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LFA-1 and associated diseases: The dark side of a receptor

L. Zecchinon, MS, T. Fett, BEng, P. Vanden Bergh, DVM, D. Desmecht, DVM, PhD*

Department of Pathology, Faculty of Veterinary Medicine, University of Liège, FMV Sart Tilman B43, B-4000 Liège, Belgium

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Abstract

Lymphocyte function-associated antigen-1 (LFA-1, $\alpha_L\beta_2$, CD11a/CD18) plays a critical role in the complex and well-orchestrated molecular interactions responsible for cell adhesion events required for normal and pathologic functions of the immune system. This review focuses on the diseases from various etiologies (genetic, bacterial, viral, neoplastic, allergic, and autoimmune) that are associated to lymphocyte function-associated antigen-1 with a tremendous impact on human and animal health.

Keywords: LFA-1; Disease; Bacteria; Virus; Allergy; Cancer

1. Introduction

Lymphocyte function-associated antigen-1 (LFA-1, $\alpha_L\beta_2$, CD11a/CD18) is a protein made of the union of the CD11a and CD18 subunits that each possesses a large extracellular domain but short transmembrane and cytoplasmic regions. The N-terminal parts of both subunits associate to form the integrin headpiece, which contains the ligand-binding site, whereas the C-terminal segments traverse the plasma membrane and mediate interactions with the cytoskeleton and with signaling proteins [1,2].

LFA-1 plays an important role in cell migration and in interactions between T cells and antigen presenting cells (APCs). However, LFA-1 is also associated to several diseases from various etiologies that exhibit a tremendous impact on human and animal health.

Abbreviations: CDT, cytolethal distending toxin; ICAM, intercellular adhesion molecule; LAD, leukocyte adhesion deficiency; LFA-1, lymphocyte function-associated antigen-1; LKT, leukotoxin (from *M. haemolytica*); LTX, leukotoxin (from *A. actinomycetemcomitans*); mAb, monoclonal antibody; MIDAS, metal ion dependent adhesion site; TCR, T-cell receptor.

* Corresponding author. Tel.: +32 4 366 4075; fax: +32 4 366 4565.

E-mail address: daniel.desmecht@ulg.ac.be (D. Desmecht).

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2. Diseases associated to LFA-1

2.1. Genetic diseases

2.1.1. Human leukocyte adhesion deficiency

Human leukocyte adhesion deficiency-1 is a genetic disease caused by mutations in the CD18 subunit. As the α and β subunits pair as precursors intracellularly and their transport to the cell surface is CD18 dependent, this leads to the lack of β_2-integrin cell surface expression and hence, clinically, to recurrent severe bacterial infections and other immune deficiencies [3–10]. Transfection of the β_2-integrin subunit complementary DNA into B lymphoblastoid cells from leukocyte adhesion deficiency (LAD) patients restored normal levels of LFA-1 to the cell surface as well as adhesion to intercellular adhesion molecule (ICAM)-1 [11]. The disease has been identified in man [3,8,12,13], dog [14,15], and cattle [16] and is viewed primarily as a failure of neutrophils function as (1) they are essential first arrivals at sites of tissue injury, and (2) they rely most heavily on β_2 integrins because they lack significant levels of other integrins, contrary to monocytes and lymphocytes that are able to express $\alpha_4\beta_1$ when β_2 integrins are missing [17]. In addition, a CD18 gene–targeted mouse model has been generated, displaying a disease phenotype similar to the human form of the disease [18,19].

Most LAD-1 cases involve single point "missense" mutations; most of them are located in the βI domain, a region coded for by exons 5–9, which is highly conserved in all β subunits and that has been modeled to ressemble the ligand-binding αI domain [9,17,20–22]. A second region of mutation of β_2 subunit centers on the last 2 cysteine-rich repeats coded for by exon 13, a heavily disulphide cross-linked region that is thought to provide structural rigidity for the β subunit and may influence either interdomain movement or the quaternary relationship between integrin subunits [17,22]. Rarer "nonsense" mutations can occur leading to an absence of transcription of the CD18 gene, to incorrect mRNA splicing and/or unstable mRNAs, and to truncated and often unstable proteins [8,21,22]. Two variant patients have also been described. The first one proved to be a heterozygote with one nonexpressing allele and the second allele that allowed normal expression but no β_2 integrin function due to a mutation in the metal ion dependent adhesion site motif [23]. The second variant was suspected to exhibit a mutation in a component of a critical signaling pathway leading to the activation of the β_2 integrins because no mutation was found in the CD18 gene [13].

Furthermore, a rare autosomal-recessive LAD-2 syndrome exists, which is characterized by a defect in fucosylation of glycoconjugates (such as ligands for the selectin family of adhesion molecules) caused by mutations in the gene for a GDP-fucose transporter of the Golgi [7,24–27]. LAD-2 is thus the first developmental and immune defect that is based on a malfunctioning nucleotide sugar transporter. LAD-2 patients suffer from problems with leukocytes adhesion and trafficking, severe psychomotor and growth retardation and have dysmorphic features, hypotonia, seizures, and strabismus [24,25,28,29].

Recent findings also indicate presence of a third form of LAD, a rare autosomal-recessive disease associated with severe defects in integrin activation by chemokine signals, despite normal ligand binding of leukocyte integrins. LAD III is caused by defects in G protein–coupled receptor–mediated integrin activation [10,30,31] as the small GTPase Rap1,

a key regulator of inside-out integrin activation, is abnormally regulated in LAD Epstein–Barr virus lymphocyte cells. Both constitutive and chemokine-triggered activation of Rap1 were abolished in LAD lymphocytes despite normal chemokine signaling. Nevertheless, Rap1 expression and activation by phorbol esters were intact, ruling out a LAD defect in Rap1 guanosine triphosphate loading. Thus, a defect in constitutive Rap1 activation results in an inability of ligand-occupied integrins to generate high-avidity binding to ligand under shear flow [32].

2.1.2. Bovine leukocyte adhesion deficiency

LAD has also been identified in Holstein cattle as an autosomal-recessive congenital disease characterized by recurrent bacterial infections, delayed wound healing and stunted growth, and is also associated with persistent marked neutrophilia. Affected cattle suffer from severe ulcers on oral mucous membranes, severe periodontitis, loss of teeth, chronic pneumonia, recurrent or chronic diarrhea, and die at an early age due to the infectious complications [33].

At the molecular level, 2-point mutations have been observed in the CD18 gene, 1 silent and 1 causing an aspartic acid to glycine substitution at amino acid 128 in the highly conserved extracellular βI domain, where several mutations have been found to cause human LAD [16]. The mutation has been perpetuated though selective breeding practices because it was traced back to a unique founder bull named Osborndale Ivanhoe and known as the Father of the Holstein breed through the use of artificial insemination in the 1950s and 1960s. After 5 generations, the carrier frequency for the aspartic acid to glycine substitution at amino acid 128 allele among Holstein cattle in the United States was approximately 15% among bulls and 6% among cows. Moreover, this mutation was also prevalent among Holstein cattle throughout the world, placing this disorder among the most common genetic diseases known in animal agriculture [10,16,34].

Peripheral blood leukocytes of normal, heterozygous, and homozygous for bovine leukocyte adhesion deficiency (BLAD) cows have been analyzed by flow cytometry for the intensity of their β_2 integrin expression: BLAD homozygotes revealed no or a very weak expression of the β_2 integrins and had a 10-fold and 4- to 5-fold increase in absolute number of neutrophils and monocytes, respectively, whereas the absolute number of lymphocytes remained normal, revealing that the LFA-1 expression could allow a ready identification of BLAD heterozygotes by flow cytometry [35]. Leukocytes from homozygous calves seem to upregulate alternative host defense capabilities (e.g., respiratory burst activity) to partially compensate for the lack of typical adherence-dependent host defense functions [36]. In contrast, the heterozygous BLAD genotype does not cause detectable functional differences in leukocytes, compared with those of clinically normal calves [36], and the mean fluorescence intensity of CD18 in heterozygous animals was 56–90% of that in the normal cows [35].

