The dynamic regulation of integrin adhesiveness

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The integrins are a family of transmembrane heterodimeric adhesion molecules that play important roles in wound healing, immune system function and organ development. Recent studies indicate that adhesion of integrins to their ligands is not constitutive but is dynamically regulated by intracellular signal transduction pathways.

Introduction

Cells adhere to each other and to substrates by way of specific, regulated biophysical interactions that govern the most fundamental biological processes, including development and differentiation. Several families of molecules have evolved to serve specialized adhesive functions in tissue organization and cell migration. One of these families, the integrins, functions in cell–cell and cell–substrate adhesion and communication, and is critical in the function of the immune system, in wound healing, tumor invasion and metastasis, and in embryogenesis. The importance of this protein family is underscored by the discovery that mutation of individual integrins in humans, mice or lower organisms results in developmental arrest or profound, debilitating, clinical consequences.

Integrins must be activated by cells in order to bind to the appropriate ligands; by itself, expression of an integrin on the cell surface is inadequate for adhesion. Within minutes of receiving an inflammatory stimulus, the integrins that are expressed on the surfaces of leukocytes acquire, through still poorly defined signal transduction pathways, the ability to attach to cell-surface and extracellular ligands. In some cases, the activation is transient, so that within minutes the integrins lose their adhesiveness. This dynamic cycling between adhesive and non-adhesive states endows a cell with the ability to regulate rapidly adhesion to ligands on apposing cell surfaces and matrices. This ability may be important in cell movement, which requires a rapid flux of adhesive interactions. To date, regulation of an integrin's binding strength for ligand has been demonstrated with integrins of most families. This review explores the current state of our knowledge of the biochemical and structural changes that permit an integrin to switch to a state in which it is competent to bind to a ligand.

In humans, the integrin family of glycoproteins consists of more than 20 non-covalently bound αβ heterodimers [1,2]. One or more integrins are expressed on the surface of every type of cell in the body except for mature erythrocytes [3]. At present, 14 α subunits and 8 β subunits have been identified in humans by biochemical or genetic means (Table 1; see also [2]). If individual α subunits associated freely with any β subunit, a large repertoire of integrins would be generated on each individual cell type. In reality, the diversity is restricted, as most α subunits are found to associate with only one type of β subunit; thus, subfamilies are designated according to individual β subunits. However, some α subunits — α^4, α^6 and α^v — associate promiscuously with multiple β subunits.

Integrin α subunits have 950–1100 amino-acid extracellular domains, and fall into three categories on the basis of structural motifs (Fig. 1). Members of one group — α^{IIb}, α^5, α^6, α^v and α^3 — have four putative divalent-cation binding sites, each about 60 amino acids long, and a protease-cleavage site. Members of a second group — α^M, α^L, α^X, α^1 and α^2 — each have three putative divalent-cation-binding repeats, lack a protease-cleavage site, and have a 180–200 amino-acid insert, the I domain, which is not found in other integrin α subunits. The I domain is homologous to domains found in several other proteins, namely von Willebrand factor, cartilage matrix protein and the complement regulatory proteins, factor B and C2, and it has recently been shown to contain a divalent-cation-binding site [4] and to participate in ligand recognition by β_2 integrins [4–6]. The α^4 integrin subunit is the only member of the third group of α subunits: it has three putative divalent-cation-binding repeats, lacks an I domain, and has a distinct protease-cleavage site [7]. The cytoplasmic domains of α subunits are short, ranging from 15 to 77 amino acids, and in general are not strongly related to one another at the sequence level, though each is well conserved across species [8].

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Table 1. The integrin superfamily.

Integrin	Characterized ligands	Recognition sequence or domain*	Distribution
$\alpha^1\beta_1$	Collagen I, collagen IV, laminin	DGEA (collagen)	Broad
$\alpha^2\beta_1$	Collagen I, collagen IV, laminin	RGD (?)	Broad
$\alpha^3\beta_1$	Laminin, collagen I, fibronectin, epiligrin	Domain 1 and 4 (VCAM-1), EILDV (CS-1, fibronectin)	Broad
$\alpha^4\beta_1$	VCAM-1, fibronectin	RGD	B and T lymphocytes, macrophages, neural crest cells
$\alpha^5\beta_1$	Fibronectin		Broad
$\alpha^6\beta_1$	Laminin		Broad
$\alpha^7\beta_1$	Laminin		?
$\alpha^8\beta_1$	?		?
$\alpha^V\beta_1$	Vitronectin, fibronectin	RGD (fibronectin)	Epithelial cells
$\alpha^L\beta_2$	ICAM-1, ICAM-2, ICAM-3	Domain 1 (ICAM-1)	Leukocytes
$\alpha^M\beta_2$	iC3b, ICAM-1, fibronectin, factor X	Domain 3 (ICAM-1), 30kD plasmin fragment (fibrinogen)	Granulocytes, macrophages, natural killer cells, cytotoxic T lymphocytes
$\alpha^X\beta_2$	Fibrinogen, iC3b	GPRP (fibrinogen)	Macrophages, granulocytes, activated B lymphocytes
$\alpha^{IIb}\beta_3$	Fibrinogen, fibronectin, vWF, vitronectin, thrombospondin	RGD, KQADGV (fibrinogen)	Platelets
$\alpha^V\beta_3$	Vitronectin, fibrinogen, vWF, thrombospondin, fibronectin, osteopontin, collagen	RGD	Endothelial and tumor cells
$\alpha^6\beta_4$	Laminin (?), basement membrane protein (?)		Epithelial cells
$\alpha^V\beta_5$	Vitronectin	RGD	Carcinoma cells
$\alpha^V\beta_6$	Fibronectin (?)	RGD	?
$\alpha^4\beta_7$	VCAM-1, fibronectin, MAdCAM-1	EILDV (fibronectin)	Activated B and T lymphocytes, macrophages
$\alpha^E\beta_7$	?		Intraepithelial lymphocytes
$\alpha^V\beta_8$	?		?

