The Leukocyte Integrins*

Published, JBC Papers in Press, May 4, 2000, DOI 10.1074/jbc.R000004200

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Integrins on Leukocytes

Leukocytes are marrow-derived cells of diverse form and function that circulate in the blood in a quiescent state of low adhesive- ness before migrating into tissues to defend against invading mi- crobes, participate in immune functions and wound repair, or become fixed extracellular residents. Some, such as T-lymphocytes, circulate and traverse blood, organ, and lymphatic compartments during long cycles of immune surveillance. Others, notably poly- morphonuclear leukocytes (PMNs, neutrophils), are rapid re- sponse cells specialized for acute spatially targeted defensive actions that can be mounted in minutes. Leukocytes are also effectors of pathologic inflammation when their accumulation and actions are disregulated. Integrins on their surfaces, together with other plasma membrane adhesion molecules, are required for interac- tions of leukocytes with endothelial cells and other cell types and with matrix structures (1). The functional state, density, and topography of integrins on leukocytes are regulated by lipid, cyto- kine, and chemokine signaling molecules and by “cross-talk” from other surface adhesion molecules (1–6).

Each class of leukocyte displays a particular pattern of integ- rins that can change in a signal- and time-dependent fashion. For example, resting human T lymphocytes (T cells) express β1, β2, and β7 integrins, but this varies with the subclass and is altered by immune stimulation (5). Freshly isolated human monocytes express β1 and β2 integrins, but their culture and/or differentiation into macrophages changes the pattern and induces αMβ2 (5, 7). Human PMNs, once thought to express only β2 integrins, display β1 and β2 heterodimers and use them in motility and migration (8–10). A common feature, however, is that each leukocyte subtype expresses one or more members of the β2 integrin family. Further, the β2 heterodimers are restricted to cells of the leukocyte lineage. The remainder of this minireview will focus on the structure and function of the β2 or “leukocyte” integrins, which were among the first adhesion molecules to be studied at the molecular level. The most recently identified member of the subfamily, αMβ2, is still being characterized (13–16).

Structure and Distribution

The gene for the β2 chain (M, 95,000) is located in band q22 on human chromosome 21 and encodes a cysteine-rich transmembrane protein with six N-linked extracellular glycosylation sites. The cytoplasmic tail contains sequences critical for inside-out signaling and cytoskeletal association. Cytoplasmic residues are differentially phosphorylated in an agonist-dependent fashion in neutrophils, but the effect on adhesive function is not clear. Each of 56 cysteine residues in β2, including four repeated cysteine motifs, is conserved in the β1, β3, and β2 integrin chains and may be important for a rigid tertiary structure. The extracellular portion of β2 contains a 241- amino acid “I-like” domain near the N terminus (Fig. 1) that is highly conserved in other β subunits and is critical for ligand recognition (17, 18) (see below).

The genes for human α2 (M, 180,000), α4 (M, 180,000), αL (M, 160,000), and αM (M, 150,000) are located in a cluster on chromosome 16 (19). Their sequences are similar with αM, α4, and αL having 60–66% amino acid identity and sharing 35% identity with αL. Each contains a distal N-terminal extracellular “I domain” (signifying “inserted” or “interactive”); also called the “A domain” because of homology to the A motif in von Willebrand factor) of approximately 200 amino acids that is critical for ligand binding (17, 20). It is also present in α2, α4, and αL. The N- terminal extracellular regions of the α subunits include seven repeats that fold into a β propeller configuration. The I domains lie within the third repeat (Fig. 1) and are predicted to be exposed and mobile. The three membrane-proximal N-terminal repeats resemble EF hand Ca2+-binding motifs and are situated on the lower face of the propeller away from the ligand contact sites, where they may contribute to orientation of the propeller and/or interaction with the β2 subunit (20).

The cytoplasmic tails of the α chains are constitutively phospho- rylated in some leukocyte types, but the contribution of phospho- rylation to function is unclear (1). The membrane-proximal cyto- plasmic domains of each α chain contain a GFFKR motif common to all integrin α subunits that putatively serves as a “hinge” that locks the heterodimers into a low affinity conformation in the absence of activating signals and is involved in αβ subunit association (21).

