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Anatomy of the lymphocyte function-associated antigen-1

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Abstract

The β_2-integrin lymphocyte function-associated antigen-1 (LFA-1, $\alpha_L\beta_2$, CD11a/CD18) is made of the association of the CD11a and CD18 subunits that each possesses a large extracellular region and short transmembrane and cytoplasmic parts. A general comparison among species enlights the importance of especially conserved functional regions, as well as their role in folding and heterodimerization. This review also focuses on providing insights into structural aspects that lead to lymphocyte function-associated antigen-1 ability, central to its critical role in the molecular interactions responsible for leukocyte adhesion and migration in the immune system, to modulate dynamically its adhesiveness through avidity (affinity and valency)-based mechanisms.

Keywords: LFA-1; Integrin; Interspecies; Folding; Modulation

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, as the formation of tight associations with neighboring cells is a prerequisite to build cell layers, tissues, and organs [1–3]. Therefore, multicellular organisms express specialized surface receptors (not found in prokaryotes or unicellular eukaryotes) that not only support the structural integrity of cells and tissues, but also contribute to the transduction of signals. These receptors can be subdivided into several groups, most importantly the

Abbreviations: EGF, epidermal growth factor; ICAM, intercellular adhesion molecule; LFA-1, lymphocyte function-associated antigen-1; MIDAS, metal ion-dependent adhesion site; PMA, phorbol myristate acetate; PSI, plexin semaphorin integrin.

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integrins, the cadherins, the immunoglobulin superfamily cell adhesion molecules, and the selectins [3,4].

2. β_2-integrins

The historical perspective of the emergence of integrins has been recently reported [5]: they are transmembrane receptors that play an important role in cellular adhesion even if their recognition as surface receptor family is only dated from 1987 [4,6]. Because they were extensively studied (more than 30,000 articles to date) and appear to be implicated in many biological, physiological, and pathological processes. These cell surface glycoproteins typically function as receptors for extracellular matrix molecules or recognize membrane-bound counter receptors [7]. As heterodimeric receptors, they consist of a 120–180 kDa α subunit and a 90–110 kDa β subunit that are noncovalently associated single-pass transmembrane proteins [2,7,8], both containing a large extracellular domain, a single transmembrane stretch and, with the exception of integrin β_4, a short cytoplasmic tail [3]. Integrins are expressed by all multicellular organisms but their diversity varies widely among species; for example, in mammals, 19 distinct α subunits can combine with 8 β subunits to form 25 receptors, whereas the *Drosophila* and *Caenorhabditis* genomes encode only 5 and 2 integrin α subunits, respectively [8].

In mammals, the 8 β subunits define 8 subfamilies, each one associating with different α subunits. The β_2-integrins, that share the common β_2 subunit CD18 [9], include 4 different heterodimers [2,10,11]: the lymphocyte function-associated antigen-1 (LFA-1), also known as CD11a/CD18 or $\alpha_L\beta_2$, that predominates [12,13]; CD11b/CD18 ($\alpha_M\beta_2$ or Mac-1 or CR3) [14,15]; CD11c/CD18 ($\alpha_X\beta_2$ or P150/95 or CR4) [9,16], and CD11d/CD18 or $\alpha_D\beta_2$ [17,18]. The CD11a chain is expressed on virtually all leukocytes and many leukocyte-derived cells, such as macrophages. T and B lymphocytes normally express only CD11a/CD18 [19], whereas polymorphonuclear neutrophils express CD11b/CD18 at a significant higher level than CD11a/CD18 and CD11c/CD18 [20,21]. The most prominent CD11 chains on activated granulocytes and tissue macrophages are CD11b and CD11c, respectively [19]. Lastly, CD11d/CD18 is abundant on the $CD8^+$ lymphocyte subpopulation in peripheral blood and on macrophages present in specialized tissue compartments [17,18].

The CD11a–d/CD18 heterodimers whose expression is restricted to white blood cells mediate high-affinity adhesion to a variety of cell types that display one or more of the β_2-integrins ligands, intercellular adhesion molecules (intercellular adhesion molecule [ICAM]-1 to -5) [20,22–36]. The adhesion process mediated is a critical step of a wide range of immunological activities, including cytolysis of target cells, cross-interaction and cross-stimulation between lymphocytes, phagocytosis of complement-coated targets, neutrophils clearance from inflamed tissue, and the regulation of leukocyte traffic between the bloodstream and tissues [37–40].

3. LFA-1 (CD11a/CD18, $\alpha_L\beta_2$)

As stated above, LFA-1 (CD11a/CD18, $\alpha_M\beta_2$) is the predominant β_2-integrin. The receptor plays a critical role in the complex and well-orchestrated molecular interactions

responsible for homotypic and heterotypic cell adhesion events required for normal and pathologic functions of the immune system. LFA-1 is made of the association of the CD11a and CD18 subunits that each possesses a large extracellular domain but short transmembrane and cytoplasmic regions (Fig. 1). 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 [41,42].

3.1. CD11a

The CD11a protein is made of a putative signal peptide of 23–112 residues (depending on the species), a large extracellular domain of 1060–1195 residues, a single hydrophobic transmembrane region of 24 residues and a cytoplasmic tail of 53–62 residues.

3.1.1. I domain

The extracellular domain contains an inserted (I) or A domain of ~200 residues (Fig. 1, Table 1) quite similar to those found in 9 of the18 α subunits sequenced to date and homologous with repeated domains found in von Willebrand factor and cartilage matrix protein [2,43]. The I domain is located between the β sheets 2 and 3 of a 7 bladed β-propeller region [8,44,45] and is crucial for the ligand-binding specificity of LFA-1 [46]. It folds independently and can thus be expressed as an isolated domain in recombinant form. Its 3-dimensional structure reveals of a classic α/β Rossmann fold, a topology that is found in several intracellular enzymes, in which the core of the module is made up of a 5-stranded parallel β-sheet core surrounded peripherally on both faces by 7 α-helices, with a short antiparallel strand occurring on one edge of this sheet [47]. The residues on top of the I domain whose side chains coordinate the metal ion (a DXSXS-containing sequence) are called the metal ion-dependent adhesion site (MIDAS) motif and are completely conserved (Asp137, Ser139, Ser141, Thr206, Asp239), but the manner in which the metal is coordinated differs slightly [47–49]. It has been proposed that during ligand binding in vivo, the sixth cation-coordinating residue is provided by an acidic glutamate residue such as Glu34 of ICAM-1 or Glu37 of ICAM-2, implying that cations directly contribute to ligand binding by connecting both partners [45]. The I domain also contains an I-domain allosteric site that plays a functional role in ICAM-1 binding [50–52].