*Amino-acid sequences are denoted by the single-letter code. Additional information can be found in previous reviews [2, 3, 16].
Abbreviations: VCAM-1, vascular cell adhesion molecule-1; ICAM, intracellular adhesion molecule; iC3b, inactivated complement component C3; vWF, von Willebrand factor; MAdCAM-1, mucosal addressin cell-adhesion molecule.

However, a membrane-proximal sequence motif, GFFKR (single-letter amino-acid code), is conserved among all α-subunit cytoplasmic domains and is thought to participate in signal transduction or α–β subunit association [8,9].

Integrin β subunits (Fig. 1) have a 675–700 amino-acid extracellular domain, a hydrophobic transmembrane region and a cytoplasmic tail of varying length (the tail is relatively short, 40–60 amino acids, except in the case of β_4, in which the tail is more than 1000 amino-acids long). The cytoplasmic domains of the β_1, β_2, β_3, β_6 and β_7 subunits are strongly homologous in a region in which deletion or mutation affects ligand binding by β_2 integrins [10]. All β subunits have a cysteine-rich domain in the carboxy-terminal half of the extracellular region that has internal disulfide bonds [11] and may contribute rigidity to the molecule [12,13].

Integrins bind to a diverse array of ligands (Table 1), including extracellular matrix proteins, plasma proteins that are deposited at sites of injury in hemostasis (fibrinogen) and complement activation (iC3b), and integral membrane proteins [2,14]. Many integrins bind to more than one of these ligands, and some ligands bind to more than one integrin, using either the same or distinct recognition sites (see [2]).

The functional significance of adhesion by integrins was initially documented in experiments that perturbed integrin function with antibodies or peptide antagonists. More recently, the physiology of integrins has been assessed by the investigation of natural or induced genetic deficiencies of individual subunits that result in an array of pathological sequelae. Integrin-mediated adhesion has functional roles in a wide variety of biological and pathological settings, including hemostasis, inflammation, tumor metastasis and development. For example, in primary hemostasis, platelet attachment to blood vessel walls and aggregation at the site of injury are mediated by integrins. Adhesion and signal transduction by integrins are essential elements of a sequence of intercellular interactions leading to the antigen-specific activation of T lymphocytes [15]. And in inflammation, integrins mediate the critical attachment-strengthening step in the adhesion cascade that permits leukocytes to traffic from the vasculature, across the blood-vessel-lining endothelium, into the parenchyma [16]. The subsequent migration of cells through the parenchyma depends on the transient nature of integrin

adhesiveness, and may require a sequence of attachment to, and detachment from, a ligand by rapidly activated and inactivated integrin sub-populations that are located at the leading and trailing edges of the cells, respectively.

Regulation of integrin function

Dynamic regulation

Adhesion by integrins is dynamically regulated by the cells on which they are expressed. This allows cells that display integrins on their surfaces to convert rapidly from a non-adherent to an adherent phenotype. For example, non-adhesive, circulating leukocytes rapidly respond to inflammatory stimuli by activating integrins and adhering to counter-receptors on the endothelium. This phenomenon can be reproduced *in vitro* [17–21] — $\alpha^L\beta_2$ integrin on the surface of resting T lymphocytes and neutrophils binds only weakly to cellularly expressed or purified intercellular adhesion molecule-1 (ICAM-1), its ligand. This suggests that the affinity for ICAM-1 of $\alpha^L\beta_2$ on unstimulated cells is too low to sustain cell binding in conventional adhesion assays.

Activation of leukocytes with phorbol esters or chemoattractants, or by cross-linking functionally relevant surface receptors — such as the antigen receptor/CD3 complex [17,19], CD2 [19], immunoglobulin [22], or major histocompatibility (MHC) class II molecules [23] — induces $\alpha^L\beta_2$ to bind to ICAM-1 within minutes. In some instances, the increased adhesion is transient, and by 20 minutes after activation, cells lose their ability to bind to ICAM-1 [17]. Regardless of how the cells become activated, the level of surface expression of $\alpha^L\beta_2$ does not change over the time course of the measured increase in adhesion [17,19,24,25]. Thus, a qualitative change in the $\alpha^L\beta_2$ molecule is hypothesized to mediate the change in adhesiveness [17,19].

The integrin $\alpha^M\beta_2$ that is found on the surface of myeloid cells also requires an activation step in order to adhere to its cellular and soluble ligands. Activation of neutrophils with chemoattractants, chemotactic peptides or phorbol esters rapidly induces $\alpha^M\beta_2$ binding to particles coated with the inactivated complement component C3 (iC3b) [26–32], or to fibrinogen [33] or ICAM-1 [34,35]; in the resting state, ligand binding is not appreciably detected. The increase in adhesiveness is transient, as within minutes of stimulation there is a sequential deactivation and reduction in binding to several ligands [27,36,37]. Although the mechanism appears similar to that used in regulating $\alpha^L\beta_2$ on lymphocytes, there is an additional level of regulation: a greater than ten-fold quantitative increase in levels of $\alpha^M\beta_2$ present on the cell surface after stimulation [28,36,38–40]. This dramatic up-regulation occurs within minutes and is the result of the translocation to the cell surface of secretory granules that contain reserve pools of $\alpha^M\beta_2$ integrin [39–42].