2.1.3. Canine leukocyte adhesion deficiency

Canine leukocyte adhesion deficiency is a fatal immunodeficiency disease found in Irish setter dogs that is very similar to LAD in humans and BLAD in cattle because it is characterized by a single missense mutation in the βI domain of the CD18 subunit that results in

very low levels of CD11/CD18 expression [10,14,15,37,38]. The mutation has been in the Irish Setter population for many generations spanning more than 2 decades [39].

The canine leukocyte adhesion deficiency is also present in a significant minority of Irish red and white setters, a related breed that has a history of interbreeding with Irish Setters and shares a common ancestry with the Irish Setter breed [39,40].

2.2. Bacterial diseases

Several studies have shown or suggest that LFA-1 (CD11a/CD18, $\alpha_L\beta_2$,) is centrally involved in the pathogenesis of diseases caused by repeats-in-toxin–producing Gram-negative bacteria such as *Actinobacillus actinomycetemcomitans* and *Mannheimia haemolytica* [41].

2.2.1. Actinobacillus actinomycetemcomitans

Periodontitis, the result of the response of the periodontium to the presence of certain members of the oral microbiota, is mankind's most common chronic inflammatory disease. One of its more severe form is localized aggressive periodontitis, which affects only the incisors and premolars [42]. The disease tends to afflict younger individuals and is associated with rapid destruction of the periodontal ligament and alveolar bone, which support the teeth. The main causative agent of this disease is *A. actinomycetemcomitans* [43] that produces both inflammation and damage to the extracellular matrices of ligament and bone. Although less well researched than enteric pathogens, this organism clearly packs a lot of virulence potential into its 2-Mb genome and interacts in a wide variety of ways with its cellular and extracellular host environments. The bacterium is also occasionally responsible for nonoral infections including endocarditis, bacteremia, pericarditis, septicaemia, pneumonia, infectious arthritis, osteomyelitis, synovitis, skin infections, urinary tract infections, and abscesses [44]. Moreover, there is currently great interest in the possibility that periodontal diseases may be a contributing risk factor for development of cardiovascular disease [45] because *A. actinomycetemcomitans* has been detected in 18% of atherosclerotic plaque samples [46].

A number of interesting virulence factors are produced by this organism. Although many of them still await identification, leukotoxin (LTX), and the newly discovered cytolethal distending toxins (CDTs) are in the limelight. Leukotoxin is the first and most intensively studied of these toxins: it belongs to the repeats-in-toxin proteins family, so named because of the C-terminal amino acid repeats, that have channel forming activity and kill cells either by osmotic lysis or by induction of apoptosis [41,47]. The *A. actinomycetemcomitans* leukotoxin kills lymphoid and myeloid cells from human and some nonhuman primate [41], and the basis of this cell/species specificity is due to the binding to human cells via LFA-1 [48], which leads to perturbation of mitochondrial function. Indeed, after exposure to LTX, Epstein–Barr virus transformed B cells (JY cell line) exhibited the classical morphological features of apoptosis including decreased cell size, plasma membrane blebbing, selective alterations in plasma membrane permeability, and condensation of nuclear DNA. The morphologic changes were accompanied by swelling of the mitochondria, a decrease in mitochondrial transmembrane potential, hyperproduction of reactive oxygen intermediates, and release of cytochrome ***c*** from the intermembrane space. Subsequently, activation of the cysteine aspartate–specific proteases (caspases)-3 and -9, cleavage of the nuclear DNA repair enzyme, poly(ADP-ribose)polymerase, and internucleosomal DNA fragmentation were also

detected, indicating that perturbation of mitochondrial structure and function, in concert with activation of specific caspases, initiate the effector phase of LTX-induced apoptosis [49]. The second important virulence factor is known as the CDT produced by a small but diverse group of bacterial pathogens such as *Escherichia coli*, *Campylobacter jejuni*, and *Haemophilus ducreyi* [50]. This newly discovered toxin family has the ability to control cell cycle progression in eukaryotic cells by irreversibly blocking the G_2 phase of the cell cycle [50]. Recent studies have established that CDT is in fact a tripartite AB toxin in which CdtB is the active "A" subunit, and CdtA and CdtC constitute the heterodimeric "B" subunit required for the delivery of CdtB into the target cell [51]. CdtB has been identified as the active subunit of the CDT holotoxin which causes, through its nuclease activity, limited DNA damage, thereby triggering the DNA-damage response that ultimately results in the observed arrest of the cell cycle in G_2 [50,52]. However, it is not known what role the 2 main *A. actinomycetemcomitans* toxins play in bone resorption: the leukotoxin targets myeloid cells causing their apoptosis and could therefore affects osteoclasts and their precursors, which could inhibit osteoclastic bone resorption. Moreover, in preliminary studies, all 3 *A. actinomycetemcomitans* CDTs have been shown to stimulate bone resorption in vitro [42].

2.2.2. *Mannheimia haemolytica*

The bacterium *M. haemolytica* plays a major role as secondary pathogen in the final progression to severe pleuropneumonias in cattle, sheep, and goats. Its pathogenesis involves many predisposing agents such as viruses (Parainfluenza virus 3, Bovine Herpes virus 1, Bovine Respiratory Syncytial virus), bacteria (*Pasteurella multocida*, *Mycoplasma bovis*, *Arcanobacterium pyogenes*), environment (excessive temperatures, change of feed, dust…), or stress associated to weaning, dehorning, and shipping [53–57]. These factors seem to alter the upper respiratory tract epithelium allowing *M. haemolytica* to colonize it, escaping clearance, and to move from nasopharynx to lungs, leading to a broncho-alveolar kind of pneumonia, which is accompanied by high morbidity and mortality [58–60]. Pulmonary lesions are lobar, anteroventrally distributed, and are characterized by extensive infiltration of neutrophils (that fail to combat infection) [61,62] and exudation of fibrin into airways and alveoli. In these latter, multifocal areas of coagulation necrosis are observed, wrought by the cytolysis of many neutrophils and macrophages that pour a variety of toxic compounds in situ, which in turn aggravate pulmonary damage [53,55].

Several virulence factors have been described for *M. haemolytica*, the main of which being the lipopolysaccharide and the leukotoxin. The latter is cytotoxic only for ruminant leukocytes, a phenomenon that is correlated with its ability to bind and interact with the ruminant LFA-1 (CD11a/CD18, $\alpha_L\beta_2$), suggesting that the most widespread cattle respiratory disease, that with the most important impact on beef production worldwide, is probably due to a tiny ruminant-specific focal variation in the CD18- and/or CD11a-expressing genes [63]. The precise identification of the subunit that binds LKT appeared controversed [64–67] until the recent cloning, sequencing and characterization of the *Bos taurus* CD11a [68] give the first opportunity to express homologous and heterologous LFA-1 in vitro to definitely answer the question. As a result, recombinant expression of bovine LFA-1 has shown that LKT binds to both the CD18 and CD11a subunits [69,70] even though CD18 plays the crucial role because LKT do not bind heterologous LFA-1 made of bovine CD11a

and human CD18 [71]. In this way, comparing ruminant vs. nonruminant CD18 subunits has open the way to the identification of the precise molecular motif responsible for the species specificity of *M. haemolytica* [72], whereas apparently contradictory results were recently published, mapping the binding site for *M. haemolytica* LKT within amino acid residues 500–600 [71] or 1–291 [73], respectively, with bovine × human and bovine × murine CD18 chimeric constructs.

