Monoclonal antibodies as probes of integrin priming and activation

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Abstract

Integrins are a family of heterodimeric, transmembrane receptors that mediate a range of cell–cell and cell–extracellular matrix interactions in an array of physiological and pathophysiological situations. Integrin-mediated cell adhesion is dynamically regulated in vivo to facilitate cell anchorage and movement, but prevents aberrant trafficking and aggregation. Following ligand engagement, integrin signalling imposes a spatial constraint on the assembly of signalling complexes and controls the transduction of mechanical force to the cytoskeleton. This transmembrane passage of signals via integrins is achieved both by clustering of receptors, which makes the ligand and effector engagement more favourable kinetically, and by induction of conformational changes, that theoretically creates ligand and effector binding sites de novo. Clustering and conformational changes can be triggered both from the inside of the cell (resulting in acquisition of ligand-competent conformers) and from the outside (ligand-induced signalling). In this paper, these processes will be described and distinguished by the terms priming and activation, respectively. Although both clustering and conformation are important for integrin function, the latter will be the main focus of this article; in particular, the importance of monoclonal antibodies for the study of integrin shape changes.

Introduction

In 2001, the first structure of the extracellular part of an integrin dimer was solved by X-ray crystallography (for αVβ3; [1]). That report was followed closely by the structure of an RGD (Arg-Gly-Asp) ligand–receptor complex formed by soaking the ligand-mimetic peptide into pre-existing crystals [2]. As shown in Figure 1, the main contact point between the integrin subunits was found to be in the ‘head’ region, where a β-propeller fold in the α subunit was complexed with a von Willebrand factor A domain in the β subunit (termed the βA domain to distinguish it from an A domain that is found in some integrin α subunits). The integrin head was supported by two rod-like ‘legs’. The α subunit leg comprised three β-sandwich domains, and the β subunit leg a PSI (plexin-semaphorin integrin) domain, an immunoglobulin fold termed the hybrid domain, four EGF (epidermal growth factor)-like repeats and a cystatin-like fold. Two highly flexible ‘knees’, which adopted a striking, approx. 135° bend in the crystal structure, were found between the ‘thigh’ domain and the first of two ‘calf’ domains in the α subunit, and at the conjunction of the hybrid domain, two EGF repeats, and the PSI domain in the β subunit. The location of the ligand-binding pocket was found to be at the junction of the β-propeller and the βA domain in the head, with the aspartate carboxy group of the RGD peptide directly co-ordinated to an Mn+2 ion.

This cation-binding site has been termed MIDAS (metal-ion-dependent adhesion site). The integrin X-ray structures raise a number of questions relevant to integrin signalling and, in particular, the importance of conformational changes. For example, does the bent form of integrins occur naturally? Is the bent form an inactive version of an extended integrin? How are conformational changes propagated through the molecule from the ligand-binding pocket? Are inside-out and outside-in signalling (i.e. priming and activation) different processes? Is signalling graded or an all-or-none event? mAbs (monoclonal antibodies) have provided invaluable information in addressing some of these issues and this paper reviews these advances.

Evidence that conformational changes take place in integrins

Gross conformational changes in integrins have been monitored by a variety of techniques, including proteinase sensitivity, fluorescence resonance energy transfer, intramolecular cross-linking, light scattering and analytical ultracentrifugation, but it is the expression of specific structural epitopes recognized by mAbs that has received most attention. The earliest studies reporting changes in mAb binding to integrins were performed on platelet αIIbβ3 [3–5], and further analyses identified a subset of mAb epitopes that were specifically induced by ligand binding [6,7]. The acronym LIBS was coined to describe these epitopes as ligand-induced binding sites [8]. Previously, LIBS mAbs have been used to show that different peptidic ligand mimics trigger distinct conformational alterations in αIIbβ3, suggesting that
The structure of α5β1 was derived by homology modelling based on the αVβ3 crystal structure co-ordinates and is depicted using WebLab Viewer software. The PSI domain has been modelled on an EGF module and this is not intended to be a true reflection of its structure. The α subunit is shown in grey, the β subunit β strands are shown in cyan and α helices are in red, yellow (α7) and orange (α2). The bivalent ion at the MIDAS site is depicted by a purple sphere. Site-directed mutations (D130A and L358G) are indicated, as are the residues contributing to the epitopes for mAbs 12G10 (K218, R154, R155) and HUTS-4 and 15/7 (S370, E371, K417). Blue arrows indicate secondary structure element and module movements.

