Anti-integrin monoclonal antibodies

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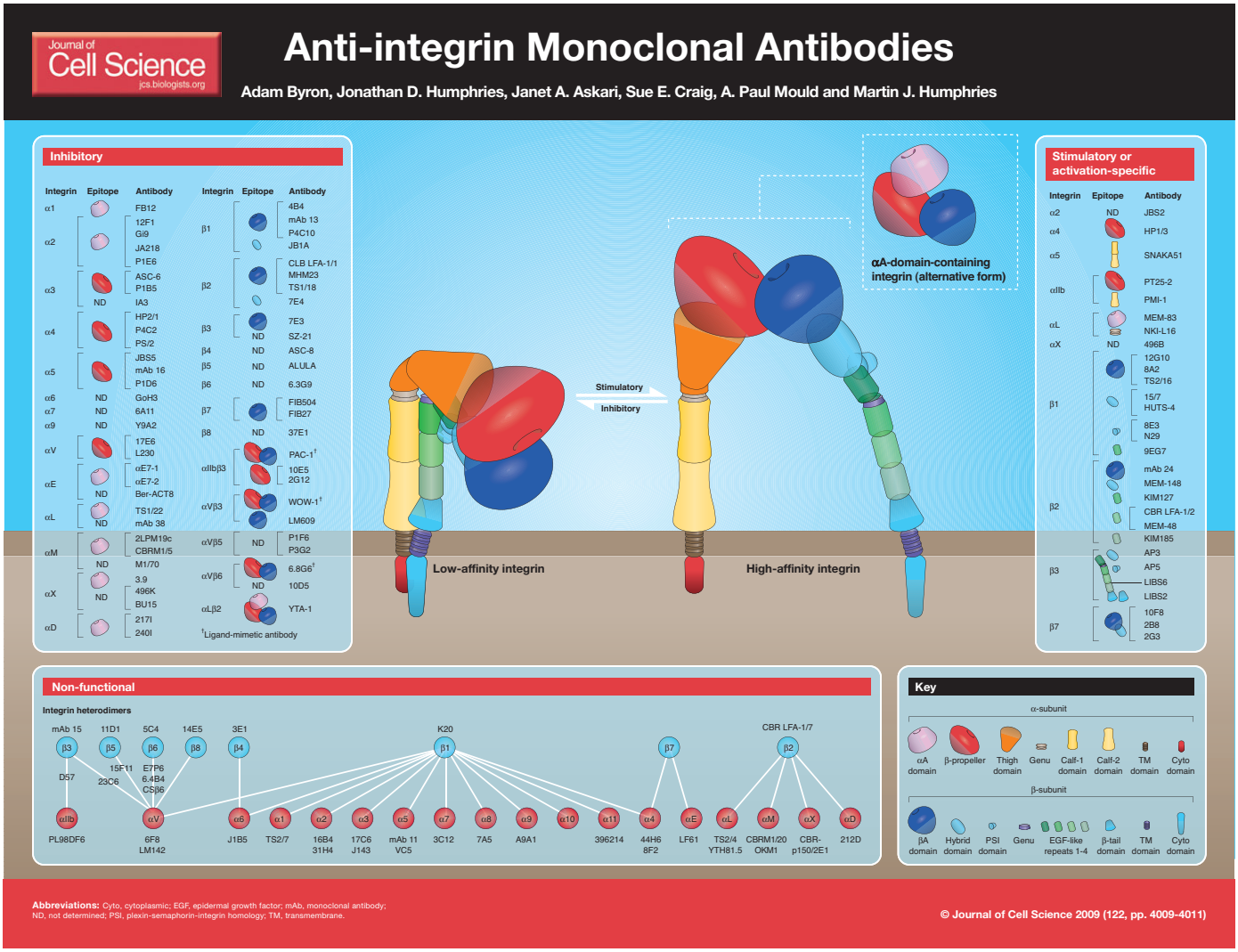
Introduction

Integrins are a family of 24 heterodimeric transmembrane receptors that support cell-cell and cell-ECM (extracellular matrix) interactions in a multitude of physiological and disease situations (Humphries, 2000; Hynes, 2002). Adhesion that is mediated by integrins is controlled dynamically to allow cell positioning and migration and to prevent abnormal trafficking and anchorage. Integrin signalling in response to ligand binding is achieved by a combination of receptor clustering and conformational changes. Both of these processes can be elicited from the inside of the cell (resulting in the acquisition of a high-affinity receptor conformation and priming of the receptor for ligand binding) and from the outside (to mediate ligand-induced activation and signalling).

The study of changes in integrin shape has been greatly aided by the availability of monoclonal antibodies (mAbs) that detect conformation-dependent epitopes. These mAbs have not only helped to pinpoint the intramolecular changes that determine the integrin activation state, but have also proven useful for regulating function. In recent years, major advances have been made in our understanding of the mechanisms that regulate integrin affinity (Arnaout et al., 2005; Luo et al., 2007) and, accordingly, we now also have an improved knowledge of the mechanisms of action of function-regulating mAbs. As these mAbs work in different ways, there is a danger that researchers might select the wrong reagent for their studies and/or misinterpret the data that they obtain. In this Cell Science at a Glance article, we have therefore attempted to explain briefly the mechanisms of antibody regulation of integrins. The accompanying poster lists three classes of key reagents: those that inhibit ligand engagement; those that stimulate ligand engagement or report a high-affinity integrin state (‘activation specific’); and those that serve as equally important negative controls. Partly owing to space constraints and partly owing to a lack of available information, we have restricted our selection of mAbs to those that recognise human integrins. Furthermore,



(See poster insert)

we only report mAbs that either affect ligand binding and cell adhesion or, to our knowledge, are non-functional controls. Although we have not catalogued mAbs for use in immunoprecipitation, immunoblotting, flow cytometry or ELISA-type experiments, many of the reagents listed are suitable for these purposes. The poster is not intended to recommend one mAb in favour of another, but to represent some of the best-characterised examples. The researcher must therefore determine the most appropriate reagent for their specific purpose.