The CD11a I domain has been shown to bind the highly homologous ligands ICAM-1 and ICAM-3 with comparable avidity in a selective manner of activation that is believed to be accompanied by a conformational change in the integrin, most likely involving the I domain [47,53]. More, T-cell binding to ICAM-3 was inducible both from the exterior of the cell by Mn^{2+} and from the interior by phorbol dibutyrate, an agonist of the "inside-out" signaling pathway [53]. The ability of manganese ion to induce the active state and the requirement for ligand binding indicate that the Mn^{2+}-bound form of the CD11a I domain is likely to represent a high-affinity state of the molecule, which is consistent with the presence in the I domain structure of a strained hydrophobic ridge with main-chain contacts to the loop primarily responsible for coordinating the metal ion [47]. By contrast, the conformation of the CD11a I domain that was solved in the absence of cation or with bound magnesium is not

Table 1
The important regions of CD11a and their functional role(s)

Extracellular region	
I domain	Ligand-binding specificity of LFA-1
MIDAS motif	Coordinate the metal ion that could contribute to ligand binding by connecting both partners
I-domain allosteric site motif	Functional role in ICAM-1 binding
β-propeller	Implicated in both cation and ligand binding
Transmembrane region	
Cytoplasmic part	
Conserved GFFKR motif	Lock LFA-1 in a low avidity state (role in ligand-binding affinity and heterodimerization)
	Role in deadhesion from ICAM-1
Lys 1097/1099	Critical for Rap1-dependent LFA-1 activation and affinity up-regulation

active state and the Mn^{2+} form the conformation in the inactive state of the integrin [48]. To answer these apparently contradictory data, Baldwin et al. [55] set out to independently determine the structure of the CD11b I domain and to evaluate the structural effects of divalent ion binding to this protein in the absence of added metal ions that were easily introduced into this crystal form allowing the straightforward assessment of the structural effects of divalent cation binding at the MIDAS. The overall protein conformation and metal-ion coordination of the I domain were the same as that observed for all previously reported CD11a I-domain structures and a CD11b I-domain complex with Mn^{2+} but addition of the cations Mg^{2+}, Mn^{2+}, and Cd^{2+} to the metal-free I domain did not induce conformational changes in the crystalline environment. Moreover, Ca^{2+} bound poorly to the I domain, which serves to explain its failure to support adhesion, and the authors proposed that the active conformation previously reported [48,49] is likely to be a construct artifact because their data do not support a dramatic structural transition for the I domain during counter-receptor binding [55].

Besides, the presence of an invariant isoleucine residue at position 331 in the I domain of CD11a and CD11b has been reported to play an important role in LFA-1 and Mac-1 activation, respectively [50]. The hydrophobic side chain finger of this isoleucine was described as fastened in the closed conformation by a conserved hydrophobic socket [56]. This "socket for isoleucine," was postulated to be a key component in allosteric regulation, controlling affinity and shape shifting in the Mac-1 I domain. By inference, the socket for isoleucine was suggested as the universal mechanism that regulates integrin conformation and function because similar findings were reported for the I domain of CD11c [43,57]. However, mutation of the isoleucine in full-length CD11a does not lead to an active LFA-1, although mutation of the equivalent residue in CD11b conveys constitutive activity to Mac-1. Ile331 does thus not appear as a critical residue contributing to allosteric regulation of LFA-1 but, of interest, recent reports state that the I domain exists in an inactive, closed conformation and requires the presence of activating agents for ligand binding, whereas the isolated wild-type I domain exists in a constitutive active state [43]. This could be explained by the fact that the

CD18 could play a role in constraining the CD11a I domain in an inactive conformation in intact LFA-1, but more structural data are required to shed light on the mechanism if integrin activation involves the I domain [43].

3.1.2. β-propeller

Following the I domain, the β-propeller (a topology also found in several enzymes) domain consists of seven 4-stranded β-sheets, each of which made of approximately 60 amino acids, arranged in a torus around a pseudo-symmetry axis, like the blades of a propeller, as seen in heterodimeric G proteins (Fig. 1, Table 1). The I domain is tethered to the top of the β-propeller domain by a hinge that may allow movement of the domains relative to one another [58]. The β-propeller 7 repeats are located on the lower face of the β-propeller [58] and are quite similar in sequence, with the exception that the final 3 contain inserted sequences that are analogous to the EF-hand, a 13-residue bivalent cation-binding motif found in proteins such as calmodulin and parvalbumin. The integrin sequences differ from classical EF-hands in that they lack a coordinating residue at position 12 [8,59]. This region is implicated in both cation and ligand binding [8], and one hypothesis to explain integrin-ligand binding is that aspartate-containing recognition sequences in integrin ligands, which bind at or near to the EF-hand–like sequences, may take the place of the missing residue and coordinate directly to the bound cation [59].

Three repeats with a divalent cation-binding motif (Fig. 1) are found at amino acid residues 468–476, 530–538, and 590–598 (human numbering).

3.1.3. Intracellular region

The short cytoplasmic tail (Fig. 1, Table 1) has been shown to contain a conserved GFFKR motif that plays an important role in ligand-binding affinity and heterodimerization (Section 3.4). Indeed, J-β2.7 and K562 transfectants expressing the truncation mutants (1) before the conserved GFFKR motif (which appears to lock LFA-1 in a high avidity state, resulting in constitutive ICAM-1 binding) and (2) after the GFFKR motif (which appears to lock LFA-1 in a low avidity state, resulting in defective ICAM-1 binding and reduced stimulation by phorbol myristate acetate [PMA] or stimulatory Abs), failed to induce transendothelial chemotaxis [60,61]. Taken together, these data suggest that transendothelial chemotaxis may require changes in LFA-1 avidity, rather than a sustained increase in avidity or low avidity that is unresponsive to stimulation by cellular signaling pathways [61]. Contradictory results, showing that deletion of the 12 cytoplasmic amino acids after the conserved GFFKR motif in COS cells did not affect ICAM-1 binding [62], seem to be an artifact due to the choice of COS cells, a cell type in which the integrin is not normally expressed, that is, in a physiologic transmembrane and cytoplasmic environment [60]. On the other hand, the disruption of this well conserved GFFKR motif (by internal deletion or point mutations of the Gly, the 2 Phe, or the Arg residues to Ala) increases ligand-binding affinity and makes CD11a constitutively active, whereas mutation of the Lys residue did not affect LFA-1 adhesion to ICAM-1, suggesting that this motif maintains a default, low-affinity state of the integrin, possibly by restraining the receptor conformation [60,63].

Very recently, impaired deadhesion from ICAM-1 that resulted in defective migration was observed for a GFFKR-deleted LFA-1, whereas signaling through the receptor was not

affected [64]. On the contrary, T-cell activation by superantigen-loaded and allogeneic antigen-presenting cells, cytotoxic T-cell activity, T-dependent humoral responses, and neutrophil recruitment during aseptic peritonitis were impaired in GFFKR-deleted mice, enlightening the role of the GFFKR motif in deactivation of LFA-1 and hence in the generation of normal immune response [64].

Lastly, Lys1097 and Lys 1099 are critical for Rap1-dependent LFA-1 activation and affinity up-regulation [65].