integrins may transduce ligand-specific signals with different functional consequences [9]. Similarly, the therapeutic integrin antagonist tirofiban has been reported to induce a receptor conformation that differs from both resting and ADP-primed receptors [10]. The conformational changes reported by mAbs can be propagated to and from the cytoplasmic face of integrins, suggesting that conformational regulation is an important feature of bi-directional signalling by integrins. Notably, ligand binding induced a transmembrane conformational change reported by binding of mAb anti-LIBScyt1, which recognizes the αIIb cytoplasmic domain [11], and overexpression of the head domain of the cytoskeletal adaptor talin reduced extracellular LIBS epitope expression [12].

Mechanism of action of regulatory mAbs
An intriguing aspect of the integrin literature is the number of reports of mAbs that stimulate receptor function. Although all stimulatory mAbs appear to increase binding affinity by decreasing the ligand off-rate, two distinct subclasses of antibodies have been defined. One subclass, represented by the LIBS mAbs, recognizes epitopes that are regulated by ligand and cation binding. Since LIBS are present in the absence of ligand, the name is not strictly accurate; however, it is clear that ligands can induce these epitopes. The epitopes for the other subclass of antibodies are not affected by a ligand or cation. It is probable that LIBS mAbs recognize conformers of integrins that are induced in either the primed or ligand-occupied state, and that they therefore displace a conformational equilibrium in favour of these forms and away from inactive and unoccupied conformers (reviewed in [13]). The binding of non-LIBS activating mAbs, in contrast, probably induces a primed conformation in the integrin rather than stabilizing naturally occurring conformations. Thus the expression of LIBS epitopes, but not non-LIBS epitopes, provides useful information about the distribution of different conformational classes in an integrin population. Most stimulatory mAbs recognize sites throughout the β subunit, implying that global changes take place during integrin priming (Figure 2). Key regions recognized by LIBS mAbs include the extreme N-terminus of the subunit within the PSI domain, the βA domain, the hybrid domain and the EGF repeats (reviewed in [14]).

Generally, it might be expected that inhibitory mAbs sterically interfere with ligand binding and therefore act as competitive inhibitors. Surprisingly, most function-blocking anti-integrin mAbs act allosterically [15]. Inhibitory mAb
epitopes appear to be the converse of LIBS epitopes in that their exposure is lowered in the ligand-occupied form of the integrin. For this reason, such an epitope has been termed as a ‘ligand-attenuated binding site’ (LABS). LABS mAbs prevent a conformational change necessary for ligand binding and/or stabilize the unoccupied state of the receptor. As many LABS mAbs bind very close to ligand-contact sites, it appears that their epitopes are structurally linked to ligand-binding sites (Figure 2).

Mechanisms of intramolecular priming and activation

The βA domain
The sites of specific mutations and mAb epitopes that are involved in the stimulation of ligand binding offer particularly interesting insights into the mechanisms of integrin conformational change that underlie priming and activation. These mechanisms are still not fully understood, but in recent years some key features have been elucidated.

Comparison of liganded and unliganded αA domain crystal structures identified a large downward displacement of the C-terminal α7 helix that was triggered by a rearrangement of the cation co-ordination at the ligand-binding site, a consequent inward movement of the α1 helix, and a squeezing of the hydrophobic core of the domain [16,17]. In the unliganded state, a conserved isoleucine residue was buried in a hydrophobic pocket, but ligand binding caused its displacement and subsequent helix reorientation. A series of mutations, both of the isoleucine and the hydrophobic residues forming its binding pocket (which lie in the lower half of the A domain), caused priming by constitutively moving the α7 helix [18].

The mAb 12G10 recognizes a unique cation-regulated epitope on the β1 A domain, induction of which parallels the activation state of the integrin (i.e. competency for ligand recognition; [19]). The ability of Mn$^{2+}$ and Mg$^{2+}$ to stimulate 12G10 binding was abrogated by mutation of the MIDAS motif, demonstrating that the MIDAS is an Mn$^{2+}$/Mg$^{2+}$-binding site and that occupancy of this site induced conformational changes in the A domain [20].
The cation-regulated region of the 12G10 epitope mapped to Arg\textsuperscript{155}/Arg\textsuperscript{155} in the \(\alpha_1\) helix (Figure 1). These results demonstrate that the \(\alpha_1\) helix undergoes conformational alterations during integrin priming and suggest that Mn\textsuperscript{2+} acts as a potent activator of \(\beta_1\) integrins because it can promote a shift in the position of this helix.