2.3. Viral diseases

Several cell adhesion molecules are subverted as virus receptors [74] and this may be more than coincidental. First, virus–cell and cell–cell adhesion are in principle very similar as both (1) require accessible cognate sites on the receptor, (2) involve multivalent interactions, and (3) may be facilitated by receptor redistribution to the site of adhesion. Second, binding to molecules that participate in the immune response has important consequences for the host–virus relationship [75]. In this way, several cell adhesion molecules have been shown to mediate viral budding and transfer in human immunodeficiency virus (HIV)-1 infection [75–77]. HIV-1 infects and kills or renders anergic $CD4^+$ T-helper cells, and alter the expression of cell adhesion molecules in patients; for example, $CD4^+$-lymphocyte expression of L-selectin and very late antigen-4 is down regulated, whereas ICAM-1 and LFA-1 are upregulated [78]. HIV-1 binds with its surface protein gp120 to CD4 whose expression is down regulated [79–82]. HIV-1 is also known to incorporate host-derived ICAM-1 on the viral particle surface, which can engage to LFA-1 on the T-cell surface, leading to a significant increase in viral infectivity [75,83–86]. Moreover, the ICAM-1/LFA-1 interaction plays an active role in syncytia formation (cell fusion) induced by HIV-1, which is a major cytopathic effect that may also represent an important mechanism of $CD4^+$ T-cell depletion in individuals infected with HIV. Indeed, syncytia formation requires not only the interaction of CD4 on the surface of uninfected cells with gp120 expressed on HIV-infected cells but also the high affinity form of LFA-1 [87–89] and more exactly a peptide region in the N-terminal portion of CD18 (including the region between residues 200–206) that has been identified through phage-display technology [90]. To examine the role of LFA-1 expressed on target cells in HIV infection, Jurkat-derived Jbeta2.7 T-cell lines that express either wild-type LFA-1, a constitutively active mutant LFA-1, or no LFA-1 were used [91]. The presence of wild-type LFA-1 enhanced the initial processes of HIV infection, as well as the subsequent replication and transmission from cell to cell. In contrast, the constitutively active LFA-1 mutant failed to promote virus replication and spread, even though this mutant could help HIV enter cells and establish the initial infection. Consequently, LFA-1 contributes in the different stages of HIV infection and not only is LFA-1 expression important for initial HIV–cell interaction, subsequent replication, and transmission, but its activity must also be properly regulated [91]. Furthermore, it has been recently demonstrated that activation of primary human $CD4^+$ T lymphocytes increases LFA-1 affinity and avidity states. Confocal analyses suggest that HIV-1 is concentrated in microdomains rich in LFA-1 clusters that also contain CD4 and CXCR4 molecules [92]. Therefore, one possible target for new Acquired Immune Deficiency Syndrome therapy is preventing the HIV from using adhesion molecules such as ICAM-1 and LFA-1 for budding and transfer processes [93].

Rhinoviruses (which cause common colds), by contrast, rather than thwarting the immune response, use it to their own ends as mucous secretions and sneezing induced by the immune response facilitate infection of other individuals. Moreover, although they have evolved more than 100 noncrossreactive variants in an attempt to evade the immune response, 90% of rhinoviruses, bind ICAM-1. If this latter has a signal transducing function on antigen presenting cells, this may be mimicked by the virus and may serve its ends by stimulating production of cytokines, which increase nasal secretions carrying the virus [75].

Furthermore, it is of considerable interest that viruses have evolved to bind to the same regions of CD4 and ICAM-1 as do their cell adhesion counter-structures. Indeed, HIV binds to the first and part of the second immunoglobulin-like domains of CD4 [75,94–96], the region that binds to major histocompatibility complex class II molecules [95]. In the same way, LFA-1 and rhinoviruses bind to overlapping but distinct regions of the first immunoglobulin-like domain of ICAM-1 [97–100]. A ''canyon'' on the rhinovirus surface that is too narrow to admit an antibody, has been hypothesized to be the binding site of LFA-1 [101], suggesting that rhinoviruses mimic LFA-1 in binding to the most membrane distal, and thus most accessible, site of ICAM-1 [97].

2.4. Cancer diseases

For tumor metastasis to occur through the blood and lymphatic vessel pathway, the tumor cells must first adhere to endothelial cells and thus tumor cells with high levels of cell adhesion molecules (the types of which varying with malignancy, site of metastasis, and tumor origin) are selected to form metastatic lesions [93,102]. In this way, the high expression of LFA-1 (CD11a/CD18) and ICAM-1 is associated, respectively, with lung cancer [103], pancreatic cancer, and pulmonary adenocarcinoma tumor progression [104,105], whereas gastric cancer cells and tumor cells from adenoid cystic carcinoma of the head and neck patients have lower ICAM-1 expression than normal cells, suggesting that decreased ICAM-1 expression may in turn decrease immune response mediated through LFA-1–dependent effector cell adhesion and that this escape from the immunosurveillance system may be one of the factors inducing lymph node metastasis [93,106,107].

On the other hand, recent studies have shown that high expression of the widely expressed cell surface glycoprotein CD44 in certain types of tumors is associated with the hematogenic spread of cancer cells. Indeed, cross-linking of CD44 results in a marked induction of the expression of LFA-1 and very late antigen-4 (VLA-4 or $\alpha_4\beta_1$) by exocytosis, and increases integrin-mediated adhesion to endothelial cells, resulting in the transendothelial migration of breast cancer cells [108]. Because these induced integrins promote tumor cell migration, they could be targeted by pharmacological means to cause a reduction in invasive capability and metastasis. Moreover, Jak tyrosine kinases that act downstream of LFA-1 are essential for lymphoma and metastasis and contribute in multiple ways to the induction of malignant behavior [109].

2.5. Allergic diseases

Allergic asthma is characterized by eosinophil migration in the airways, which is strictly dependent on the expression of adhesion molecules such as LFA-1 [110]. Accordingly,

eosinophils from atopic asthmatic subjects show increased expression of LFA-1, but not of Mac-1 or VLA-4. In addition, LFA-1 expression correlated positively with blood eosinophil number, while no correlations were observed between Mac-1 or very late antigen-4 (VLA-4; CD49d/CD29) expression and blood eosinophil number. Eosinophils migration through human ombilical vein was totally inhibited by preincubating eosinophils with anti–LFA-1, whereas anti–Mac-1 had no effect, suggesting that an early recruitment of blood eosinophils overexpressing LFA-1 occurs in atopic asthmatics in the first hours after allergen challenge [111,112]. Moreover, activated macrophages are present in increased numbers in the airways inflammation observed in asthma and it has been shown that expression of ICAM-1 and LFA-1 on alveolar macrophages was significantly increased in asthmatic patients. There was also a significant correlation with the percentage of cells expressing both markers in asthma, highlighting the importance of macrophages in the inflammation of asthma and suggesting that macrophage interactions with other cells play a role in this inflammation [113].

Furthermore, manipulation for inducing selective inactivation of antigen-specific Th2 cells provides a rational approach to the control of allergic inflammation [114,115] as induction of Th2 cell tolerance to a soluble antigen by blockade of the LFA-1–dependent pathway in vivo prevents antigen-induced eosinophil recruitment into the tissue and antigen-specific IgE production [115,116]. Indeed, an ICAM-1/LFA-1 interaction is involved as a costimulatory signal in inducing T-cell tolerance to a soluble antigen, because in vivo pretreatment with the combination of anti–ICAM-1 monoclonal antibody (mAb), anti–LFA-1 mAb, and a soluble antigen, but not with a soluble antigen alone, induced T-cell tolerance and the subsequent inhibition of allergic inflammation [115]. This finding is consistent with the notion that the stimulation of T cells through the T-cell receptor in the absence of a costimulatory signal leads to T-cell tolerance [117,118] and that LFA-1 is a costimulatory molecule for T-cell receptor–mediated T-cell activation [119–121]. Moreover, in vivo coadministration of mAbs against LFA-1 and ICAM-1 suppresses the progression of experimental allergic encephalomyelitis in rats by preventing the infiltration of encephalitogenic cells into the central nervous system, suggesting that the administration together of mAbs against adhesion molecules including LFA-1 and ICAM-1 might provide a new immunotherapeutic approach for the treatment of multiple sclerosis [122].

Additionally, ICAM-1 expression was induced in allergic patients on epithelial cells of conjunctiva and in the nose, following allergen-specific challenge or both conjunctivitis and rhinitis due to pollen. ICAM-1 could be an activation marker on epithelial cells, or could enhance their susceptibility to bind offending cells such as eosinophils. As ICAM-1 is also a receptor for the vast majority of rhinoviruses, which are known to provoke, mainly in children, asthma attacks, allergy may be considered as a primary event leading to asthma (through rhinovirus infection) and nonspecific hyperreactivity [123]. Moreover, antibodies against ICAM-1 and LFA-1 significantly inhibit the development of the clinical and histologic signs of allergic conjunctivitis [124].