The time-course of the increase in the level of $\alpha^M\beta_2$, however, does not parallel the kinetics of adhesiveness

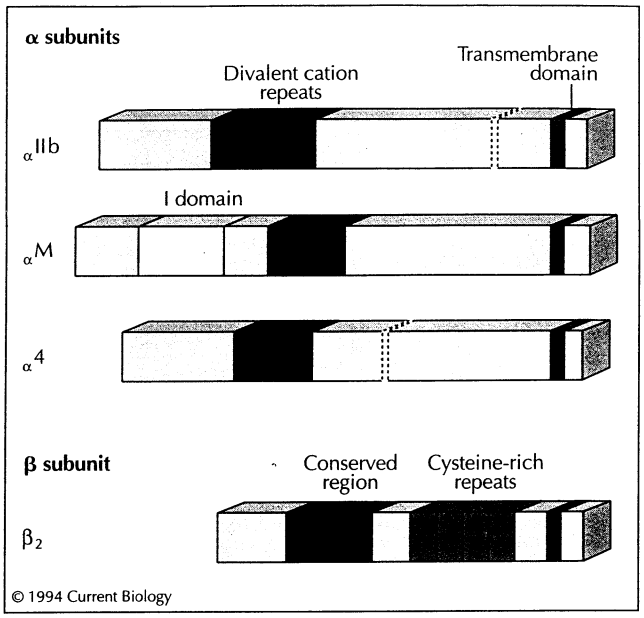

Fig. 1. A schematic representation of integrin α and β subunits. The structures of a representative example of each of the three major classes of integrin α subunits are depicted: α^{IIb} is representative of an integrin that contains four EF-hand repeats (divalent-cation-binding motifs) and a membrane-proximal protease-cleavage site (dotted lines); α^M of an integrin that contains an I domain; and α^4 of its own subclass, with a membrane-distal protease-cleavage site (see text). A representative β subunit (β_2) is shown, with its cysteine-rich and conserved regions indicated.

[36,37], as ion-channel antagonists [43] and temperature conditions [44] that prevent the increase in $\alpha^M\beta_2$ expression do not greatly affect the increase in $\alpha^M\beta_2$-dependent adhesion. Recently, monoclonal antibodies have been described that recognize active forms of $\alpha^M\beta_2$; expression of the 'neoepitopes' that these antibodies recognize requires myeloid-cell activation. Because a change in expression at the cell surface is insufficient to promote adhesion or neoepitope expression [45], $\alpha^M\beta_2$ is believed to require additional, conformational changes in order for adhesion to increase [36,37,43,45,46].

An activation signal is also required for the $\alpha^{IIb}\beta_3$ integrin found on the surface of platelets to bind to its ligand. On resting platelets, $\alpha^{IIb}\beta_3$ does not bind fibrinogen. Injury to the blood-vessel endothelium and the release of inflammatory modulators prompt a rapid arrest and aggregation of platelets that is mediated in part by $\alpha^{IIb}\beta_3$. *In vitro*, the $\alpha^{IIb}\beta_3$ on platelets becomes structurally competent to bind to fibrinogen after exposure to collagen, epinephrine, thrombin, ADP, platelet activating factor (PAF) or thromboxane A_2, with only a small change in receptor-site density [47]. Analogously, $\alpha^{IIb}\beta_3$ integrin expressed on the surface of transfected Chinese hamster ovary (CHO) cells requires a structural change in order to bind fibrinogen, with no requirement for increased surface expression [48].

Hematopoietic cells modulate the ability of their β_1-subunit-containing integrins to adhere to extracellular

and cell-surface ligands; this property may permit their attachment to, and detachment from, the extracellular matrix and endothelial-cell proteins, as occurs during diapedesis and cell migration during inflammation. Cross-linking of the cell-surface molecules CD2 or CD3 on lymphocytes, or treatment with tumor-promoting phorbol esters, increases the adhesiveness of $\alpha^4\beta_1$, $\alpha^5\beta_1$ and $\alpha^6\beta_1$ integrins for laminin and fibronectin [49]. Separate studies have consistent results: $\alpha^2\beta_1$ and $\alpha^6\beta_1$ on murine T-cell clones and human T-cell leukemic lines show increased binding to proteins of the extracellular matrix after the cells have been triggered with antigen or phorbol esters [50]. Additionally, phorbol esters induce the $\alpha^4\beta_1$ integrin on peripheral B cells to bind ligand [51], and $\alpha^6\beta_1$ on macrophages to bind to laminin [52,53]; the chemoattractant MIP-1β induces $\alpha^4\beta_1$ on subsets of naive T lymphocytes to bind to its ligand, vascular cell adhesion molecule-1 (VCAM-1) [54].

Intracellular signal transduction pathways

What are the molecular steps that couple cytoplasmic signal transduction to a change in the ability of an integrin to bind ligand, leading to altered adhesiveness? One approach to addressing this question has been to assess the effect on adhesion of pharmacological agents that block or activate specific protein kinases. Depletion of intracellular Ca^{2+} in T-cell clones, presumed to affect the activity of the Ca^{2+}/calmodulin-dependent protein kinase, prevents the increase in adhesiveness that is triggered by engagement of CD2, CD3 or MHC class II molecules, but has no effect on the adhesion induced by protein-kinase-C-activating phorbol esters [55]. Tyrosine kinase inhibitors strongly block the adhesion triggered by engagement of CD3 and MHC class II molecules, partially lower the adhesion triggered by cross-linking of CD2, but only weakly affect adhesion stimulated by phorbol esters ([55]; and L. Petruzzelli and T.A.S., unpublished observations). These studies suggest that an increase in the intracellular Ca^{2+} concentration and the activation of protein tyrosine kinases are earlier steps in the integrin activation pathway, and protein kinase C activation is a later step in a signal transduction pathway that regulates adhesion by the $\alpha^L\beta_2$ integrin.