3.1.4. General comparison among species

Overall, the general organization of human [66], ovine [67], bovine [68], caprine [69], porcine [69], murine [70], canine (GenBank NW_876320), and rat (GenBank NW_047562) CD11a proteins is quite similar. Comparison between human CD11a sequence and its bovine, ovine, caprine, porcine, murine, canine, and rat counterparts shows overall 77%, 77%, 77%, 76%, 71%, 78%, and 58% identity, respectively, with the highest identity for the MIDAS, the transmembrane region and the cation-binding motifs and the lowest identity for the cytoplasmic domain (Table 2). The high conservation of the MIDAS and the putative cation-binding motifs is consistent with an involvement of these regions in the functional activity of the CD11a subunit, as suggested by the requirement of Mg^{2+} and Ca^{2+} for LFA-1–dependent cellular interactions [71] or binding to purified ICAM-1 [72,73]. The transmembrane region shows also a high degree of conservation that could be explained by (1) physicochemical and (2) functional constraints. Indeed, (1) residues lying in the membrane have to possess rather hydrophobic character to allow liposolubility, which is confirmed by the presence of many leucine residues and (2) bidirectional integrin signaling (inside-out and outside-in) is accomplished by transmission of information across the plasma membrane [74]. By contrast, the low conservation of the cytoplasmic tail suggests that most of it is not required to guarantee adequate functioning of LFA-1. This is in agreement with the observation that truncation of the CD11a subunit cytoplasmic domain has no effect on binding to ICAM-1, whereas binding is markedly diminished by the CD18 subunit cytoplasmic domain truncation [62]. Residue Glu 332 that is located in the linker following the I domain and that is known to be critical for communication to the β_2 I-like domain, rolling, integrin extension and activation by Mn^{2+} of firm adhesion [39] is strictly conserved.

The mature protein contains 21 cysteine residues that are strictly conserved in the other species (except that caprine and rat CD11a each lack, respectively, 2 and 1 of these residues that therefore do not seem to be indispensable), which is consistent with a role in maintaining the global structure of the protein. The bovine, murine, canine, and rat versions distinguish, respectively, by additional cysteine residues in the cytoplasmic domain at position 1145 (bovine numbering), in the I domain at position 199 (mouse numbering), in the I domain and the extracellular part (at positions 200 and 361, canine numbering), and at positions 287 (I domain), 877, 908, and 1250 (extracellular region, rat numbering).

Interestingly, in human (GenBank NM_002209 and AY892236), ovine [67], caprine [69], and porcine [69] CD11a, an allelic variant with a triplet insertion, resulting in an additional Glu744 in the extracellular domain was consistently identified, which suggests an allelic

Table 2
Between-species percent identities of CD11a constitutive blocks

Block	Hum vs.						
	Bov	Ovi	Cap	Por	Mur	Can	Rat
Overall	77	77	77	76	71	78	58
Putative signal peptide	56	52	56	56	32	40	3
Extracellular region/overall	78	78	78	78	73	80	63
Extracellular region/I domain	84	86	86	79	73	86	76
Extracellular region/MIDAS domain	100	100	100	85	85	100	85
Extracellular region/putative cation-binding motif 1	88	88	88	77	77	88	66
Extracellular region/putative cation-binding motif 2	77	77	77	77	77	100	88
Extracellular region/putative cation-binding motif 3	88	88	88	88	66	77	77
Transmembrane region	91	95	95	91	75	75	83
Cytoplasmic tail	56	56	58	51	59	60	59

Abbreviations: Hum, human; Bov, bovine; Ovi, ovine; Cap, caprine; Por, porcine; Mur, murine; Can, canine; Rat, rat CD11a.

polymorphism that might be biologically relevant. Studies of genomic sequences will permit to know if this addition represents 2 alleles or an alternative splicing.

Finally, one has to note that the lowest between-species identities are observed with the rat CD11a sequence, which has been derived from an annotated genomic sequence. Cloning and characterization of rat CD11a from rat peripheral blood mononuclear cells would probably give a higher identity.

3.2. CD18

The CD18 molecule also contains a putative signal peptide of 13–23 residues, a large extracellular domain of 676–685 residues, a single hydrophobic transmembrane region of 23 residues, and a short cytoplasmic tail of 46–48 residues (Fig. 1, Table 3).

3.2.1. Extracellular region

Starting from the N-terminal end, the extracellular region successively encloses a cysteine-rich repeats containing analog of the so-called plexin semaphorin integrin (PSI) domain (residues 23–74 in human) [75], an inserted I-like domain [76] of 240–248 amino acids (residues 124–363 in human) and a series of 4 cysteine-rich repeats that display a significant degree of similarity with the epidermal growth factor (EGF)-like domains in laminin [8,77–79] (449–496, 497–540, 541–581, and 582–617 in human).

In the absence of a tertiary structure, structure predictions performed for the PSI and the βI domain reveal, respectively, an α-helical character [75] and a similarity, but not an identity, with the αI domain [76,80]. A putative MIDAS-like D*X*S*X*S motif [81] is predicted within the I-like domain (residues 134–138 in human).

The cysteine-rich repeats vary from classical EGF repeats by the presence of 8 (7 in the first repeat), rather than 6, cysteine residues. The positions of these 2 additional residues

Table 3
The important regions of CD18 and their functional role(s)

Extracellular region	
PSI domain	
I-like domain	MIDAS-like motif
Four cysteine-rich repeats	Are supposed to maintain an intramolecular constraint whose release is needed for LFA-1 activation
Transmembrane region	
Cytoplasmic part	
Tyrosine-based sequences	Role in internalization
K_{724}AL$\underline{X}$HL$\underline{Z}$D	Efficient formation and expression of CD11a/CD18 and CD11b/CD18 heterodimers on the cell surface, as well as their maintenance in the inactive state (X = I in human and T in other species, and Z = T in murine and S in other species)
Arg733–Lys742 (noncompletely conserved region)	Cytoskeletal association, endoplasmic reticulum retention, assembly, transport to the plasma membrane of the mature LFA-1
Arg736–Gln746	α-Actinin binding region
Asn748–Ser 769	Prevents association with α-actinin
Y735RRF motif	Sorting signal for LFA-1 recycling
F754	Role in adhesion to ICAM-1
S756	Major residue phosphorylated in response to PMA
T758TT	Control, in part, binding to ICAM-1 and stimulation with PMA
F766	Key residue for binding to ICAM-1

within the integrin repeats differ from the laminin EGF-like modules, suggesting that each may have a distinct structure [82]. The EGF-like repeats are not required for either dimerization or subunit selection as construction of a chimera of CD18 containing the EGF-like repeats of the β_1 subunit led to association with CD11a [83]. Moreover, unlike cells displaying wild-type LFA-1 (CD11a/CD18), those expressing the chimaera are constitutively active in ICAM-1 adhesion, suggesting that activation of LFA-1 involves the release of an intramolecular constraint, which is maintained, in part, by the cysteine-rich region [83].

Furthermore, a structure and function map of the CD18 has been established by mapping the epitopes of a panel of monoclonal antibodies including function blocking, nonblocking, and activating antibodies using human/mouse β_2 subunit chimeras [76]. Activating antibodies recognize the C-terminal half of the cysteine-rich region, those that do not affect ligand binding map to residues 1–98 and residues 344–521, and monoclonal antibodies to epitopes within the predicted I-like domain strongly inhibit LFA-1–dependent adhesion, which is consistent with the observation that most mutations in leukocyte adhesion deficiency are predicted to be buried in the CD18 I-like domain [84–87].