Besides, epidermal dendritic cells isolated from psoriasis or atopic dermatitis possess greatly enhanced T lymphocyte–activating properties, suggesting in psoriasis expression of surface antigens such as β_2 integrins and ICAM-1 [125].

LFA-1 plays also a significant role in the elicitation of allergic contact dermatitis reactions, as a specific anti–LFA-1 mAb injected into the ears of mice after sensitization but prior to

challenge with dinitrofluorobenzene dose dependently suppress ear swelling, which was accompanied by a reduction in dermal edema and leukocyte infiltration into the dermis [126].

2.6. Autoimmune diseases

The pathology of many autoimmune diseases such as multiple sclerosis, thyroiditis, rheumatoid arthritis, and insulin-dependent diabetes mellitus involve the infiltration of autoreactive lymphocytes to a certain organ or site in the body [93,127–129]. In this way, LFA-1 has been identified as a key contributor in the development of these disorders. Indeed, ICAM-1/LFA-1 interaction is essential for T-cell activation and for migration of T cells to target tissues. This interaction also functions, along with signal-1, as a costimulatory signal (signal-2) for T-cell activation, which is delivered by the T-cell receptor–major histocompatibility-peptide complex. Therefore, blocking ICAM-1/LFA-1 interaction can suppress T-cell activation in autoimmune diseases [93,130–132].

3. Conclusion

LFA-1 ($\alpha_L\beta_2$, CD11a/CD18) not only plays a central role in major immune functions such as cell adhesion and migration, but is also implicated in several viral, neoplastic, allergic, and autoimmune diseases. Moreover, LFA-1 is able to engage bacterial repeats-in-toxins that induce cell death and thus promote disease progression in an "Achille's heel mechanism." In this way, extensive study of the interaction between LFA-1 and its natural or bacterial ligands could open new avenues in the therapeutic field.

4. Summary

LFA-1 ($\alpha_L\beta_2$, CD11a/CD18) is a cell adhesion receptor associated to several diseases from various etiologies with a tremendous impact on human and animal health; for example, (1) genetic diseases are caused by mutations in the CD18 subunit that lead to the lack of β_2-integrin cell surface expression and hence, to recurrent severe bacterial infections and other immune deficiencies in human, cattle, and dog, (2) LFA-1 contributes in the different stages of HIV infection, and (3) is centrally involved in the pathogenesis of diseases caused by repeats-in-toxin–producing Gram-negative bacteria such as *A. actinomycetemcomitans* and *M. haemolytica*. Moreover, the interaction between LFA-1 and its ligand ICAM-1 is implicated in the progression of (4) cancers, as well as (5) allergic, and (6) autoimmune diseases.

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Bind another day: The LFA-1/ICAM-1 interaction as therapeutic target

L. Zecchinon, T. Fett, P. Vanden Bergh, D. Desmecht*

Department of Pathology, Faculty of Veterinary Medicine, University of Liège, FMV Sart Tilman B43, B-4000 Liège, Belgium

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Abstract

Lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18, $\alpha_L\beta_2$) actively contributes to the molecular interactions responsible for normal functions of the immune system but is also associated to several diseases from various etiology (genetic, bacterial, viral, neoplastic, allergic, and autoimmune). In this way, the interaction between lymphocyte function-associated antigen-1 and its major ligand intercellular adhesion molecule-1 (ICAM-1 or CD54) has been extensively studied, leading to the development of therapeutic antibodies, peptides, and small inhibitory molecules.

Keywords: LFA-1; Disease; Therapeutic; ICAM; Binding

1. Introduction

Cell adhesion receptors are known to play an essential role in multicellular organisms by mediating the direct association of cells with each other and with proteins of the extracellular matrix [1,2]. Among these receptors is lymphocyte function-associated antigen-1 (LFA-1, CD11a/CD18, $\alpha_L\beta_2$) that actively contributes to the molecular interactions responsible for cellular adhesion and migration in the immune system [3–6].

Abbreviations: APC, antigen presenting cell; ICAM, intercellular adhesion molecule; IL, interleukin; JAM, junctional adhesion molecule; LFA-1, lymphocyte function-associated antigen-1; mAb, monoclonal antibody; MTX, methotrexate; NK, natural killer.

* Corresponding author. Tel.: +32 4 366 4075; fax: +32 4 366 4565.
E-mail address: daniel.desmecht@ulg.ac.be (D. Desmecht).

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In this context, LFA-1 needs to be tightly regulated, which occurs through conformational changes [7–11] and controlled association with the cytoskeleton [12,13] and various proteins such as its ligands, intercellular adhesion molecules (ICAMs)-1 to -5 and junctional adhesion molecule (JAM)-A.

2. Intercellular and Junctional Adhesion Molecules

2.1. Intercellular adhesion molecule-1

The first counterreceptor identified for LFA-1 was the ICAM-1 (CD54), a member of the immunoglobulin superfamily, providing the first example of an interaction between a member of the integrin family and a member of the immunoglobulin superfamily [14,15]. ICAM-1 is a cell surface glycoprotein that promotes adhesion in immunological and inflammatory reactions [15]. It is constitutively expressed on some tissues and induced on other by inflammatory cytokines such as interleukin-1 or interferon-γ [16]. It could thus be expressed on nonhematopoietic cells such as vascular endothelial cells, thymic and certain other epithelial cells, fibroblasts, and on hematopoietic cells such as tissue macrophages, mitogen-stimulated–T lymphocyte blasts, and germinal center dendritic cells in tonsils, lymph nodes, and Peyer's patches [17]. In these different cell types, ICAM-1 displays Mr heterogeneity with a Mr of 97,000 on fibroblasts, 114,000 on the myelomonocytic cell line U937, and 90,000 on the B lymphoblastoid cell JY [17]. Electron micrographs show that ICAM-1 is a bent rod, 18.7-nm long, suggesting a model in which its 5 immunoglobulin-like domains are oriented head to tail at a small angle to the rod axis. The amino-terminal 2 immunoglobulin-like domains appear to interact conformationally and domain 1 contains the primary site of contact for LFA-1 [18–21].

ICAM-1 has been shown to also bind Mac-1 (CD11b/CD18) [22] through its domain 3 [23]. These findings provide a function for the tandem duplication of immunoglobulin-like domains in ICAM-1 and have implications for other immunoglobulin superfamily members. Mutations at 2 sites in the third domain that destroy consensus sequences for N-linked glycosylation enhance binding to purified Mac-1, and agents that interfere with carbohydrate processing provide evidence that the size of the N-linked oligosaccharide side chains on ICAM-1 affects binding to Mac-1 but not to LFA-1, suggesting that the extent of glycosylation on ICAM-1 may regulate adhesion to LFA-1 or Mac-1 in vivo [23].