Because the time course of β_2-subunit modification in response to phorbol esters parallels the induction of adhesion [17,56], phosphorylation of the cytoplasmic domain of the β_2 subunit by protein kinase C has been proposed to activate $\alpha^L\beta_2$ directly [57–59]. Mutagenesis studies, however, do not support this idea, as substitution of the major phosphorylation site on the β_2 subunit has no effect on adhesion of $\alpha^L\beta_2$ to ICAM-1 [10] or adhesion of $\alpha^M\beta_2$ to erythrocytes coated with the complement protein iC3b ([60] and M.S.D., L.B. Klickstein and T.A.S., unpublished observations). Although mutation of the three threonine residues in the cytoplasmic domain of the β_2 subunit reduces basal binding to ICAM-1, these mutants still respond to phorbol ester treatment with increased adhesiveness [10]; thus, the activation of integrins is dissociable from the direct phosphorylation of the β_2 subunit. Phosphorylation or dephosphorylation of a regulatory protein, instead of the cytoplasmic domain of an integrin subunit, may nevertheless determine adhesiveness [61].

An additional level of complexity arises because cells use many different signaling pathways to regulate individual integrins. Differences in the expression of regulatory proteins among cells may translate into identical stimuli having opposing effects in different cells. For example, in T lymphocytes, staurosporine, an inhibitor of protein kinase C, completely blocks the phorbol ester-induced increase in adhesion due to $\alpha^L\beta_2$, but only partially blocks CD3-triggered adhesion [17]. In contrast, in B lymphocytes, staurosporine paradoxically triggers $\alpha^L\beta_2$ to bind to ICAM-1 [61]. Preincubation of either cell type with the protein-phosphatase inhibitor okadaic acid blocks the adhesion triggered by phorbol esters, but not that triggered by activating monoclonal antibodies (L. Petruzzelli and T.A.S., unpublished observations; and [61]). The apparently opposing roles of protein kinase C in different types of lymphocytes may reflect single or multiple cell-specific isoforms of protein kinase C that serve as positive or negative regulators of integrin adhesiveness. In contrast, in both B and T lymphocytes, unidentified serine phosphatases (possibly protein phosphatases 1 or 2A) activate the adhesiveness of $\alpha^L\beta_2$ at a step downstream of protein kinase C. Clearly, future biochemical and genetic experiments are required to define additional steps on the signal transduction pathways that couple cell activation with a change in integrin adhesiveness.

Structural changes in integrin molecules

Although the intracellular biochemical pathway that activates integrins remains poorly defined, recent experiments indicate that structural changes in the extracellular domains of integrins participate in the change in adhesiveness. Here we discuss evidence that integrins cycle through multiple conformations on the cell surface. We describe several types of immunological and biochemical probes, which either report on or stabilize an activated integrin conformation.

Reporter monoclonal antibodies

Probes that detect physical changes in integrin structure that occur after cell activation are available for the platelet integrin $\alpha^{IIb}\beta_3$ and the leukocyte integrins $\alpha^L\beta_2$ and $\alpha^M\beta_2$ (Fig. 2a). For example, the PAC-1 monoclonal antibody recognizes a neoepitope on the $\alpha^{IIb}\beta_3$ integrin that is present on platelets treated with ADP, epinephrine or thrombin, but not on resting platelets [48,62]. Transfected CHO cells that express the $\alpha^{IIb}\beta_3$ integrin do not bind the PAC-1 monoclonal antibody until a structural change in the integrin occurs [48]; thus, it is hypothesized that PAC-1 binds only to an activated form of the $\alpha^{IIb}\beta_3$ complex. The NKI-L16 monoclonal antibody also reports a change in conformation of an integrin after activation. It recognizes the $\alpha^L\beta_2$ integrin on resting T lymphocytes only poorly [63,64], but binds well to T lymphocytes that are activated with phorbol esters, IL-2 or monoclonal antibodies that cross-link

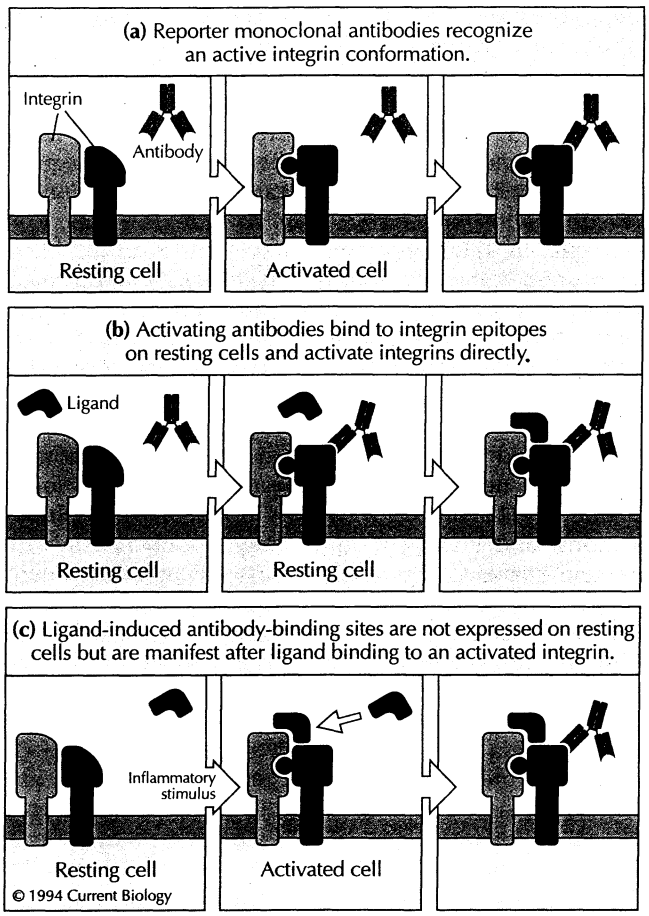

Fig. 2. A model for the different types of structural changes in integrins detected by monoclonal antibodies.