3.2.2. Cytoplasmic domain

Many lines of evidence indicate that the CD18 cytoplasmic region (Fig. 1, Table 3) plays an important role in modulating cell adhesion because it was found to be required in transendothelial chemotaxis and in constitutive binding of COS transfectants to ICAM-1 and stimulation with PMA [61,62,88]. Moreover, deletion of the CD18, but not the CD11a,

cytoplasmic tail has been shown to abolish colocalization of LFA-1 (CD11a/CD18) with actin and its coprecipitation with vinculin and α-actinin in COS cells, indicating its role in cytoskeletal associations of LFA-1 [89]. In this way, a noncompletely conserved region (Arg733–Lys742) in the CD18 cytoplasmic domain seems to be critical not only for its cytoskeletal association, but also for endoplasmic reticulum retention, assembly, and transport to the plasma membrane of the mature LFA-1 [89]. Distinct domains that regulate binding to α-actinin have been identified: an α-actinin binding region between residues 736 and 746 in the membrane proximal half of the tail and an inhibitory region between residues 748 and 769 that prevents association of α-actinin to the membrane-proximal binding region [90].

A sorting signal can be found in the membrane-proximal Y735RRF motif, which appears to be selectively required for the recycling of spontaneously internalized receptors to the cell surface and to growth factor-induced membrane ruffles. Notably, the disruption of this motif by the nonconservative substitution Y735A diverts internalized integrins from a recycling compartment into a degradative pathway, without impairing the ability of the mutated receptor to recruit cytoskeletal proteins and to affect cell adhesion and spreading onto immobilized ligand [91].

The F754 in the membrane-proximal NPLF sequence is a key residue for binding to ICAM-1 as the F754A mutation inhibits adhesion to ICAM-1 in CHO cells and spreading without affecting receptor recycling [91]. The conserved T758TT motif and residue F766 are also important for binding to ICAM-1 [61,88,92]. As these sites are well conserved in the β_1, β_3, and β_7 integrin subunits, they may be of broad importance in regulating adhesiveness of integrins [88].

On the other hand, CD18 is phosphorylated in response to PMA, whereas the corresponding α subunits (CD11 a–c) are constitutively phosphorylated, suggesting that phosphorylation of the CD18 subunit is responsible for the increased avidity of integrins on stimulated cells [93–95]. Mutation of all the potential phosphorylation sites in the cytoplasmic domain of the CD18 together with the phosphoamino acid analysis of the CD18 from wild-type PMA-stimulated cells has allowed the identification of S756 as the major residue phosphorylated in response to PMA [88]. Although the most obvious protein kinase responsible for PMA-induced phosphorylation is protein kinase C, the sequence surrounding S756 would not be predicted to be a good potential protein kinase C phosphorylation site [96] and it may be phosphorylated only under the influence of the potent activator PMA, or by a second protein kinase that is itself activated by protein kinase C [88]. However, residues that influence phosphorylation of S756, presumably by forming part of the kinase recognition site or contributing to its formation, have been identified. So, residues NH2 terminal to K755, as well as T758 but not T760 contribute to PMA-stimulated phosphorylation of S756 [88]. Furthermore, multiple mutations demonstrate that serine 756 phosphorylation can be dissociated from phorbol ester-stimulated binding of LFA-1 to ICAM-1 because a dramatic increase in ICAM-1 binding in response to PMA could be observed with these mutants [88].

3.2.3. General comparison among species

The general organization of CD18 proteins is quite similar. Sequence comparisons between human CD18 [97,98] and its bovine [99], water buffalo (GenPept AAW29104), ovine

[100], caprine [101], porcine [102], murine [103], canine [104], rat (GenBank XM_228072), chicken [105], common carp (GenBank AB031070), and channel catfish [106] counterparts show overall 83%, 83%, 83%, 83%, 83%, 81%, 80%, 77%, 63%, 49%, and 48% identity, respectively, with the highest identity for the MIDAS-like motif (100%), the cytoplasmic tail, and the I-like domain and the lowest identity for the signal peptide, the PSI domain, and the EGF domains 3 and 4 (Table 4). The very high interspecies conservation of the putative MIDAS-like and I-like domains and the cytoplasmic tail is consistent with an involvement of these regions in the functional activities of β_2-integrins. Overall, the high evolutionary conservation of the I-like domain confirms its importance in β_2-integrins functions, which is compatible with the observation that monoclonal antibodies the epitopes of which were mapped within this region inhibit binding of LFA-1 to ICAMs 1–3 [76]. The maximum conservation being observed for the CD18 MIDAS-like motif is consistent with its importance in ligand recognition [107,108]. The high degree of conservation in the cytoplasmic tail of CD18, with many Ser, Thr, and Tyr residues, is compatible with the important role that phosphorylation of these residues plays in regulating adhesive activity [93] and with the observation that cytoplasmic domain truncation of CD18 markedly diminishes binding of LFA-1 to ICAM-1 [62]. The mature protein contains 56 (58 for the rat) conserved cysteine residues in the extracellular region, which is consistent with a role in maintaining the global structure of the protein. Two additional cysteine residues are present in rat CD18 I domain. Four to 6 N-linked putative glycosylation sites (Asn-X-Thr/Ser) are observed all located within the extracellular region, except carp and catfish that possess one in the cytoplasmic portion.

3.3. Folding

Using conformation-dependent monoclonal antibodies as probes, the folding of particular regions of CD11a [44] and CD18 [109] has been approached and shown to be mutually dependent on the partner subunit. The I domain of CD11a folds before association with the CD18 subunit (as shown by immunoprecipitation of the unassociated CD11a) but by contrast the β-propeller domain requires CD18 for folding because it is not folded in unassociated CD11a after a chase as long as 12 h after synthesis. This suggest that the I domain and the β-propeller domains fold independently of one another and that the latter bears an interface for association with CD18 [44]. Similar results were observed with CD11b [110]. Furthermore, I domain-deleted LFA-1 (CD11a/CD18) and Mac-1 (CD11b/CD18) expressed on the surface of transfected cells bind to all the monoclonal antibodies mapped to the putative β-propeller and C-terminal regions of CD11a and CD11b, suggesting that the folding of these domains is independent of the I domain [111]. In contrast, I-less LFA-1 did not bind its ligands ICAM-1 and -3, whereas I-less Mac-1 is still able to bind iC3b and factor X, in a reduced way compared with wild-type Mac-1, but not fibrinogen. Thus, the I domain is not essential for the folding, heterodimer formation, and surface expression of LFA-1 and Mac-1 but is required for binding to some ligands, but not others [111].

On the other way, folding of the conserved βI domain but not of flanking regions in the CD18 subunit requires association with CD11a, suggesting that this domain is a distinct folding and hence structural unit, and is intimately associated with the α subunit [109].

Table 4
Between-species percent identities of CD18 constitutive blocks

Block	Hum vs.										
	Bov	Buf	Ovi	Cap	Por	Mur	Can	Rat	Chic	Car	Caf
Overall	83	83	83	83	83	81	80	77	63	49	48
Putative signal peptide	63	63	68	63	59	60	30	13	16	29	25
Extracellular region/overall	83	82	82	82	83	81	81	78	64	49	48
Extracellular region/PSI domain	76	76	78	78	75	80	78	76	61	48	55
Extracellular region/I-like domain	94	94	94	93	95	94	94	86	78	64	63
I-like domain/MIDAS-like motif	100	100	100	100	100	100	100	100	100	100	100
Extracellular region/EGF domain 1	81	79	79	81	75	81	77	83	54	37	26
Extracellular region/EGF domain 2	81	81	81	81	81	79	72	77	79	50	52
Extracellular region/EGF domain 3	70	70	70	70	70	73	65	70	58	46	48
Extracellular region/EGF domain 4	77	77	75	75	72	63	66	75	61	44	38
Transmembrane region	86	86	82	86	82	82	82	82	65	60	60
Cytoplasmic tail	95	95	95	95	91	95	89	93	64	52	52

Abbreviations: Hum, human; Bov, bovine; Buf, buffalo; Ovi, ovine; Cap, caprine; Por, porcine; Mur, murine; Can, canine; Rat, rat; Chi, chicken; Car, carp; Caf, catfish CD18.