Further analyses of the \(\beta_1\) A domain subunit have revealed additional similarities with the \(\alpha_1\) domain, in that mutation of a conserved leucine residue in the same position as that in the \(\alpha_1\) domain caused integrin priming; however, unlike in \(\alpha_1\) domains, mutation of residues in the hydrophobic core did not activate [21]. This finding, coupled with the observation of an outward movement of the hybrid domain using negative-staining electron microscopy [22], suggests that there is a propagation of conformational changes from the \(\beta_1\) domain to the rest of the integrin molecule.

**Inter-domain movement**

Interestingly, there is abundant evidence that inter-domain movement takes place during integrin priming and activation, including the potential outward swing of the hybrid domain, separation of the legs and transmembrane domain and a closing up of the \(\beta_1\) domain/\(\beta_2\) propeller interface (Figure 1; reviewed in [23]). Mutational and mAb epitope mapping results are consistent with these possibilities, as mutations at the \(\beta_1\) domain/hybrid domain interface block priming, and this region is the site for binding of some function-blocking mAbs. Similarly, the anti-LIBS mAbs 15/7 and HUTS-4 map to a region of the hybrid domain that interfaces closely with the \(\alpha_1\) subunit (the three residues at the bottom of the image in Figure 1; [21]). Changes in the expression of these epitopes are induced by conformational changes in \(\beta_1\) caused by bivalent cations, function-perturbing mAbs, or ligand recognition. Recombinant truncated \(\alpha_7\beta_1\) with a mutation L358A in the \(\alpha_7\) helix of \(\beta_1\) had constitutively high expression of the 15/7 and HUTS-4 epitopes, mimicked the conformation of the ligand-occupied receptor, and had high constitutive ligand-binding activity. Taken together, these results suggest that the transduction of conformational changes through \(\beta_1\) involves shape shifting in the \(\alpha_7\) helix region, which is linked to a swing of the hybrid domain away from the \(\alpha_1\) subunit.

Mutations and epitopes that activate \(\beta_2\) integrins have been mapped to EGF repeats 2 and 3, a site where the bend in the dimer takes place [24]. This led to the hypothesis that integrin priming employs a switchblade mechanism, involving straightening at this site and stimulatory mAb epitope exposure. Recent analyses of \(\alpha_1\)VB3 integrin structure by negative staining in the presence of different bivalent cations has demonstrated a predominance of bent conformers and extended conformers in Mn\textsuperscript{2+} [22]. As Ca\textsuperscript{2+} tends to favour the adoption of an inactive state, and Mn\textsuperscript{2+} tends to prime integrins, these findings support the unbending hypothesis.

Although most activating mAbs recognize the \(\beta_1\) subunit, activating anti-(\(\alpha_1\) subunit) mAbs do exist. For example, the anti-\(\alpha_IIb\) mAb D33C, which recognizes the \(\beta_3\)-propeller, has been reported to induce fibrinogen binding and platelet aggregation [25]. In addition, the anti-\(\alpha_IIb\) mAb PMI-1 binds close to the heavy-light chain border [18,26] and the epitope of the anti-\(\alpha_\text{L}\) mAb NKI-L16 is located close to the transmembrane domain [27]. Finally, as there are sites within the C-terminal \(\alpha_1\) subunit calf-2 and \(\beta_3\) subunit cystatin-like domains that bind activating mAbs, this is evidence for long-range conformational propagation within integrins.

In conclusion, structure–function studies employing regulatory mAbs that are based on the X-ray crystal structures of integrin \(\alpha_1\)VB3 are beginning to reveal the mechanisms of intramolecular signal propagation. The specific conformational changes that take place in the integrin head are now well defined, and there is substantial evidence to support a swinging apart of the integrin legs. It remains to be determined whether leg separation is the major mechanism triggering effector binding within cells, or whether more subtle conformational reorganizations take place within the dimeric cytoplasmic domains. In the future, resolution of this issue will address the question of how graded signalling events are triggered by integrins.

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**References**


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