The factors that dictate leukocyte-specific expression of the α and β chains remain incompletely defined (1). In myeloid leukocyte subtypes, specific β2 heterodimers are differentially targeted to subcellular storage granules in addition to the plasma membrane. Cellular activation then leads to translocation of granular β2 heterodimers to the surface. Activation of constitutive surface β2 integrins can occur without treatment of additional heterodimers from granules; treatment of neutrophils with ceramide or cytochalasins dissociates these two events (1, 4). There is differential activation of constitutive surface and newly translocated β2 integrins on migrating PMNs and redistribution of heterodimers to specialized regions of the plasma membrane (1, 22). β2 integrins dynamically associate with other plasma membrane proteins. These interactions alter adhesive and signaling functions (1) (see the last minireview in this series by Woods and Couchman (83)).

Ligands

Each of the β2 integrins recognizes one or more members of the intercellular adhesion molecule (ICAM) family. αMβ2, α4β1, and

* This minireview will be reprinted in the 2000 Minireview Compendium, which will be available in December, 2000. This is the third article of four in the “Integrin Minireview Series.” This work was supported by National Institutes of Health Grants HL44525, RO8 HL03799, CA59548, and P50 HL50153, an Asthma Research Center funded by the American Lung Association, the Eccles Program in Human Molecular Biology and Genetics, and the Huntsman Cancer Foundation.

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1 The abbreviations used are: PMN, polymorphonuclear leukocyte; ICAM, intercellular adhesion molecule; MIDAS, metal ion-dependent adhesion site; PI, phosphatidylinositol; PKC, protein kinase C; PH, pleckstrin homology; FAK, focal adhesion kinase; LAD I, leukocyte adhesion deficiency type I.

2 The β2 integrins are comprehensively reviewed in Ref. 1. In the last decade dozens of reviews and hundreds of primary reports on leukocyte function, leukocyte integrins, and specific aspects of the biology of integrins have appeared. We used many that could not be cited because of space limitations and will provide a list of these on request.

3 The designations for the β2 integrin heterodimers and their individual peptide subunits can be confusing. The trivial names LFA-1, MAC-1, and GP150,95 were used when the more recent designations of αLβ2, α5β1, and α6β1 respectively are still often used. αMβ2 integrin was also earlier called MO-1 and complement receptor 3 (CR3). The CD designations for the individual subunits are CD11a (α2), CD11b (αM), CD11c (αL), and CD18 (β2). The αMβ2 chain will likely be assigned as CD11d (1).

4 M. Feldhaus, G. A. Zimmerman, and T. M. McIntyre, submitted for publication.
the α propeller in concert with simultaneous conversion of the α subunit I domain to an active conformation via tertiary changes (28). Thus, dynamic structural alterations in β2 heterodimers are involved in ligand recognition, as with other classes of integrins (see the first minireview in this series by Plow et al. (84)). Modulation of avidity is also involved (see below).

**Inside-out Signaling**

Activation of leukocytes by agonists that bind to diverse classes of receptors triggers ligand recognition by β2 integrins. This process is termed "inside-out signaling," integrin "activation," and "functional up-regulation" (1). Rapid, regulated modulation of ligand recognition is critical for leukocytes, because they must circulate in a non-adhesive state before targeting and arrest at specific sites. The molecular mechanisms that mediate inside-out signaling of integrins have been elusive (see the second minireview in this series by Ginsburg and co-workers (85)). More than one pathway may trigger inside-out signaling of an individual β2 integrin heterodimer (29, 30). Transfected and mutated cell systems are now popular for analysis of integrin signaling (30–32). Such models suggest that inside-out signaling of β2 integrins occurs via mechanisms dependent on the small GTPase Rho (33, 34) and that there is differential intracellular regulation of the activity of β2 versus β1, β3, and β2 integrins in the same transfected cell type (35–37). There are cell-specific aspects of integrin regulation, however, that may operate in model cell systems but not in primary leukocytes and vice versa (38).

Analysis of inside-out signaling of β2 integrins is most detailed for α5β2. Changes in both affinity and avidity are involved, depending in part on the cellular system and the stimulus chosen to alter its adhesive function. Low and high affinity states of α5β2 occur on lymphocytes (39) and other leukocytes (1). Manipulation of the "extracellular face" of α5β2 using divalent cations and function-perturbing antibodies induces rapid dynamic changes in recognition of ICAM-1 and other ligands (1, 40). Recent experiments with soluble recombinant peptides based on the I domain of α5 indicate that this region is required for ICAM-1 recognition when α5β2 shifts to the high affinity state on cultured T cells treated with Mg2+ (41). In contrast, treatment of T cells with phorbol esters or agents that increase intracellular Ca2+ causes clustering of α5β2 and increased avidity of binding without detectable change in affinity (41–44). Lateral motion of α5β2 in the plasma membranes of Epstein-Barr virus-transformed lymphoblasts is enhanced by phorbol esters and by cytochalasins, suggesting that conversion from the non-adhesive to the adhesive state involves signals that alter cytoskeletal interactions and allow the integrin to segregate into clusters or adhesive patches (45). Clustering of membrane "rafts" containing α5β2 occurs in murine thymocytes and activated T cells and confers adhesion to ICAM-1 (46). There is additional evidence for avidity modulation of α5β2 in surrogate cell models (47, 48). Lateral segregation of α5β2 in the plane of contact occurs in T cells adherent to cellular targets or purified proteins in model membranes (49, 50). Differential regulation of affinity versus avidity of α5β2 is triggered by specific signals and may involve a two-step mechanism in which increased affinity, which can be induced by ICAM-1 and potentially by other binding partners, and altered avidity occur in sequence (41, 48).