Furthermore, an activating monoclonal antibody to the C-terminal cysteine-rich region of CD18 preferentially bound to the unassociated β_2 subunit, suggesting that it may bind to an epitope that is in a $\alpha\beta$ interface in unactivated LFA-1 [109].

3.4. Heterodimerization

The extracellular and the cytoplasmic parts of LFA-1 have been both described as key area of dimerization because the α and β subunits each possess retention elements, in the extracellular and the cytoplasmic domains, that keep them intracellularly, until their association allow the CD18-dependent transport to the cell surface [87,89]. Consistent with this interpretation, a LFA-1 mutant lacking the entire cytoplasmic domain of CD11a ($\alpha_L^{\Delta 1088}\beta_2$) is retained partially as an immature dimer in a pre-Golgi compartment, whereas the $\alpha_L^{\Delta 1088}\beta_2^{\Delta 733}$ cotransfectant displays expression levels comparable with the wild-type heterodimer. Furthermore, when the central RN region of CD18, spanning residues D300 to C459, was replaced by the equivalent sequences from β_1 to β_7 to give the chimeras β_2RN1 and β_2RN7, the former construct failed to form heterodimer at the cell surface with CD11a, whereas the latter of these could be expressed together with the CD11a subunit to form a variant LFA-1. The RN region consists of 2 parts, one is the C-terminal end of the putative I domain (RB, residues D300 to A359), and the other the mid-region (BN, residues Y360 to C459). Chimeras exchanging the 2 component regions were made and of the 4 resultant constructs, only the β_2RB1 chimera failed to support LFA-1 expression. Thus, the β_1 specific residues of this region affect the interaction with the CD11a. Whereas the $\alpha_L\beta_2$RB7 LFA-1 variant is wild-type like with respect to ICAM-1 adhesion, the $\alpha_L\beta_2$BN1, and $\alpha_L\beta_2$BN7, as well as the $\alpha_L\beta_2$RN7, variants are more adhesive than the wild type, suggesting that the β_2 mid-region is, in part, required for maintaining the LFA-1 in a resting state [112].

Moreover, a membrane-proximal region of 8 residues (K_{724}ALXHLZD, with X = I in human and T in other species, and Z = T in murine and S in other species) in the CD18 cytoplasmic domain is required for efficient formation and expression of CD11a/CD18 and CD11b/CD18 heterodimers on the cell surface and for maintenance of these integrins in the inactive state [113]. Similarly, the conserved GFFKR motif, located in the membrane-proximal region of CD11a cytoplasmic domain, is required for efficient formation of LFA-1 αβ heterodimers, to ensure stable association of α and β subunits, and probably contributes topologic information for the correct membrane insertion of CD11a [60,62,63,89,114]. As a matter of fact, the CD11a cytoplasmic domain maintains a default low-affinity state in the adhesion receptor, possibly by interacting with CD18 via the GFFKR-containing region [89]. Indeed, complete truncation of the CD11a cytoplasmic domain or internal deletion of this sequence constitutively activates LFA-1 [60,62]. Thus, extracellular and cytoplasmic domain segments of both α and β subunits regulate not only the heterodimer formation and surface expression but control, in part, ligand-binding activity.

4. LFA-1 modulation

As circulating leukocytes are continually exposed to ICAMs on the vasculature, the ligand-binding activity of LFA-1 (CD11a/CD18) has to be kept under tight control to avoid inappropriate adhesion while these cells are flowing in blood or migrating through tissues and thus needs to be highly regulated [115]. The overall strength of cellular adhesiveness (i.e., avidity) is governed by the intrinsic affinity of the individual receptor-ligand bonds, and the number of these bonds (valency). Valency depends on the density of receptor and ligand on the interacting surfaces, the geometric arrangement of those surfaces, and the ability of the receptor and ligand to move, either passively by diffusion or actively, from other parts of the cell into the zone of cell adhesion. The dynamic regulation of integrin-mediated adhesiveness is thought to involve modulation of all of these parameters [116]. So, integrins are dynamic molecules that react to ligand(s) binding but that are also modulated through conformational changes initiated from the cytoplasmic domain to the extracellular ligand-binding sites [38], by internalization [91,117] and cytoskeletal restraint [65,90], leading to the fact that not only can a cell modulate the type and number of integrins expressed on its surface, but also the activation state of the receptors can be altered, allowing flexibility in the specificity and strength of adhesive contact. More, cells often express multiple integrins with sometimes overlapping and/or redundant adhesive properties [3].

4.1. Structural basis of regulation

The CD11a and CD18 subunits form a globular, ligand-binding headpiece connected to the plasma membrane by 2 "stalks" (1 formed by each subunit) that continue by a short cytoplasmic domain, which mediate interactions with the cytoskeleton and with signaling proteins (Fig. 1). Structural analyses has led to a general model in which conformational changes affecting integrin affinity occur after ligand binding or when triggered by

inside-out signaling [116,118,119]. Three affinity states are described: the low, the intermediate, and the high-affinity forms (Fig. 2). In the low-affinity conformation, the stalk region is acutely bent at the genu (knee), with the ligand-binding headpiece in close proximity to the membrane-proximal stalk region as LFA-1 extends only 5 nm over the surface [120]. In this bent conformation, extensive interfaces totaling over 4000 $Å^2$ of solvent accessible surface are buried between the headpiece and tailpiece and between the α and β tailpieces. These interfaces stabilize the bent conformation, and are important in regulating the equilibrium between the bent and extended integrin conformations [121–123]. Indeed, a switchblade–like extension of the stalk regions shifts the molecule to higher-affinity conformation, extending 25 nm over the cell surface and thus becoming accessible to ligand immobilized on a counter surface [120] (Fig. 2). Therefore, it is proposed that global changes occurring in the extracellular domain through extension of the genu, and the presence of the closed headpiece, generate a conformation of intermediate affinity and that separation of the stalks (which is translated through the I-like domain of CD18 into a downward movement of the seventh α-helix of the I domain that alters the affinity of the magnesium-containing ligand-binding site) leads to the high-affinity conformation [50,116,119,124–126]. Downward displacements by 1 or 2 turns of α7-helix lead to intermediate- and high-affinity conformations with ~500- and ~10,000-fold increased affinity, respectively [126]. Moreover, forced separation of the transmembrane and cytoplasmic regions of CD11a and CD18 (that are close to each other in the bent, inactive conformation), occurring following either inside-out signaling or ligand binding [74], can trigger extension and conformational changes in the ligand-binding domain, generating the extended and hence high-affinity conformation [127,128]. It is also clearly established that the membrane-proximal GFFKR motif of the cytoplasmic region of CD11a, which constitutes its hinge domain, functions as a negative-regulatory sequence by suppressing integrin activation [129] as its deletion converts inactive into constitutively active LFA-1. In fact, the arginine residue of this motif forms a salt bridge with an aspartic-acid residue at the corresponding position in CD18, which places both cytoplasmic regions in juxtaposition [119]. Moreover, replacement of the cytoplasmic tails of CD11a and CD18 with complementary α-helices that formed a heterodimeric α-helical coiled-coil kept the receptor in a low-affinity state. By contrast, replacement with non-complementary α-helices resulted in constitutive activation of LFA-1 [113,130].