CD3. Our laboratory has generated two monoclonal antibodies that recognize active conformations of $\alpha^M\beta_2$, namely CBRM1/5 and CBRM1/19. These antibodies bind to neutrophils and monocytes stimulated with chemoattractants, cytokines or phorbol ester, but not to resting cells ([45] and M.S.D. and T.A.S., unpublished observations). CBRM1/5 recognizes only 10–30% of $\alpha^M\beta_2$ molecules on activated myeloid cells, but it completely abolishes $\alpha^M\beta_2$-dependent cell adhesion to both ICAM-1 and fibrinogen. Thus, the increase in binding strength of $\alpha^M\beta_2$ may be due to a conformational change in the ligand-binding region on a sub-population of $\alpha^M\beta_2$ molecules that occurs after activation, and it is these structurally competent molecules that mediate adhesion. This hypothesis is supported by a recent study which shows that phorbol esters induce a 15–30% subset of $\alpha^L\beta_2$ molecules on T cells to convert to a high-affinity state that binds ICAM-1 [18].

Activating ligands

Reagents of a second class (Fig. 2b) directly induce integrins to bind ligand in the absence of an activating signal. Although the exact mechanism is unknown, these monoclonal antibodies or peptide ligands presumably promote adhesion by stabilizing an integrin conformation that exposes cryptic ligand-binding sites — this implies that integrins naturally shift among several conformations and that cellular activation or the addition of certain protein ligands preferentially sustains an adhesive state (Fig. 2). Several monoclonal antibodies have been described that directly activate the β_2- and β_1-subunit-containing integrins. KIM 127 recognizes the β_2 subunit and promotes adhesion mediated by $\alpha^L\beta_2$ and $\alpha^M\beta_2$ integrins [65]. NKI-L16 recognizes the α^L subunit and induces homotypic adhesion of B-cell lines and T-cell clones [66], and partially restores binding to purified ICAM-1 of monkey COS cells transfected with a wild type α^L chain and a CD18 β subunit defective in its cytoplasmic domain [67]. MEM-83, which recognizes the α^L integrin subunit, directly induces T lymphocytes to bind to ICAM-1 in the absence of an activating signal [68].

We have generated a similar monoclonal antibody against the β_2 subunit, CBRLFA1/2, which activates $\alpha^L\beta_2$ binding to both ICAM-1 and ICAM-3 in transfected K562 erythroleukemic cells, in which $\alpha^L\beta_2$ is constitutively inactive and recalcitrant to stimulation with phorbol esters (L. Petruzzelli and T.A.S., unpublished observations). Activating monoclonal antibodies raised against the β_1 subunit induce $\alpha^2\beta_1$ to adhere to collagen and laminin [69–71], $\alpha^4\beta_1$ to adhere to fibronectin and VCAM-1 [72], $\alpha^5\beta_1$ to adhere to fibronectin [73–75], and $\alpha^6\beta_1$ to adhere to laminin [70]. In some of these assays, Fab antibody fragments can substitute effectively for intact antibodies, suggesting that antibody-induced receptor cross-linking is not responsible for the change in ligand binding. An additional activating monoclonal antibody, L25, raised against the α^4 subunit, promotes rapid homotypic aggregation of T and B lymphocytes [76]. Although the ligand for $\alpha^4\beta_1$ on lymphocytes remains uncharacterized, this aggregation occurs with Fab fragments also, and is blocked by monoclonal antibodies to either α^4 or β_1 subunits [77,78].

Divalent cations have been suggested to act as ligands that directly activate the adhesiveness of integrins [79–81]. Integrin α subunits contain repeated motifs that have extensive homology to the divalent-cation binding sites on Ca^{2+}-binding regulatory proteins [82–85], and the I domain of the α^M subunit contains an additional divalent-cation-binding site [4]. Occupancy of the divalent-cation-binding sites alters the adhesiveness of integrins *in vitro* [79,80,86]. Chelation of divalent cations by EDTA abolishes adhesion [2], and addition of Mn^{2+} stimulates ligand binding in the absence of activating signals [80,81,86–89] — Mn^{2+} may lock molecules in an avid state [87,90] or may increase their affinity for ligand, as does an increased Mg^{2+} concentration [91].

Mutation of residues that disturb divalent-cation binding leads to a disruption of ligand recognition [4,92]. Furthermore, the detection of several neoepitopes present on active integrins requires the presence of divalent cations [45,64,79,86]. Because the local extracellular concentration of divalent cations varies little among cells, it is unlikely that integrin adhesiveness is dynamically regulated by perturbations in divalent cation concentration. Instead, activation of cells may influence the ability of divalent cations to bind to integrins

and confer conformational changes. Support for this hypothesis is provided by the observation that the divalent cation concentration required for adhesion increases ten-fold when deletions are made in the cytoplasmic domain of α_2 and α_4 subunits, and decreases ten-fold with the addition of an activating monoclonal antibody that recognizes an extracellular epitope of the β_1 subunit (P. Kassner and M. Hemler, personal communication). To confirm such a regulatory role for divalent cations, future studies must show that activation induces a change in affinity for divalent cations that directly converts integrins to an adhesive state.

Ligand-induced binding sites

Proteins of a third class (Fig. 2c) recognize an integrin conformation that reflects the state of ligand occupancy [93–95]. In β_2 and β_3 integrins, the ligand-occupied state is induced by the binding of monoclonal antibodies, peptides or adhesive proteins. For $\alpha^{IIb}\beta_3$, at least three different ligand-induced binding-site epitopes have been identified [93] (Fig. 2c). A similar monoclonal antibody also has been reported to recognize a common epitope on the three integrin α subunits that associate with the β_2 subunit [96]. The significance of the conformational changes that occur after initial ligand binding is unclear: these secondary changes may stabilize receptor interaction with ligand [47] or, alternatively, may induce distinct effector functions. Future studies that map the structural epitopes recognized by the monoclonal antibodies that detect ligand-induced binding sites may address the functional implications of these conformational changes.