ICAM-1 domain 1 residues Glu34 and Gln73 have been identified as critical for binding of LFA-1 as an intact receptor [18], and interaction between isolated I domain and domain 1 of ICAM-1 is inhibited partially by mutation of Glu34 but not by Gln73, which correlates with divalent cation dependence (Mg^{2+} and Mn^{2+} promote binding), indicating that this residue might be in direct contact with the metal ion-dependent adhesion site. On the cell surface, ICAM-1 exists predominantly in a dimeric form that binds more efficiently to LFA-1 than does its monomeric form [24]. However, a single ICAM-1 monomer contains the "fully competent" LFA-1–binding surface [25]. The X-ray crystal structure of domains 1 and 2 of ICAM-1 shows that the N-terminal of domain 1 functions as the interface of the dimer formation [26]. Also, the residues that bind to LFA-1 are oriented with the critical Glu34 residue pointing away from each other in the dimer, which is ideal for simultaneous binding of

2 LFA-1 molecules. The LFA-1–binding surface is relatively flat, runs diagonally across the GFC β-sheet face, and includes residues on the CD edge of the β-sandwich on domain 1 [21]. This site is proposed to dock at a groove in the I domain of LFA-1 [27]. Moreover, the anti–ICAM-1 monoclonal antibody (mAb) RR1/1, which maps to Gln73 [18] enhances I-domain binding, suggesting potential allosteric control or coordinate binding by this region [28]. When 35 point mutants of ICAM-1 were generated, residues important for binding of purified LFA-1 were identified. Nineteen of these mutants bind recombinant LFA-1 equivalently to wild type, whereas 16 mutants show a 66- to 2500-fold decrease in LFA-1 binding. These mutants, along with modeling studies, define the LFA-1–binding site on ICAM-1 as residues Glu34, Lys39, Met64, Tyr66, Asn68, and Gln73 that are predicted to lie on the CDFG β-sheet of the immunoglobulin fold. The mutant Gly32Ala also abrogates binding to LFA-1, possibly indicating a direct interaction of this residue with LFA-1. These data have allowed the generation of a highly refined model of the LFA-1–binding site of ICAM-1 [29]. Presumably, other residues including the conserved residues between the members of the ICAM family (Leu51, Thr53, and Leu55), play some critical role in the ICAMs/β_2-integrin interactions [22].

2.2. Intercellular adhesion molecule-2

ICAM-2 has also been identified as a ligand for LFA-1 (CD11a/CD18) [30–32] and for Mac-1 (CD11b/CD18) [22]. It is constitutively expressed by leukocytes, platelets [33], and endothelial cells [34] whose basal expression is high, unlike that of ICAM-1. The crystal structure of the extracellular region of ICAM-2 shows that the glutamic acid residue at position 37 is critical for LFA-1 binding and is proposed to coordinate the Mg^{2+} ion in the I domain; this Glu37 is surrounded by a relatively flat recognition surface and lies in a beta strand. This finding suggests that there are differences in the architecture of recognition sites between integrins that contain or lack I domains. A bend between domains 1 and 2 of ICAM-2 and a tripod-like arrangement of N-linked glycans in the membrane-proximal region of domain 2 may be important for presenting the recognition surface to LFA-1 [35]. Furthermore, a peptide region from the first immunoglobulin domain of ICAM-2 has been shown to be specifically involved in binding to LFA-1 (CD11a/CD18) as a synthetic peptide from this part of ICAM-2, covering residues 21–42, binds to purified CD11a/CD18, and inhibits the adhesion of endothelial cells to this integrin. Several shorter peptides from the same region showed less or no activity [36].

2.3. Intercellular adhesion molecule-3

ICAM-3 is another LFA-1 ligand that has been shown to also bind CD11d/CD18 [37–39]. ICAM-3 is a glycosylated protein of 124,000 Mr that is constitutively highly expressed on resting leukocytes and antigen presenting cells. It appears thus to be potentially the most important ligand for LFA-1 in the initiation of the immune response because the expression of ICAM-1 on resting leukocytes is low. ICAM-3 is closely related to ICAM-1 and consists of 5 immunoglobulin domains. It binds LFA-1 through its 2 N-terminal immunoglobulin-like domains [37,39–41]. The binding site has been further resolved by rational targeting of

14 point mutations throughout immunoglobulin-like domains 1 and 2, coupled with modeling studies. Within domain 1 a cluster of residues (Glu37, Leu66, Ser68, and Gln75), that are predicted to lie on the CC'FG face of the immunoglobulin fold, play a dominant role in LFA-1 binding [41]. Furthermore, deletion of individual immunoglobulin-like domains of ICAM-3 and ICAM-3 chimeras with CD21 (CR2) showed that there is a single LFA-1–binding site in ICAM-3 and that domain 1 is necessary and sufficient for LFA-1 binding. Epitope mapping and functional studies performed with 17 anti–ICAM-3 monoclonal antibodies demonstrated that only some monoclonal antibodies, with epitopes wholly within domain 1, were able to block binding of ICAM-3 bearing cells to purified LFA-1, in agreement with the data obtained from the domain deletion mutants and CD21 chimeras. Analysis of a panel of 45 point mutants of domain 1 of ICAM-3 identified 5 residues that may contact LFA-1 as part of the binding site, Asn23, Ser25, Glu37, Phe54, and Gln75. These 5 residues are predicted by molecular modeling, based on the structure of vascular cell adhesion molecule-1 (VCAM-1), to cluster in 2 distinct locations on domain 1 of ICAM-3 on the BED face (N23 and S25 that comprise a consensus N-linked glycosylation site) and on the C strand or CD loop (Glu37), the E strand (Phe54), and the FG loop (Gln75) [42].

2.4. Intercellular adhesion molecule-4

The Landsteiner-Wiener (LM) blood group protein or ICAM-4 is specifically expressed on red cells and has been shown to also bind LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) [22,43,44]. The 3-dimensional structure of ICAM-4 is composed of 2 immunoglobulin-like domains that are predicted to form an elbow angle between them [22]. The divalent cations Ca^{2+} and Mg^{2+} promote maximal binding of LFA-1 and Mac-1 to ICAM-4, whereas Mg^{2+} cations without Ca^{2+} failed to support binding [22]. This observation is in agreement with the previous results suggesting that Mg^{2+}, but not Mn^{2+} and Ca^{2+}, is required for the ICAM-4/LW blood group antigen expression [45]. The interactions between the ICAM-4 and the β_2 integrins are specific, because the binding to LFA-1 and Mac-1 is inhibited by characterized antibodies [22]. The study of domain deletion mutants also demonstrated that domain 1 of ICAM-4 is necessary and sufficient for expression of the LFA-1–binding site. However, the domain 2 may have a role in presentation of the binding site to β_2 integrins when ICAM-4 is expressed at the cell surface in the context of the cell glycocalyx [22]. In contrast, the Mac-1–binding site required the presence of both domains of ICAM-4, indicating that there are protein recognition sequences between domain 2 and Mac-1 [22]. The key residues Glu and Gln (at positions 34 and 73 in ICAM-1) are absent in the binding surface of ICAM-4 [22]. Moreover, the single and double mutants of the corresponding positions on ICAM-4 (Arg52 and/or Thr91 by Glu and Gln) did not enhance the interaction with LFA-1 and even reduced the interaction with Mac-1, suggesting that the presence of an acidic residue at position 52 is not essential for ICAM-4/β_2-integrin interactions and may actually alter the protein interaction surface [22]. Presumably, other residues including the conserved residues between ICAM-4 and the other members of the ICAM family (Leu51, Thr53, and Leu55), play some critical role in the ICAMs/β_2-integrin interactions [22]. Having established the importance of domains 1 and 2 of ICAM-4 for β_2-integrin interaction, site-directed mutagenesis of 6 predicted surface-exposed residues identified on the

3-dimensional of ICAM-4 (Leu80, Trp93, Arg97, Glu151, Thr154, and Trp77) suggested that LFA-1– and Mac-1–binding sites are distinct but overlapping [22]. Furthermore, at least 2 other ICAM-1 critical residues for binding of LFA-1, Gln27, and Tyr52, are also common to ICAM-2 and ICAM-4 (Gln45 and Tyr69) [22].

2.5. Intercellular adhesion molecule-5

ICAM-5 or telencephalin is expressed in the central nervous system by subsets of neurons but not by glial cells and may act as a major adhesion molecule for leukocyte binding to neurons in the central nervous system [22,46–49]. ICAM-5 serves as a ligand for LFA-1 as protein constructs containing the first immunoglobulin domain of ICAM-5 were able to support LFA-1 interaction, whereas deletion of the first domain abolished binding. Monoclonal antibodies reacting with the first domain of ICAM-5 also completely blocked the interaction. The soluble first domain of ICAM-5 inhibited the binding of T cells to immobilized ICAM-5 at concentrations of 50 nM and higher. Interestingly, the 6th domain of ICAM-5 was also able to support leukocyte binding, but this binding activity may not involve integrins [47,49].