4.2. Outside-in signaling

The outside-in signaling can be defined as the ligand-induced propagation of intracellular signals, which result from changes in integrin conformation (Fig. 2) or cell surface distribution (Fig. 3) or both [116,131]. The outside-in signal can be triggered via binding of LFA-1 (CD11a/CD18) to outside stimuli (i.e., ICAM-1) that produce a signaling cascade including activation of the tyrosine kinase ζ-associated protein-70 (Zap-70), which initiates the activation of other LFA-1 molecules to form clusters (Fig. 3) [132]. These clusters of LFA-1 with high avidity to multivalent ICAM-1 result in signaling that leads to F-actin polymerization and higher order F-actin bundling [133,134]. In fact, LFA-1 receptor clustering does not precede ligand binding, and instead functions in adhesion strengthening after binding to multivalent ligands. Indeed, (1) stimuli that activate adhesion through LFA-1 fail to alter

on LFA-1 [65,89,90] because it is known that the CD18 cytoplasmic region can associate with talin, filamin, vinculin, and α-actinin [90,151]. Indeed, in unactivated nonadherent leukocytes, the β_2 integrins mobility is constrained by the actin cytoskeleton via the protein talin. Activation of cells induces proteolysis of talin and dissociation from the CD18 tail in a transient phase that results in free mobility of the integrins in the membrane, followed by rapid reattachment to actin filaments as a result of α-actinin binding to a previously cryptic binding site in the membrane proximal half of the cytoplasmic domain [90]. The association of α-actinin with clustered integrins [152] may stabilize the cytoskeleton and promote firm adhesion to and migration across the endothelium. Distinct domains that regulate binding to α-actinin have been identified: a region between residues 736 and 746 in the membrane proximal half of the tail is necessary and sufficient for α-actinin binding, a distinct region in the membrane distal end of the tail has a unique role in regulating α-actinin binding and an inhibitory region resides between residues 748 and 769 and prevents association of α-actinin to the membrane-proximal binding region [90]. Moreover, the ability of point mutations at any of several residues in this inhibitory domain (residues N748, N749, D750, P752, T758, T759, T760, M762, and Ala767) to apparently unmask the previously cryptic α-actinin binding site in the CD18 tail suggest that the conformation of the tail is important in regulating α-actinin binding [90]. In addition, the Arg733–Lys742 residues seem to be critical not only for the cytoskeletal association, but also for endoplasmic reticulum retention, assembly, and transport to the plasma membrane of the mature LFA-1 [89].

5. Conclusion

LFA-1 plays a central role in cell adhesion and migration. In this context, the receptor needs to be tightly regulated, which occurs through conformational changes and controlled association with the cytoskeleton and various proteins. Exploring this stimulant research field consequently open new avenues to deal with the numerous disorders associated to LFA-1.

6. Summary

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. Among these receptors is LFA-1 (CD11a/CD18, $\alpha_L\beta_2$) that actively contributes to the complex and well-orchestrated molecular interactions responsible for normal and pathologic functions of the immune system.

LFA-1 is made of the association of the CD11a and CD18 subunits, whose general organization is well conserved among species, 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 signaling proteins.

Moreover, LFA-1 needs to be tightly regulated and is so a dynamic molecule that reacts to ligand(s) binding but that is also modulated through conformational changes initiated from the cytoplasmic domain to the extracellular ligand-binding sites, by internalization and/or cytoskeletal restraint, leading to the fact that not only can a cell modulate the type and number of integrins expressed on its surface, but the activation state of the receptors can be altered, allowing flexibility in the specificity and strength of adhesive contact.

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Key roles of LFA-1 in leukocyte migration and immune response

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Abstract

To patrol the body effectively for infectious organisms, the cells of the immune system must both circulate as nonadherent cells in the blood and lymph and, in the presence of a foreign antigen, be able to congregate in lymphoid organs, cross endothelial and basement membranes to aggregate at sites of infection, and adhere to cells bearing foreign antigen. In this context, lymphocyte function-associated antigen-1 (LFA-1, $\alpha_L\beta_2$, CD11a/CD18) plays a critical role (1) in forming stable bonds with counter receptors in the vascular walls to allow leukocytes to leave the circulation and (2) in validating the interaction between an antigen presenting cell and a T lymphocyte. Both roles are described in detail in this review.

Keywords: LFA-1; Integrin; Adhesion; Migration

1. Introduction

Lymphocyte function-associated antigen-1 (LFA-1), also known as CD11a/CD18 or $\alpha_L\beta_2$, is the predominant β_2-integrin, expressed on virtually all leukocytes and many leukocyte-derived cells [1–3]. The CD11a–d/CD18 heterodimers mediate high-affinity adhesion to a variety of cell types that display one or more of the β_2-integrins ligands, intercellular adhesion molecules (ICAMs) (ICAM-1 to -5) [4–19]. The adhesion process mediated is a critical step

Abbreviations: APC, antigen presenting cell; DC, dendritic cell; ICAM, intercellular adhesion molecule; LFA-1, lymphocyte function-associated antigen-1; MHC, major histocompatibility complex; TCR, T-cell receptor.

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of a wide range of immunological activities, including cytolysis of target cells, cross-interaction and cross-stimulation between lymphocytes, phagocytosis of complement-coated targets, neutrophils clearance from inflammation sites, and the regulation of leukocyte traffic between the bloodstream and tissues [20–23].

2. Roles of LFA-1

2.1. Cell migration

Cell migration plays a key role in a wide variety of biological phenomena including leukocyte function and the inflammatory response. Indeed, to patrol the body effectively for infectious organisms, the cells of the immune system must both circulate as nonadherent cells in the blood and lymph and, in the presence of a foreign antigen, be able to congregate in lymphoid organs, cross endothelial and basement membrane barriers to aggregate at sites of infection, and adhere to cells bearing foreign antigen. Hence, rapid transition between adherent and nonadherent states is of key importance to the dual functions of immune surveillance and responsiveness [2,24].