Truncations, mutations, and deletions of the GFFKR motif of αL (Fig. 1) in a Jurkat T cell line and in K562 transfectants cause constitutive ICAM-1 recognition; there is also additional evidence that the membrane-proximal region of the α cytoplasmic tail exerts a general inhibitory effect on ligand recognition that is independent of the β1 chain (1, 21, 30, 51). The β2 cytoplasmic tail is, however, critical for modulation of α5β2 adhesive strength (1, 38, 47, 51). The β2 chain associates with a variety of cytoskeletal and regulatory proteins, including α-actinin, talin, filamin, vinculin, and Rack 1. Certain of these interactions have been demonstrated to be altered when leukocytes of different classes are activated by physiologically relevant stimuli and to regulate α5β2 function (1, 52). Inside-out signaling of α5β2 on B lymphoblastoid cells is regulated by Rho acting downstream of protein kinase C (PKC) (52), consistent with studies in transfected cells mentioned above.6

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6 PI 3-kinases, PKC, and integrins are reviewed in Kolanus and Seed (86).
A member of the Sec7 family, cytoshein-1, modulates αb2 in lymphocytic and monocytic cell lines (35, 54–56). Cytoshein-1 was identified using the intracellular domain of β2 in a yeast two-hybrid screen; it has a C-terminal pleckstrin homology (PH) domain and an N-terminal motif similar to the yeast Sec7 domain, conferring membrane association and guanine nucleotide exchange factor activity (35, 54). Cytoshein-1 weakly coimmunoprecipitated with αb2 in lysates of a Jurkat T cell line, but not with αb2 (35). Overexpression of cytoshein-1 or its Sec7 motif in Jurkat cells induced αb2-dependent adhesion to immobilized ICAM-1 without increasing αb2 heterodimers on the surface, whereas overexpression of the PH domain inhibited adhesion stimulated by T cell receptor engagement (35). Expression of a constitutively active chimeric phosphatidylinositol 3-kinase (PI 3-kinase) (PI 3-kinase) induced adhesion of Jurkat cells to ICAM-1 and increased membrane association of cytoshein-1, both of which were inhibited by overexpression of the PH domain but not by a control PH motif (54). This suggests that PI 3-kinase regulates recruitment of cytoshein-1 to the plasma membrane utilizing the PH domain where it mediates inside-out signaling of αb2, potentially via the Sec7 domain (54, 56). Additional evidence in other systems indicates that signals delivered via PI 3-kinases activate integrins, potentially converging with signals from PKC.8 It is unknown if inside-out signaling of αb2 on primary leukocytes is mediated by PI 3-kinase-dependent cytoshein-1 translocation. A recently identified factor that is structurally similar to cytoshein-1, GRP1, also regulates αb2 in leukocyte cell lines (57).

Inside-out signaling and ligand recognition of αb2 follows paradigms outlined for αb1 (1, 26–29). Two functional states of the crystallized αb1 I domain indicate a basis for changes in conformation (17, 26, 27). Avidity modulation involving clustering of αb2 or cell spreading also occurs (1, 29, 30, 58). L-plastin, an actin-organizing protein, regulates αb2b3 on human monocytes and neutrophils based on experiments with cell-permeant peptides (59). In human neutrophils, inside-out signaling of αb2b3 was dependent on PI 3-kinase and actin cytoskeletal reorganization when triggered by engagement of cell-surface receptors like TNF-α receptor I when the 2 integrin is activated through the receptors for inflammatory peptides (29). An inhibitor of PI 3-kinase also failed to block αb2b3-dependent adhesive interactions of neutrophils in earlier experiments (60). These findings are consistent with differential regulation of αb2b3 and αb1 via the α cytoplasmic tails (30) and also suggest alternative mechanisms for signaling of cytoshein-1 interaction with the cytoplasmic tail of β2 if it is central to activation of αb2b3, as proposed for αb1 (see above). Pak1, a serine/threonine kinase, may be an intermediary in a pathway leading to inside-out signaling of αb2b3 in neutrophils (29).