2.6. Junctional adhesion molecule-A

JAM-A, formerly named JAM-1, is an immunoglobulin superfamily member that contains 2 immunoglobulin-like folds in the extracellular domain. JAM-A is expressed on leukocytes and at tight junctions of endothelial and epithelial cells, where homophilic JAM-A interactions promote cell–cell contact and the organization of the junctional complex [50–53]. Robust evidence has been provided for a key role of JAM-A in leukocyte transmigration and inflammatory extravasation through interaction with LFA-1 [50,54,55], mediated by the membrane-proximal immunoglobulin-like domain 2 of JAM-A [54].

3. Ligand-binding site

The identification of the molecular motifs involved in ligand binding has been the subject of intensive work because it is of great importance, for example, for drug design. In the absence of tertiary structures of an intact LFA-1 complexed with its natural ligands, several alternatives, each with its advantages and drawbacks, have been used: generation of ligand-binding integrin chimera, ligand cross-linking, epitope mapping of mAbs, and mutation of residues [56].

The major ligand-binding site in the CD11a molecule has been clearly found in the I domain as shown by several distinct experiments: (1) recombinant I domain bound directly to purified recombinant ICAM-1 in a Mg^{2+}-dependent manner and inhibited LFA-1–dependent T-cell adhesion to ICAM-1 [28,57], (2) I domain–deleted LFA-1 showed no recognition of LFA-1 ligands but displayed the hallmarks of a constitutively active receptor mediating signals into the cell, suggesting a key role for the I domain in controlling integrin activity [58], (3) replacement of 2 noncontiguous regions in the I domain with the corresponding mouse sequences abolished most binding to human ICAM-1 without affecting αβ subunit association or expression on the surface [59], (4) mutation of Glu34 but not Gln73 inhibits

partially the interaction between the I domain and ICAM-1 [28], (5) specific residues found to be important for ICAM-1 binding (Met140, Glu146, Thr243, and Ser245) are shown to be located on the surface of the I domain surrounding the site to which Mg^{2+} is chelated, and fine a ligand-binding interface, which is confirmed by epitope mapping [59], (6) recombinant I domain has been shown to contain the epitopes for anti–CD11a mAbs that interfere with the interaction between LFA-1 and ICAM-1 [57], and (7) soluble LFA-1–I-domain blocks ICAM-1 binding of the high affinity Mg^{2+}-induced form [60]. Recently, the structure of the I domain of LFA-1 bound to ICAM-1 has revealed the open ligand-binding conformation. The I domain Mg^{2+} directly coordinates Glu34 of ICAM-1, and a dramatic swing of I-domain residue Glu241 enables a critical salt bridge. Liganded and unliganded structures for both high- and intermediate-affinity mutant I domains reveal that ligand binding can induce conformational change in the CD11a I domain and that allosteric signals can convert the closed conformation to intermediate or open conformations without ligand binding [61]. Moreover, considerable similarity exists in the interactions of LFA-1 with JAM-A and ICAM-1 to -3 and a coordination of binding involving divalent metal ions appears to be the principal mechanism mediating the interaction between LFA-1 and its ligands. By analogy to the interaction between LFA-1 and ICAM-1, which requires the acidic amino acid residue Glu34 on ICAM-1 [61], a yet to be identified acidic residue present on JAM-A, for example, Glu163 or Asp169, may be involved in the interaction with LFA-1 [62].

Although the I domain appears to be a dominant ligand-binding site, in vitro translated protein fragments of LFA-1 containing the CD11a–bivalent-cation binding repeats V and VI (residues 458–467 and 497–516, respectively) were also found to interact with ICAM-1 [63]. A 3-dimensional model of these domains was constructed based on the sequence similarity to known EF hands, showing that the 2 regions critical for the interaction of LFA-1 with ICAM-1 lie adjacent to each other, the first next to the nonfunctional EF hand in domain V and the second coinciding with the potential divalent cation-binding loop in domain VI. The binding of ICAM-1 with the domain V and VI region in solution was not sensitive to divalent cation chelation [63].

4. The LFA-1/ICAM-1 interaction as therapeutic target

4.1. Antibodies

Various animal models for ischemia reperfusion injury, trauma, asthma, lung injury, rheumatoid arthritis, and organ transplantation have indicated that anti-adhesion therapy can reduce the serious vascular and tissue injury mediated by infiltrating leukocytes. In this way, anti–LFA-1 antibodies inhibit immunoreactivity, including cytotoxic T lymphocyte–mediated lysis and leukocyte migration to sites of inflammation [64].

Moreover, an improved understanding regarding the pathophysiology of psoriasis has prompted the development of targeted biologic treatments for patients against LFA-1 (CD11a/CD18), because T lymphocytes play an important role in initiating the immune system and the inflammatory responses that result in the development and maintenance of psoriatic plaques. Accordingly, humanized mAb (hu 1124, Efaluzimab, Xanelin) against CD11a inhibits the interaction of LFA-1 with various ICAM molecules [65,66]. Because ICAM-1 is

expressed on activated endothelial cells and antigen presenting cells, the antibody inhibits both the antigen presenting cell–T-cell interaction and the T-cell adhesion to endothelial cells, as well as their subsequent activation, which results in a decrease of transendothelial migration. Treatment with Efaluzimab seems to be well-tolerated, provides clinical benefit, including improved quality of life, in patients with moderate-to-severe plaque psoriasis and can be safely administered for extended periods of time. Most adverse events such as mild chills, abdominal discomfort, headache, and fever are dose-related. Apart from this, transient increases in white blood cell and lymphocyte counts were observed [66]. Efalizumab is associated with an early onset of action, with improvement noted as early as 14 days. Studies with extended treatment suggest that continuing Efalizumab therapy is more beneficial in maintaining and improving responses as relapse of psoriasis is usually seen within 60–70 days after discontinuation of therapy, and rebound in approximately 5% of patients is noted. Efalizumab is associated with acute adverse events during the first and second injections, which decrease in incidence with each subsequent injection. Consequently, Efalizumab offers an interesting new therapeutic option for the treatment of psoriasis and the potential for improved and potentially safer long-term, continuous ‘‘maintenance’’ therapy [65,67,68].

Furthermore, it has been shown that the induction of antigen-specific Th2 cell tolerance by allergen immunotherapy with blockade of the ICAM-1/LFA-1 interaction would be a rational therapeutic approach to allergic inflammation such as asthma [69]. Indeed, in vivo pretreatment with anti–ICAM-1 and anti–LFA-1 mAb, and a soluble antigen inhibited antigen-induced eosinophil recruitment into the airways and IgE antibody production in mice in an antigen-specific manner. In vitro antigen-induced interleukin-2, interleukin-4, and interleukin-5 production were decreased in spleen cells of the mice pretreated with the 2 mAbs and the antigen, indicating the induction of both Th1 and Th2 cell tolerance in vivo [69]. In addition, the combination of anti–LFA-1 mAb and a soluble antigen has a weaker inhibitory effect on antigen-induced eosinophils recruitment into the tissue and IgE antibody production than the combination of anti–ICAM-1 mAb, anti–LFA-1 mAb, and a soluble antigen. The synergistic effect of anti–ICAM-1 mAb and anti–LFA-1 mAb might be explained by the redundancy of adhesion pairs. Indeed, LFA-1 has at least 5 ligands (ICAM-1 to -5) and ICAM-1 has also Mac-1 as counterreceptor, although the involvement of these adhesion molecules in the induction of T-cell tolerance remains to be determined [69].

On the other hand, the role of modulation of ICAM-1/LFA-1 interaction in controlling autoimmune diseases or inducing immunotolerance has been established because this interaction is essential for T-cell activation and for migration of T cells to target tissues. Therefore, blocking ICAM-1/LFA-1 interaction can also suppress T-cell activation in autoimmune diseases and organ transplantation [70]. In this way, the administration of anti–ICAM-1 and anti–LFA-1 antibodies prevents insulin-dependent diabetes mellitus [71,72], rheumatoid arthritis [73,74], and psoriasis [65,68]. Coadministration in vivo of mAbs against LFA-1 and ICAM-1 suppressed the progression of experimental allergic encephalomyelitis in rats by preventing the infiltration of encephalitogenic cells into the central nervous system, whereas administration of the mAb to LFA-1 alone or ICAM-1 alone did not show such suppressive effects [75]. In addition to antibody inhibition of ICAM-1/LFA-1 interaction, antibodies to other cell adhesion molecules such as VLA-4 and B-7 have been developed, and their effectiveness has been demonstrated in vivo [76,77].