T-cells are at the heart of most adaptative immune responses by performing many functions, the nature of which depends upon their maturation and activation status [25]. T-cell function and migration are in fact 2 sides of the same coin [26] and the latter process is known to be LFA-1 dependent, though $\alpha_4\beta_1$ and $\alpha_4\beta_7$ are also involved [21]. Typically, T-cells respond to pathogens only on direct contact with pathogen-derived antigen(s), and must thus migrate to sites where antigens are found. But, as antigens occur in countless shapes and forms, the immune system has to counter this diversity by generating a large army of combat-ready T-cells, each with a unique T-cell receptor (TCR) [26]. Initially, naïve T-cells (T-cells newly emigrated from the thymus that have never encountered antigen) must determine whether antigen is present and whether it poses a threat to the body. This information is provided by dendritic cells (DCs) that in the periphery capture and process antigens, express lymphocyte costimulatory molecules, migrate to lymphoid organs (the spleen and the lymph nodes), secrete cytokines to initiate immune responses, and also tolerize T-cells to antigens that are innate to the body (self-antigens), thereby minimizing autoimmune reactions [27]. Naïve T-cells migrate preferentially to these lymphoid tissues in search of antigen presented by DCs, a process referred to as homing [28], where an encounter with an antigen induces the formation of T-cell clones, yielding approximately 1000 times more descendants with identical antigenic specificity [26]. Eventually, these activated lymphocytes acquire effector functions and home to sites of inflammation, where they interact with antigen-bearing parenchymal cells and leukocytes such as (1) macrophages and neutrophils, or (2) eosinophils, mast cells, and basophils, in reactions, respectively, induced by the $CD4^+$ T helper lymphocytes 1 or 2. Their polarization, based on their profile of cytokine production, is determined by both environmental and genetic factors that act in concert. T helper lymphocyte 1 cells (which produce interferon-gamma, interleukin-2, and tumor necrosis factor-beta) evoke cell-mediated immunity and phagocyte-dependent inflammation, whereas Th2 cells (which produce interleukin-4 to -6, -9, -10, and -13) evoke strong antibody responses (including those of the Immunoglobulin E class) and eosinophil accumulation, but inhibit several functions of phagocytic cells [29].

Other effector cells orchestrate humoral responses by contacting activated B cells in lymphoid organs or persist as circulating memory cells that can confer protection by giving, upon secondary challenge, a qualitatively different and quantitatively enhanced response [26].

Because flowing blood quickly dislodges cells that touch the vessel wall by extreme fluid dynamics, T-cells need adhesion receptors to form stable bonds with counter receptors in the vascular wall and to display tissue-specific recognition signals. Accordingly, leukocytes engage several sequential adhesion pathways to leave the circulation. Initially, tethers are formed by selectins that are specialized to intervene rapidly and with high tensile strength [30,31] but, as the flowing blood exerts pressure, these selectin–mediated adhesion bonds dissociate at the cell's upstream end and new bonds form downstream, resulting in a rolling motion that is much slower than that of free-flowing cells [26,32]. To stop rolling, cells must recruit additional receptors belonging to the integrin family, specifically LFA-1, $\alpha_4\beta_1$, and $\alpha_4\beta_7$ [26,33–36]. Selectins are constitutively active but integrins become activated when rolling cells receive signals from chemokines on endothelial surface. Chemokines are secreted polypeptides that bind to heparin-like glycosaminoglycans on cell surfaces and in the extracellular matrix, which transmit signals through G proteins [26,36–40]. Actually, they act as signal lamps for trafficking of lymphocytes at the important crossing points of lymphoid tissues. As a result, T-cells adhere to the endothelium and undergo polarization, with the formation of a lamellipodium at the leading edge and a uropod at the trailing edge, allowing them to convert cytoskeletal forces into net cell-body displacement. A complex system of signal transduction molecules, including tyrosine kinases, lipid kinases, second messengers, and members of the Rho family of small GTPases is thought to regulate these cytoskeletal rearrangements, that in turn promote chemoattractant receptors, integrins, and other adhesion molecules, cytoskeletal proteins and intracellular regulatory molecules to change their cellular localization [41]. However, it is unclear whether the chemokine signal is locally transmitted at the endothelial contact zone or whether the rolling leukocyte accumulates successive chemokine signals to reach a threshold global activation. Recent in vitro and in vivo data suggest that the induction of high integrin avidity by endothelial chemokine-transduced G(i)-signals is a general mechanism that has evolved to locally enhance integrin avidity to ligand within subseconds at restricted leukocyte-endothelial contacts. In addition, a second specialized mechanism, involving stepwise signals integrated by selectin ligands on rolling cells, seems to activate integrins on the entire leukocyte surface. This G protein–coupled receptors–independent and much slower pathway (10^1–10^2 s) is transmitted through rolling engagements of neutrophils, primarily on E-selectin. It has been proposed that these 2 mechanisms are differentially used by distinct leukocyte subsets at various vascular beds, providing much larger combinatorial diversity of integrin activation on rolling leukocytes than previously predicted [36].

Accordingly, many features of the migrating T-cell can be mimicked in vitro through the binding of active LFA-1 on T-cells to immobilized ICAM-1. Within 1–2 min of contact, T-cell polarization is evident and within 2–3 min, the T-cell becomes motile and migrates randomly (by switching low and high motility behavior) with an average speed of 10.3 μm/min enabling rapid initiation of the immune response [42,43]. These events are critically dependent on dynamic changes to the actomyosin cytoskeleton under the regulation of

myosin light chain kinase operating at the leading edge and Rho-associated kinase involved in the retraction of the trailing edge, both processes operating coordinately to cause forward migration of a T-cell [43]. Chemokines are quite likely to be the key molecules directing migration of leukocytes that involve cell polarization with generation of specialized cell compartments [44] as membrane receptors associated with adhesion move either to leading or trailing of the cells [26]. For example, the chemokine receptors CCR2 and CCR5 localize at the leading edge providing stimulating signals for integrins and directional clues [44], whereas ICAM-1/ICAM-3, CD43, and CD44 move to the uropod [26,45]. The GTPase family member RhoA required in trailing end detachment and hence cell migration has been well-documented for leukocytes [43,46–48]. Rho-associated kinase that is known to have many potential downstream effectors [21,49], also prevents detachment at the rear of the migrating T-cell [43].

Furthermore, in vitro flow chamber models showed very recently that endothelium-presented chemokines triggered instantaneous extension of bent LFA-1 (in the absence of ligand engagement) that primed the integrin to a subsequent ICAM-1–induced activation of the I domain, which is immediately needed to support lymphocyte adhesion [50]. The unfolded LFA-1 is predicted to extend 25 nm over the cell surface, compared with the inactive folded state, which extends only 5 nm over the surface and is practically inaccessible to ligand immobilized on a "counter surface" [51]. Notably, soluble chemokines failed to promote this critical integrin extension and therefore poorly stimulated adhesiveness under shear flow [50]. According to Shamri et al., LFA-1 activation under shear flow in lymphocytes is an abrupt and spatially confined event that does not depend on a prior chemokine encounter during selectin-mediated rolling. Thus, stepwise activation of LFA-1 by chemokine signals does not seem necessary for firm LFA-1–dependent arrest on vascular [50,52].