Mechanisms for changing integrin adhesiveness

A change in affinity

Debate has focused on the identification of the terminal step in the cascade that leads to integrin activation. Many groups of workers are investigating the biochemical and biophysical basis for the change in integrin binding strength. The 'affinity modulation' model has received a significant amount of support among investigators in the field. Affinity modulation refers to the structural conversion of individual integrin molecules such that the transition between resting and activated states is reflected by an increase in the measurable affinity of the integrin for monomeric ligand (Fig. 3a). In this model, agonist-stimulated activation results in an as-yet undefined modification of an integrin subunit, or its association with additional molecule(s); in some way, this translates into a conformational change in the ligand-binding region of the integrin.

Two types of recent experiments provide immunological and biochemical evidence to support this model. First, as described above, monoclonal antibodies raised against β_2 and β_3 integrins recognize changes in receptor conformation; because expression of the neoepitopes correlates with the kinetics of receptor activation, and because the neoepitopes map to ligand-binding regions of the integrins and monoclonal antibodies to

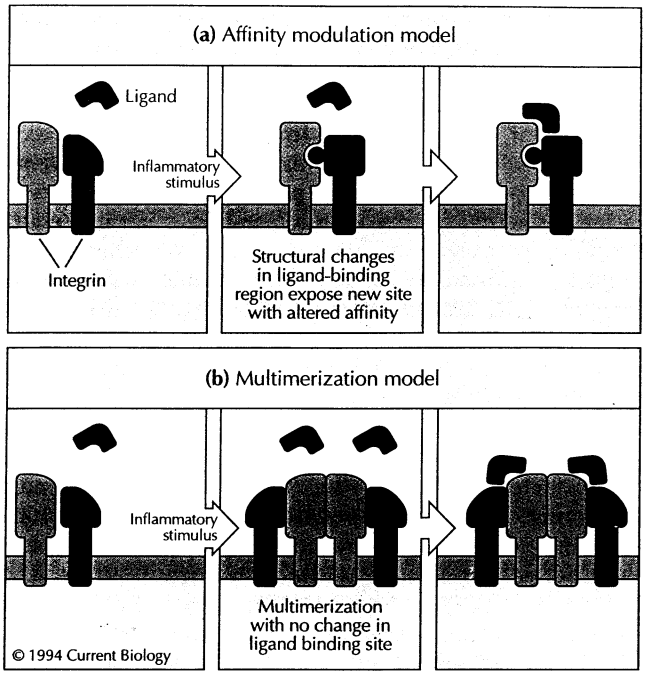

Fig. 3. Two models for how activation increases the ability of integrins to bind ligand. According to the affinity modulation model **(a)**, cell activation causes a structural change in an individual integrin molecule so that its affinity for monomeric ligand is changed. In the multimerization model **(b)**, cell activation causes a group of integrin molecules to become apposed; this higher-order receptor binds to ligand more strongly, without requiring a change in the conformation of individual molecules.

them block function [5,45], they are believed to constitute ligand-binding sites that are exposed after activation. Second, experiments with several integrins show a substantial change in their affinity for ligands after cell activation. Resting $\alpha^{IIb}\beta_3$ integrin does not measurably bind soluble fibrinogen but, when activated, it binds fibrinogen with an affinity (K_d) of 0.11 μM [48]. In addition, resting monocytes do not appreciably bind to factor X or fibrinogen, but when activated with ADP, their $\alpha^M\beta_2$ integrin binds to factor X with an affinity of 0.03 μM, and to fibrinogen with an affinity of 2 μM [80,97,98]. And finally, because the affinity of $\alpha^L\beta_2$ on activated T cells for soluble, monomeric ICAM-1 is too low to detect by direct binding, an indirect competition assay between soluble, truncated ICAM-1 and a Fab fragment from a monoclonal antibody specific for $\alpha^L\beta_2$ was designed [18]. Using this method, the affinity for soluble ICAM-1 of resting $\alpha^L\beta_2$ was estimated to be 67 μM, whereas after activation with phorbol esters a sub-population (15–30%) of activated $\alpha^L\beta_2$ binds to soluble ICAM-1 with an approximately 200-fold higher affinity of 0.36 μM. Collectively, these experiments support a model in which activation induces structural changes in integrins that cause affinity for individual ligands to be modulated.

The recognition by monoclonal antibodies of activation neoepitopes, and changes in apparent affinity between integrins and ligands after cell activation, can also be explained by a clustering or multimerization model

(Fig. 3b). Multimerization or redistribution of integrins so that the local concentration increases in the area of ligand apposition could strengthen attachment of the integrin to any ligand, without requiring a change in affinity of an individual integrin for a particular monomeric ligand. Monoclonal antibodies recognizing activation-dependent neoepitopes may recognize a novel structural feature that is manifest only on laterally juxtaposed integrin multimers. Some experimental evidence supports this model. For example, treatment of myeloid cells with phorbol esters induces a clustering of $\alpha^M\beta_2$ integrin that correlates with an increased adhesiveness for erythrocytes coated with the complement protein iC3b [99].

However, treatment of myeloid cells with the chemotactic factor f-Met–Leu–Phe does not induce receptor clustering, although it does stimulate adhesion [99,100]. In immuno-electron-microscopy studies of neutrophils treated with f-Met–Leu–Phe or phorbol esters, the monoclonal antibody CBRM1/5, which recognizes a sub-population of active $\alpha^M\beta_2$ molecules, clusters non-randomly on pseudopods and at sites of neutrophil adhesion, whereas the monoclonal antibody LM2/1, which recognizes all $\alpha^M\beta_2$ molecules, distributes randomly over the cell surface (M.S.D., D. Bainton and T.A.S., unpublished observations). Although the multimerization model has attractive features, it cannot by itself explain the experiments that document a change in affinity of integrins for monomeric ligands after cellular activation [18]. Future studies that measure the molecular size of the complexes that form between a monovalent Fab fragment, made from a monoclonal antibody that recognizes an activation dependent neoepitope, and its target integrin should address the role that multimerization plays in integrin activation.