Although antibodies are effective, their use as therapeutic agents does present a number of pharmaceutical challenges. Due to their physicochemical properties, antibodies cannot be delivered orally, are expensive to produce and are prone to physical and chemical degradation, which make them difficult to formulate. Antibodies can also generate immunogenicity upon repeated injections to produce immune reaction. Therefore, some efforts have been focused on developing peptides or small-molecule inhibitors of ICAM-1/LFA-1 interaction [70].

4.2. Peptides and peptidomimetics

4.2.1. ICAM-1–derived peptides

Peptides derived from the sequence of ICAM-1 first domain ($ICAM_{1-21}$ and $ICAM_{26-50}$) have been evaluated for their ability to modulate the binding of anti–CD11a antibody to T cells and their capacity to inhibit homotypic T-cell adhesion [70,78]. Although both of these peptides inhibited homotypic T-cell adhesion, it was interesting to find that the $ICAM_{1-21}$ peptide inhibited anti–CD11a antibody, whereas the $ICAM_{26-50}$ peptide enhanced the anti–CD11a antibody binding to LFA-1 on T cells [79]. These results suggest that these 2 fragments bound to different sites of the LFA-1 receptor and have different mechanisms of action in inhibiting T-cell homotypic adhesion. It is possible that the $ICAM_{1-21}$ peptide binds to CD11a at the same site as the antibody and inhibits both anti–CD11a antibody and ICAM-1 binding to LFA-1. On the other hand, the $ICAM_{26-50}$ peptide may bind to a different region on CD11a and induce a conformational change of LFA-1 that favors anti–CD11a antibody binding but inhibits ICAM-1 binding to LFA-1. The $ICAM_{1-21}$ peptide was further studied by fragmenting its sequence into overlapping cyclic peptides cIBL ($ICAM_{1-10}$), cIBC ($ICAM_{6-15}$), and cIBR ($ICAM_{12-21}$), that were found to have higher activities than the parent peptide ($ICAM_{1-21}$) in inhibiting homotypic and heterotypic T-cell adhesion as well as mixed lymphocyte reaction, suggesting that conformational restriction in cyclic peptides creates a more rigid structure that prevents the peptide from adopting nonproductive conformation and accommodates better binding to LFA-1 to block ICAM-1/LFA-1 interaction [79–82]. Interestingly, the overlapping sequence (Pro-Arg-Gly-Gly) of cIBC and cIBR was found to form a β turn, similar to the same sequence in the ICAM-1 X-ray structure of domain 1 that plays a role in stabilizing the angle between ICAM-1 domains 1 and 2 [21]. This β turn seems to be important for binding to LFA-1 [70].

Whereas the mechanism of inhibition of ICAM-1/LFA-1 interaction by ICAM-1 peptides was initially thought to be due solely to their ability to bind LFA-1 at the ICAM-1–binding site, it has been shown that ICAM-1 peptides cIBL, cIBR, and cIBC trigger an internalization of LFA-1 into the cytoplasmic domain of T cells and lower the adhesion properties of T cells by binding to the I domain [80,81]. This receptor-mediated internalization represents a unique strategy to selectively target drugs delivery that will be further detailed below.

4.2.2. LFA-1–derived peptides

Several peptides derived from the CD11a and CD18 subunits have been studied. Linear peptide LAB ($CD11a_{237-261}$) and its cyclic derivatives cLAB.L ($CD11a_{237-246}$), cLAB.C ($CD11a_{244-253}$), and cLAB.R ($CD11a_{251-261}$) were derived from the CD11a I domain [78,79]. cLAB.L was found to inhibit homotypic and heterotypic T-cell adhesion to

epithelial monolayers, probably by the disruption of LFA-1/ICAM-1 interaction. Its inhibitory activity is greatly reduced by low temperature (indicating that an energy-dependent process was involved in peptide activity) and the absence of cell activation [83–85]. The mechanism of binding to ICAM-1 was evaluated using docking and antibody inhibition studies that reveal that cLAB.L was found to bind at the calcium binding site of domain 1 of ICAM-1 and ICAM-3 on T cells [83–86]. Binding of FITC-labeled cLAB.L peptide to ICAM-1 was enhanced when the cells were activated with phorbol myristate acetate and anti–CD3 antibody, as well as in the presence of a mixture of calcium and magnesium ions [85]. Moreover, circular dichroism studies show that the peptide forms a 1:1 (peptide:calcium/magnesium) complex with low cation concentrations and multiple types of complexes with higher cation concentrations. Binding to divalent cations causes a conformational change in cLAB.L, which is consistent with the fact that its binding to ICAM-1 was influenced by divalent cations [86]. Interestingly, the cLAB.L peptide can be internalized by ICAM-1 into the cell cytoplasmic domain, which could be an alternative mechanism of inhibiting cell adhesion [85], as already been observed in a number of studies [87,88].

On the other hand, LAB.2 ($CD11a_{441-465}$) and its cyclic derivative cLAB.2L ($CD11a_{441-450}$) from the divalent cation-binding region of CD11a were found to inhibit ICAM-1/LFA-1–mediated T-cell adhesion by directly binding to ICAM-1 or by disrupting the interaction of both subunits of LFA-1 [78,89]. The solution structure of cLAB.2L was studied by nuclear magnetic resonance, circular dichroism, and molecular dynamics simulations, which reveal that this cyclic peptide exhibits 2 types of possible conformations in solution: structure I is a loop-turn-loop type of structure, which is suitable to bind cations such as EF hand proteins, whereas structure II is a more extended structure with beta hairpin bent at Asp4-Val5-Asp6-Gln7. Circular dichroism and nuclear magnetic resonance studies indicate that the peptide binds to calcium and forms a 1:1 (peptide:calcium) complex at low calcium concentrations and multiple types of complexes at higher cation concentrations, as cLAB.L does, but that the conformation of the peptide is not significantly altered upon binding to calcium [89].

Lastly, the LBE peptide ($CD18_{112-137}$) and its cyclic derivatives cLBE.L ($CD18_{112-122}$) and cLBE.C ($CD18_{120-129}$) were discovered from the CD18 subunit I-like domain and were determined to be active in inhibiting homotypic T-cell adhesion and suppressing mixed lymphocyte reaction [79]. Structural analysis of LBE exhibits a stable helical conformation that may be in equilibrium with other possible conformers containing loop and turn structures and may contribute significantly to the mechanism of action of LBE by promoting divalent cation-binding capability [90]. Furthermore, LBE enhances cLAB.L binding to T cells and epithelial cells. Whereas the adherence of T cells to epithelial monolayers is suppressed by the 2 peptides, the addition of LBE to the monolayers before the addition of cLAB.L produces a better inhibitory effect than the reverse procedure. LBE, but not cLAB.L, changes the ICAM-1 conformation, suggesting that LBE binds to ICAM-1 at sites that are distinct from those of cLAB.L and induces improved conformation in ICAM-1 for binding to cLAB.L [84].

4.2.3. Other peptides

A high-throughput screen of peptides by a phage display library has led to the development and characterization of a small cyclic peptide antagonist of ICAM-1–dependent cell

aggregation called IP01 [91]. This peptide can inhibit firm adhesion of neutrophils to endothelium, a critical event in inflammatory diseases, in an assay that recapitulates physiologic flow conditions. Moreover, alanine scanning of the peptide has identified at least 4 amino acids (Leu2, Leu3, Met5, and Arg6) necessary for inhibition of ICAM-1–dependent cell aggregation that may mimic the epitopes of either LFA-1 or ICAM-1, whereas IP01 does not display any homology with the primary amino acid sequences of LFA-1 subunits. It is suggested that there are several possible mechanisms of action for IP01: (1) to bind to ICAM-1 at the same binding site as LFA-1 peptides, (2) to bind to ICAM-1 at the proximal site of the LFA-1–binding region and to induce steric inhibition, and (3) to bind ICAM-1 at the allosteric site that controls the LFA-1–binding site [91].