2.2. Interactions between T-cells and antigen presenting cells

The mammalian immune system must specifically recognize and eliminate foreign invaders but refrain from damaging the host. This task is accomplished in part by the production of a large number of T lymphocytes, each bearing a different antigen receptor to match the enormous variety of antigens present in the microbial world. However, because antigen receptor diversity is generated by a random mechanism, the immune system must tolerate the function of T lymphocytes that by chance express a self-reactive antigen receptor. Therefore, both T and B cells are selected during development against overt self-reactivity although this process is not unequivocal as autoreactive cells can escape deletion and, in some cases, lead to autoimmune diseases. Central to the recognition of self- and nonself antigens is the interaction, within the lymph node, of antigen-specific T-cells and professional antigen presenting cells (APCs) (DCs, macrophages, and B lymphocytes) that present an antigenic peptide bound to cell surface major histocompatibility complex (MHC) proteins. These latter molecules are divided into 4 structurally different classes [53], the main of which being MHC class I and II receptors. MHC class I receptors are recognized by cytotoxic $CD8^+$ T-cells or killer T-cells and present peptides that are derived from pathogen proteins generated inside the APC (i.e., virally infected or cancerous cells), whereas MHC class II receptors present peptides usually derived from pathogens taken up from outside the APC

that are recognized by $CD4^+$ T helper 1 and T helper 2 cells. These cells help to activate B cells to produce antibodies and macrophages for intracellular killing of pathogens harbored inside target cells [54,55]. Primary immune responses are initiated by specific physical interaction of antigen-specific T-cells and APCs, forming a contact zone termed the "immunological synapse" [56–59]. Both this antigen-specific interaction (signal-1) and a costimulatory signal (signal-2, caused by interactions of several different pairs of molecules at the interface between T-cells and APC, including the interaction between LFA-1 on T-cells and ICAM-1 on the APC), are necessary to activate naïve T-cells during the interaction with the APC [58,60–63]. In fact, MHC-antigen binding to the TCR in the absence of a costimulatory signal not only fails to activate the cell but also leads to a state called anergy in which the T-cell becomes refractory to activation [64]. The source of this secondary signal can influence the type of response generated by T-cells as blocking ICAM-1/LFA-1 leads to a significant increase in Th2 cytokines [65], whereas costimulation by ICAM-1/LFA-1 (1) strongly inhibits interleukin-10 production, which may favor the development of T helper lymphocyte 1 rather than T helper lymphocyte 2 cells [66] and (2) functions as a threshold modulator of T helper lymphocyte 2 cell differentiation by increasing the effective concentration of interleukin-4 required to drive T helper lymphocyte 2 cell responses [67]. By inhibiting ICAM-1/LFA-1 interaction, T-cells are prevented from firm adhesion to epithelial, endothelial, or APC cells and are thus prevented from taking part in the immune response [54].

Productive interactions between T-cells and APCs seem to be a dynamic process that combines physical binding with vigorous crawling across and scanning of the APC surface, resulting in signal induction. The T-cells maintain vigorous migration upon cognate interactions to DCs, continuously crawl across the DC surface, and rapidly detach (median within 6–12 min). These dynamic and short-lived encounters favor sequential contacts with the same or other DC and trigger calcium influx, upregulation of activation markers, T blast formation, and proliferation [68]. After T-cell detachment, subsequent migratory contacts to the same or neighboring DCs allow the accumulation of sequential signals and interaction time [69]. Although DCs are remarkably efficient in evoking T-cell responses with few antigen–MHC complexes (1–100 per DC) [42,70–72], they must first encounter a T-cell with appropriate antigen specificity (one in 10^5–10^6). This presents a "needle-in-a-haystack" problem, in that DCs must rapidly scan a large portion of the T-cell repertoire to establish rare cognate interactions [42]. The mechanisms by which this is accomplished are poorly understood. Chemotaxis has been proposed to guide T-cells toward DC, thereby increasing the likelihood of productive interactions [26,73,74]. However, naïve $CD4^+$ T-cells exhibit random migration in vivo, suggesting that antigen recognition may arise instead through a stochastic mechanism [75,76] and do not migrate collectively as they might under the direction of pervasive chemokine gradients. Instead, they appear to migrate as autonomous agents, each cell taking an independent trafficking path [77]. Furthermore, DCs vigorously extend and sweep their dendrites in all directions, which increase their available surface area, and are clearly active partners in initiating and terminating contacts with T-cells. Because multiple dendrites extend from the DC body and move several times faster than T-cells, DC can survey a large percentage of T-cells in their vicinity as they are able to contact ~5000 T-cells per hour [42]. As each contact lasts ~3 min, each DC would be in contact with ~250 T-cells at any instant, a number which is close to the limit of available surface area on

DC, estimated to ~2400 μm^2, if we consider a mean contact area with a T-cell as ~8 μm^2, thereby restricting the maximum number of simultaneous T-cell contacts to ~300 [42]. As an example of scanning efficiency, assuming that a node contains 100 antigen-bearing DCs, this rate would give a 95% probability of contacting an antigen-specific T-cell present at a frequency of 1 in 10^6 within ~6 h. This simple calculation assumes an even distribution of T-cells that can each be sampled more than once and verifies that a stochastic scanning mechanism is compatible with the kinetics of immune response initiation in vivo. If T-cell/DC interactions were orchestrated by chemokine gradients, T-cells with irrelevant specificity would quickly surround DC, thereby inhibiting scanning efficiency [42].

In the early events at the T-cell contact site following antigen binding, a central zone of LFA-1 with a peripheral ring of TCRs forms. Within minutes, a dynamic rearrangement reverses this structure and the central area becomes occupied by the TCR, a mechanism that allows T-cells to distinguish potential antigenic ligands [58]. Formation of a stable central cluster at the heart of the synapse is a determinative event for T-cell activation, proliferation, and differentiation into effector cells, which can respond rapidly by migrating to locations where there is antigen challenge. These effector cells are of several subsets with functions including killing virus-infected cells, activating macrophages to kill ingested bacteria, and activating B cells to produce antibody [21,58]. The interaction of LFA-1 with ICAM-1 is believed to be a critical event in facilitating the sustained interactions between T-cells and APC that are necessary for T-cell activation [78,79]. Indeed, LFA-1 facilitates T-cell activation by lowering the amounts of antigen necessary for T-cell activation. In the absence of LFA-1, 100-fold more antigen was required for T-cell–APC conjugation and all subsequent events of T-cell activation, including TCR down-regulation, Ca^{2+}-flux, T-cell proliferation, and lytic effector cell induction. Thus, LFA-1 facilitates the functional triggering of TCRs by promoting adhesion of T-cells to APCs but does not affect T-cell activation otherwise [79].

3. Conclusion

This review emphasizes the central roles of LFA-1 in key steps such as leukocyte migration through the vessel wall and elicitation of immune response by APCs. In this context, it clearly appears that the receptor needs to be tightly regulated, either by stimuli coming from outside or inside the cell. The study of these numerous ways to modulate LFA-1 surely constitutes a promising research field.

4. Summary

The LFA-1 (CD11a/CD18, $\alpha_L\beta_2$) receptor is expressed on all leukocytes and mediates the direct association of cells with each other as well as with proteins of the extracellular matrix.

Consequently, LFA-1 plays a central role in key immune functions such as cell migration and interactions between T-cells and APCs. Indeed, the leukocytes that patrol the body effectively for infectious organisms must be able, in the presence of a foreign antigen, to cross endothelial and basement membrane barriers to aggregate at sites of infection, and adhere to cells bearing foreign antigen. In this context, LFA-1 (1) allows rolling cells to adhere firmly to the endothelium and then undergo polarization to cross the vessel wall, and (2) is needed

to strengthen the contact between APCs and T-cells by providing a second signal through the interaction with ICAM-1.

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