhesive therapies targeting the major leukocyte integrins LFA-1, Mac-1 and VLA-4 have proven to be relatively successful in the clinic. However, the current approaches block integrin ligand binding in a non-specific manner and, thus, impair the normal immune response and compromise the health of the patient. Furthermore, ligand-mimetic small molecule integrin antagonists have failed in the clinic, presumably as a result of outside-in signaling induced by the stabilization of an active integrin conformation [123,124]. Alternative therapies have been proposed that include specifically targeting the active form of the integrin [87] or regulating the adhesive function of integrins through their associated intracellular adaptor proteins [44]. The development of reagents with higher selectivity for specific integrin populations involved in the disease state may provide more efficacious treatments for both chronic and acute immune pathologies.

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Table I

Leukocyte integrins

Integrin	Expression	Ligands
LFA-1 ($\alpha_L\beta_2$)	Lymphocytes, dendritic cells, NK cells, neutrophils	ICAMs (1–5), JAM-1
Mac-1 ($\alpha_M\beta_2$)	Neutrophils, monocytes, macrophages, dendritic cells, lymphocytes, NK cells	ICAMs (1, 2, 4), fibrinogen, C3bi, factor X, others
VLA-4 ($\alpha_4\beta_1$)	Lymphocytes, eosinophils, monocytes, NK cells, neutrophils, non-immune cells	VCAM-1, fibronectin, JAM-2