Peptide-based therapy was also derived by controlling the signaling mechanisms of LFA-1, which oligomerization activates calcium-calmodulin kinase and leads to the release of perforin, granzymes, and IFN-γ in natural killer cells [92]. Treatment of natural killer cells with human immunodeficiency virus-1 protein Tat, which is known to affect several calcium-mediated events in immune cells, impairs their cytotoxic activity by inhibiting the rise in intracellular free calcium concentration upon cross-linking of CD11a and hence preventing natural killer cell degranulation [93]. Moreover, a peptide derived from the C-terminal domain of Tat was found to block calcium influx and LFA-1–mediated activation of calcium-calmoduline kinase, providing evidence that exogenous peptide treatment is able to inhibit natural killer cell activation occurring upon contact with dendritic cells. Lastly, this mechanism might contribute to the impairment of natural immunity in HIV-1 infection [92].

4.2.4. Peptidomimetics

The identification of an epitope (comprising residues Glu34, Lys39, Met64, Tyr66, Asn68, and Gln73) within ICAM-1 first domain as essential for its interaction with LFA-1 [21,29] has recently led to the design of peptidomimetic inhibitors of ICAM-1/LFA-1 interaction, resulting in the transfer of this contiguous, nonlinear epitope to a small-molecule framework [94]. This nonpeptide template was expected to overcome the pharmaceutical limitations of peptides, particularly their inefficient delivery via an oral route. The LFA-1 antagonists bound to LFA-1 and inhibited ICAM-1 binding to leukocytes as well as a mixed lymphocyte reaction with potency significantly greater than that of cyclosporine A. Moreover, in comparison to an antibody to LFA-1, they exhibited significant anti-inflammatory effects in vivo. Structure–activity relationship studies were performed (1) on the amino acid moiety concentrating on the carboxylic acid and the amino acid side chain, and (2) on the 2-bromobenzoyl moiety of a derived compound, resulting in 2 molecules, generated by combining the best elements of the amino acid structure–activity relationship and the benzoyl structure-activity relationship, that are 16-fold more potent than the original screening hit [95,96].

4.2.5. Peptide–drug conjugate for cell-specific targeting

Both the ICAM-1– and LFA-1–derived peptides previously described exhibited properties consistent with receptor-mediated internalization, which presents a unique strategy to selectively target toxic drugs delivery [81,85]. Indeed, by conjugating these peptides to an anticancer or anti-inflammatory drug, the internalization of this peptide conjugate could

selectively target and deliver drugs to cells expressing these cell adhesion molecules, thus eliminating systemic side effects of toxic drugs and also overcoming drug resistance by avoiding the efflux pumps.

In this way, the ICAM-1–derived peptide cIBR [81] was conjugated to methotrexate (MTX) to determine its ability to deliver drugs to LFA-1–expressing cells [70]. The effects of conjugation on both the activity of peptide binding to LFA-1 and MTX activity were evaluated and the MTX–cIBR conjugate, like the unconjugated cIBR peptide, was found to block anti–LFA-1 antibodies in T cells activated by phorbol myristate acetate, suggesting that the ability of the peptide to bind LFA-1 was not affected by conjugation. Importantly, the nuclear magnetic resonance solution structures of MTX–cIBR and cIBR were analyzed, and the peptide portion of MTX–cIBR had a structure similar to cIBR alone, suggesting that conjugation with MTX did not alter the structure of the peptide fragment in the MTX–cIBR conjugate. MTX–cIBR conjugate activity was compared to that of MTX in inhibiting dihydrofolate reductase, one mechanism of MTX anticancer activity, and studies have demonstrated that MTX–cIBR ($K_d = 29.8 \times 10^{-9}$ M) has a 10-fold lower affinity for dihydrofolate reductase than that of MTX ($K_d = 1.84 \times 10^{-9}$ M). This lower affinity may be due to the interference in binding between the MTX fragment and dihydrofolate reductase by the cIBR fragment. Low-dose MTX is also widely used in the treatment of rheumatoid arthritis, and its mechanism of activity is thought to be due to inhibition of the production of TNF-α. Studies have demonstrated that equimolar doses of either MTX or MTX conjugates reduce TNF-α production in human peripheral blood mononuclear cell stimulated with phorbol myristate acetate and ionomycin, indicating that MTX conjugation does not significantly reduce the activity of MTX. In comparison to MTX alone, MTX–cIBR was toxic for T cells but not for endothelial cells that do not express LFA-1, suggesting that the internalization of MTX–cIBR is mediated by LFA-1. Further studies have demonstrated that blocking LFA-1 with either anti–LFA-1 or unconjugated cIBR peptide decreases the toxicity of MTX–cIBR conjugates to T cells in a dose-dependent manner, suggesting that MTX–cIBR is internalized by LFA-1. Therefore, MTX–peptide internalization mediated by LFA-1 is a novel peptide-based–drug-delivery system that could be used to selectively target T cells involved in autoimmune diseases such as rheumatoid arthritis. This conjugation method could also be applied to other toxic anticancer drugs.

4.3. Allosteric inhibitors

Two distinct mechanistic classes of small-molecule inhibitors of LFA-1, termed αI allosteric and α/β I-like allosteric antagonist, have recently been developed. The αI allosteric antagonists bind underneath the C-terminal helix of the CD11a I domain, which stabilize the I domain in the inactive closed conformation. By contrast, the α/β I-like allosteric antagonists bind to the β_2 I-like domain metal ion-dependent adhesion site and disrupt conformational signal transmission between the I and the I-like domain, leaving the I domain in a default inactive form. Furthermore, the 2 classes of the antagonists have opposite effects on integrin conformation; the αI allosteric antagonists stabilize the bent conformation, whereas the α/β I-like allosteric antagonists induce the extended conformation with inactive I domain. The small-molecule antagonists to the β_2 integrin highlight the importance of the

structural linkages within and between integrin domains for transmission of the conformational signals and regulation of the overall conformation [97].

The tertiary structure of the CD11a I domain in complex with lovastatin, a drug used clinically for lowering cholesterol levels, that binds the I domain at the I-domain allosteric site region and inhibits ICAM-1/LFA-1 interaction by preventing the LFA-1 conformational change to the activated form via allosteric control [98], reveals that lovastatin interacts with Leu132, Phe153, Ile235, Tyr257, Lys287, Leu298, Glu301, Leu302, and Lys305 residues that help form a large hydrophobic cavity between the central β-sheet and α-helix 7 of the I domain [99]. Other small molecules such as a series of p-arylthio cinnamides and hydantoin derivatives (i.e., BIRT-377 that has been developed for oral formulation) were also found to bind to this I-domain allosteric state site and inhibit ICAM-1/LFA-1 interaction by way of an allosteric modification of LFA-1 [98,100,101]. Because of the discovery of this allosteric mechanism, some of the small molecules have been used to detect conformational changes in LFA-1 upon binding to the I domain or the I-like domain [102].

5. Conclusion

LFA-1 is able to engage several ligands expressed on many cell types, leading to various cellular normal or pathologic responses.

Studying these interactions and what they entail is thus a promising research field that has already allowed the development of therapeutic antibodies, peptides, and small inhibitory molecules for several major disorders such as cancers, allergies, and autoimmune diseases.

6. Summary

LFA-1 (CD11a/CD18, $\alpha_L\beta_2$) binds ICAM-1 to -5 and JAM-A, which elicit key immune functions. These molecular interactions could also be associated to several diseases and have thus been extensively studied, particularly the relation between LFA-1 and its major ligand ICAM-1 (CD54), leading to the development of potential therapeutic tools such as antibodies, allosteric inhibitors, peptides, and peptidomimetics.

The paper globally reviews LFA-1 ligands and the more recent findings regarding the blockade of the LFA-1/ICAM-1 interaction in the therapeutic field.

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