Mediators of the change in adhesiveness

Although the biophysical models described above account for changes in the adhesiveness of integrins, none addresses the molecular basis of the effector mechanism. Several groups of workers have struggled experimentally with this question. Mutational analyses that compare the structure of integrins with their function have been performed to assess which regions of the integrin molecule are critical for regulated adhesion. Immunohistochemical and biochemical studies have been used to characterize the extracellular and intracellular molecules that associate with integrins and affect adhesion.

Although phosphorylation of integrin cytoplasmic domains does not directly activate ligand binding, experimental evidence suggests that cytoplasmic regions of both α and β subunits may be targets for the modulation of integrin–ligand binding affinity (Fig. 4a). For example, truncation of the cytoplasmic domain of the β subunit of $\alpha^L\beta_2$ integrin in B lymphoblastoid cells or transfected COS cells abrogates cell binding to ICAM-1 [67]. Refined mutagenesis studies have delineated the region of the cytoplasmic domain of the β_2 subunit that is required for adhesion. The amino-terminal 28 amino acids are dispensable, but a segment of five amino acids, including three contiguous threonines and a single phenylalanine, located in the distal carboxy-terminal region are required to sustain adhesion of COS cells to ICAM-1. These residues are conserved in the β_1, β_3 and β_7 integrin subunits. In apparent contrast, truncation of the cytoplasmic domain of the β_3 subunit does not affect the adhesiveness of $\alpha^{IIb}\beta_3$ [101]. A role for the cytoplasmic domain of the β_3 subunit is, however, suggested by the finding of Glanzmann thrombasthenia patients with a point mutation in their β_3 integrin cytoplasmic domain and depressed integrin function [102].

The cytoplasmic domains of integrin α subunits also affect ligand binding. When expressed in CHO cells, wild-type $\alpha^{IIb}\beta_3$ integrin exhibits, in the absence of stimulation, low levels of both adhesion to fibrinogen

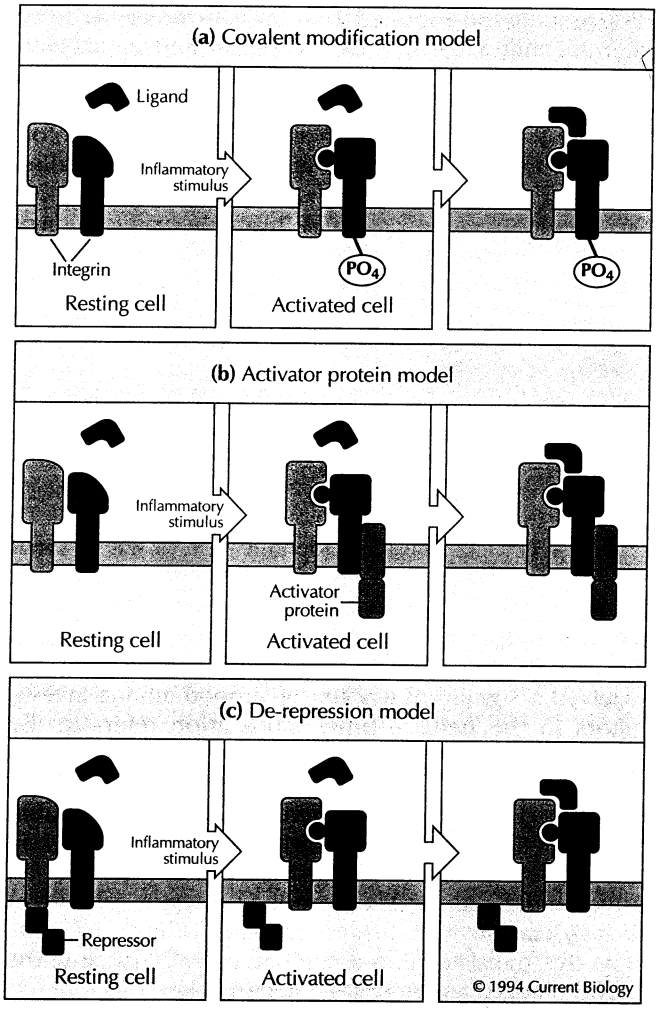

Fig. 4. Three models for mediators of changing integrin adhesiveness. **(a)** Covalent modification: activation promotes phosphorylation, or another post-translational modification, of the integrin which enhances its binding to ligand. **(b)** An activator protein: activation promotes association of the integrin with a cytoplasmic or transmembrane moiety, and this association activates the integrin's binding to ligand. **(c)** De-repression: activation causes detachment of a molecule that otherwise maintains the integrin in a resting state; after detachment of this repressor, the integrin is free to adopt a new, ligand-binding conformation.

and expression of activation-dependent neoepitopes [48]. Truncation of the cytoplasmic domain of α^{IIb} and co-expression with a wild-type β_3 subunit activates the resting integrin to bind to fibrinogen [101]. Chimeric α subunits made up of the extracellular domain of α^{IIb} and the cytoplasmic domain of α^2, α^5 or α^6 have constitutively high levels of ligand binding and activation-dependent neoepitope expression [9,101]. For $\alpha^{IIb}\beta_3$, and perhaps for all integrins, recognition sequences within the cytoplasmic domain of the α subunit regulate the integrin's activity. Deletion of the strongly conserved membrane-proximal GFFKR sequence motif resets the $\alpha^{IIb}\beta_3$ molecule to a default high-affinity state. A de-repression model of activation can explain all of these experimental results (Fig. 4c) [47].