**Outside-in Signaling**

Engagement of integrins delivers outside-in signals that trigger intracellular transduction cascades, in addition to mediating adhesion. These outside-in signals can also be integrated with signals delivered through receptors for signaling molecules to yield coordinated functional responses. Cross-linking of leukocyte integrins with antibodies against β2 or specific α subunits, engagement of β2 heterodimers with specific ligands, or engagement of β2 integrins together with other surface structures or receptors delivers outside-in signals that lead to diverse cellular responses (1).2 Signaling to gene regulatory pathways involves transcriptional events and mRNA stabilization and is differentially affected by experimental conditions when β1 versus β2 integrins are engaged (61–64). Outside-in signaling by specific β2 integrins may vary in leukocyte subtypes (65) and is impaired in leukocytes from subjects with leukocyte adhesion deficiency type I (LAD I) (see below) (66, 67).

Intracellular signaling pathways are triggered by engagement of β2 integrins (1). Activation of focal adhesion kinase (FAK) is central to many paradigms of outside-in signaling by integrins, and FAK binds peptides based on the sequence of the β2 cytoplasmic domain in addition to β1 and β2 cytoplasmic peptides (68). Nevertheless, FAK does not appear to be critical for outside-in signaling by β2 integrins, and it is inconsistently detected in human monocytes and neutrophils (63, 69) even though phosphorylation on tyrosine occurs in these cells in response to β2 integrin engagement (1, 67). In contrast, the human THP-1 monocytic leukemia cell does express FAK (70), illustrating the potential difference in integrin signaling in primary leukocytes versus transformed cell lines.

Integrin-linked kinase, a serine/threonine kinase that associates with β2 cytoplasmic domains, and calreticulin, which associates with the GFPPKR sequence of α subunits, mediate signaling functions of integrins (71, 72), but it is not known if they regulate β2 heterodimers.

**Genetically Altered Animal Models**

Mice with partial (73) and complete (74) deficiency of β2 integrins and specific deletions of αb2b3 (75, 76, 77), and αb2b6 have been produced. Animals deficient in all β2 integrins display phenotypic features of humans with LAD I (see below) including neutrophilia and a defect in accumulation of PMNs in inflamed skin. PMN emigration into lung alveoli in response to a bacterial challenge and into the peritoneum in response to a sterile irritant are preserved (74). Blocking antibodies against β2 integrins inhibit neutrophil accumulation under the same or similar conditions (1, 74), however, suggesting alternative adhesion mechanisms that compensate for deficiency of β2 integrins in mice. In contrast, absence of PMNs from inflamed or injured extravascular sites is the usual outcome in most humans with β2 integrin deficiency (1).

**LAD I and LAD I Variant Syndromes**

Humans with LAD I have absent or a greatly reduced display of all β2 integrin heterodimers on the surfaces of their leukocytes, absent or dramatically reduced accumulation of PMNs and monocytes at extravascular sites, recurrent life-threatening bacterial infections, and impaired tissue remodeling and wound healing. Cells from these subjects have defective adhesive and signaling functions when studied in vitro (1, 2, 66, 67). The search for the molecular basis of LAD I led to identification and initial characterization of β2 integrins (1). The syndrome results from a variety of mutations that prevent normal heterodimer formation and surface display. Recently, variant LAD I syndromes have been identified (80, 81).7 Each of these subjects had clinical features consistent with LAD I but, in contrast to the phenotype outlined above, had normal or only moderately reduced levels of β2 integrins (40–60% of control) on the surfaces of circulating leukocytes at the time of diagnosis. In one of the subjects there were two new mutations of the β chain; one was located in the MIDAS motif of the I-like domain (81). Phenotypic and sequence characterization of leukocytes from the other two subjects indicates a defect in inside-out signaling. These rare LAD variants, like variations in structure and signaling of integrin αb2b3 in the syndrome of Glanzmann thrombasthenia (82), may yield unique insights that are relevant both to β2 heterodimers on leukocytes and to the biology of integrins in general.

**Acknowledgments**—We thank Takashi Kei Kishimoto and Nancy Hogg for making unpublished manuscripts available to us, John McDonald for editorial comments, Michelle Bills and Diana Lim for preparation of the manuscript and figure, and the members of our group and many other colleagues for useful discussions and other contributions.

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