According to this model, in the resting state a repressor constitutively binds to the α^{IIb} subunit. Activation could release the integrin into a high-affinity conformation by modification or detachment of the repressor. The activation process can then be dissected into at least two steps: cytoplasmic-domain modification and transmembrane signaling. Virtually nothing is understood of the latter step, transmission of the signal across the membrane. How the initial step occurs is also unclear, but one possibility is that activation induces the cytoplasmic domain of the β_3 subunit to modify a repressor–α-subunit interaction. Thus, truncation of the β_3 subunit releases its regulatory effect on the repressor but is not sufficient for activation. Mutation of critical residues in the β_3 subunit's cytoplasmic domain may prevent repressor detachment and render the integrin resistant to activation. Mutations that uncouple activation of the cell from integrin adhesiveness can be overcome only by direct modulators of the extracellular conformation [102].

It is unclear how general the $\alpha^{IIb}\beta_3$–repressor model will prove to be with respect to the activation of other integrins. Studies that correlate adhesion of β_1-subunit-containing integrins with deletion of the cytoplasmic domains of β_1 have been difficult to interpret. Truncation of the entire cytoplasmic domain of β_1 does not affect its ligand-binding properties [103] but instead affects the concentration of β_1-containing integrins into focal contacts, the points of cellular adhesion to the substrate [103–105]. But deletion of five or more amino acids from the carboxyl terminus of the β_1 subunit significantly impairs both adhesion and localization into focal contacts [104]. Truncation of the cytoplasmic domains of α^1 and α^5 subunits has little effect on adhesion to their respective ligands, collagen and fibronectin [106,107], but truncation of the α^4 subunit abolishes binding to VCAM-1 or fibronectin [108].

Some of these apparent contradictions may be explained by a recent study, which shows that the divalent-cation concentration requirement for adhesion changes as the relative state of activation of the integrin changes: an integrin in a highly avid state requires lower concentrations of divalent cation (P. Kassner and M. Hemler, personal communication). Thus, whether or not truncation of the cytoplasmic domain of an integrin α subunit influences adhesion may depend on the experimental divalent-cation concentration; high concentrations could mask the effects of mutations that would decrease adhesion at lower divalent-cation concentrations.

Another hypothesis for explaining how the change in adhesiveness occurs proposes a regulated association between an intracellular or intramembrane activator and the cytoplasmic domains of integrin subunits (Fig. 4b). At present, there are several candidate molecules implicated in the activation of integrin adhesiveness. The cytoskeletal proteins talin and α-actinin co-localize with integrins to sites of adhesion [109–111] and, *in vitro*, bind to the cytoplasmic domain of the β_1 integrin subunit [112,113]. Treatment of leukocytes with cytochalasin B, an agent that disrupts actin filaments, does not affect adhesion to ICAM-1 by $\alpha^L\beta_2$ or expression of an activation neoepitope on $\alpha^M\beta_2$ (M.L. Dustin, O. Carpen, M.B. Lawrence, M.S.D. and T.A.S., unpublished observations). Thus, activation may induce the cytoplasmic domain of the α and β subunits to associate with cytoskeletal elements, but this may not regulate adhesion directly but rather might transmit distinct downstream intracellular messages.

Because the composition of the plasma membrane changes after cell activation, lipid molecules have also been suggested to act as regulators of integrin adhesion. Indeed, several studies report that, *in vitro*, lipids affect integrin adhesiveness directly. Integrin-modulating factor-1 (IMF-1), an unsaturated fatty acid or isoprenoid acid, transiently increases the binding of iC3b-coated particles, endothelial cells and fibrinogen to $\alpha^M\beta_2$ [114,115]. The binding strength and binding specificity of purified $\alpha^V\beta_3$ in liposomes changes according to the lipid composition [116,117]. In addition, phosphatidic acid promotes the dose-dependent binding of fibrinogen by $\alpha^{IIb}\beta_3$ [118]. These experiments suggest that a regulated change in the local lipid composition of the membrane can influence integrin conformation and alter integrin–ligand interactions. Future studies are required to clarify how these lipids affect ligand binding, and whether this mechanism of change is biologically relevant.

Conclusions

In this review, we have summarized the experimental evidence that supports a model of integrin activation in which extracellular signals evoke intracellular signals that, in turn, lead to a change in integrin adhesiveness. This mechanism of regulation probably developed as a result of a requirement for rapid control of specific adhesive interactions, for example, in inflammation. Intracellular signal transduction pathways that activate and inactivate integrins within seconds provide a responsive mechanism for regulating adhesion. This system may be critical in physically dynamic adhesive situations, such as occur during cell migration: subpopulations of integrin molecules at the leading edge of

a cell could be activated by a chemotactic gradient to bind ligand. As migration proceeds, these integrins will remain in the same place relative to the substrate, and will move rearward relative to the cell. As they reach the trailing edge of the cell, it is hypothesized that they are deactivated, allowing detachment and retraction of the trailing edge.

There is now good experimental evidence that the dynamism of integrin adhesion reflects a series of regulated conformational changes that converts a subset of molecules from low-affinity to high-affinity binding states. But despite a wealth of experiments, our understanding of the details of the signal transduction pathways that activate integrins and the structural changes that an integrin molecule undergoes remains, in many ways, primitive. Based on pharmacological and mutagenesis experiments, intracellular regulators such as tyrosine kinases, protein kinase C, and protein phosphatases are implicated in integrin regulation, but a precise pathway that couples a particular signal from the outside to a change in adhesion remains elusive.

Although it has not been experimentally explored, a separate cascade may be responsible for returning integrins from an adhesive to a resting state. Furthermore, it is difficult to speculate whether cells regulate the adhesiveness of integrins through a previously defined, multi-purpose cascade, or through a novel, dedicated signal transduction pathway. Genetic experiments in lower organisms, reconstitution experiments in mutant tissue culture cells lacking particular integrin subunits, and the isolation of proteins that interact with integrin cytoplasmic domains, are currently being used to identify additional elements that induce integrins to adhere to, and de-adhere from, their ligands. Elucidation of the structural changes that characterize the active adhesion state of integrins may await definitive biophysical data, including the determination of crystal structures of active and inactive integrin molecules.

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