Integrin structure and conformational changes

The first crystal structure of an integrin (αVβ3) was solved in 2001 (Xiong et al., 2001). The structure revealed a 'head' region, which was the main point of contact between the two subunits and comprised a β-propeller fold in the α-subunit and a von Willebrand factor-A domain in the β-subunit (the βA domain). The head was supported by two rod-like 'legs'. The α-subunit leg comprised three β-sandwich domains (termed thigh, calf-1 and calf-2) and the β-subunit leg included a PSI (plexin-semaphorin-integrin) domain, an immunoglobulin fold (termed the hybrid domain), four epidermal growth factor (EGF)-like repeats and a cystatin-like fold (termed the β-tail domain). A soluble form of the integrin was used for the crystallisation studies, but it is now well established that both integrin legs link to transmembrane domains and then to short cytoplasmic domains that can interact with each other or with cytoskeletal and signalling proteins (Wegener et al., 2007).

Interestingly, the initial crystal structure revealed a bent molecule, with articulation points in both integrin legs at the so-called genu. This form of the integrin is now thought to represent the conformation with low affinity for a ligand. The adoption of a high-affinity conformation involves a series of shape changes, including unbending of the receptor and various inter-module and intra-module movements, such as swing-out of the hybrid domain away from the α-subunit and α-helical movements in the βA domain. Most evidence points to a separation of the cytoplasmic and transmembrane domains as a key step in the acquisition of the high-affinity conformation. It is currently unclear how many classes of integrin conformation exist, but primed and ligand-bound integrins have similar conformations, and these are distinctly different from low-affinity receptors. The poster therefore contains two general representations of integrins (bent and extended). As yet, there are no mAbs that are able to distinguish primed from ligand-bound integrins, although the relative expression of different epitopes varies between these states.

Using the prototypic peptide ligand arginine-glycine-aspartate (RGD) in co-crystallisation studies, the ligand-binding pocket in αVβ3 integrin was located at the junction of the β-propeller and the βA domain in the head (Xiong et al., 2002). The aspartate carboxyl group of RGD was found to coordinate a divalent cation directly in a so-called metal-ion-dependent adhesion site or MIDAS. This is now accepted as a common mode of ligand binding by integrins. Half of all integrin α-subunits contain an A-domain inserted into the α-subunit β-propeller, and for those dimers that contain this αA domain, the module has evolved to incorporate the main ligand-binding site through a very similar mechanism to that of the βA domain. Intriguingly, ligand binding to the αA domain causes engagement of the βA domain by an intramolecular pseudo-ligand (a glutamate residue at the base of the αA domain) and therefore relays ligand binding to the β-subunit (Alonso et al., 2002). Thus, the conformational changes that underpin changes in integrin activation state are common to all receptors.

Mechanism of action of regulatory mAbs

Stimulatory or activation-specific mAbs

Whereas all stimulatory or activation-specific mAbs appear to increase ligand-binding affinity by reducing off-rate (i.e. the rate of dissociation), the reagents fall into two subclasses (Humphries, 2000). One subclass recognises epitopes that are regulated by ligand and cation binding. The epitopes for these mAbs are frequently termed LIBS (ligand-induced binding sites). A second subclass is not affected by ligand or cation binding (this includes the widely used anti-β1 mAb, TS2/16). It is probable that cation- and ligand-regulated mAbs recognise conformations of integrins that are found in either the primed or ligand-occupied state, and that they therefore shift a conformational equilibrium in favour of those forms. The epitopes might be created de novo as a result of movement of secondary structure elements within protein modules (e.g. within the βA domain) or by exposure of masked epitopes (e.g. as a result of leg separation, unbending or domain movement). Inevitably, any mAb that binds preferentially to the high-affinity form of the receptor will activate integrin by skewing the conformational equilibrium. However, some might appear to lack stimulatory activity either because the equilibrium might already be fully displaced towards the high-affinity state or because the cell type tends to exhibit all-or-none-type activation responses (e.g. mAb 24 and leukocyte β2 integrins).

A special subclass of cation- and ligand-regulated mAbs are those that contain ligand-mimetic peptide sequences within their complementarity-determining loops (e.g. PAC-1 and WOW-1, which recognise β3; and 6.8G6, which binds β6). These reagents are particularly useful for detecting high-affinity conformations of integrins because they effectively act as ligands. However, these mAbs also block binding because they compete directly for ligand occupancy. The binding of non-ligand-regulated mAbs probably induces a primed conformation in the integrin by forcing shape changes rather than by stabilising naturally occurring conformations. Most stimulatory mAbs recognise sites throughout the β-subunit, implying that major changes take place in this region of the receptor. Key sites that are recognised by ligand-regulated mAbs include the PSI domain (which is partly buried in the bent form), the βA domain (in which movement of its α1 and α7 helices create new epitopes), the hybrid domain (in which swing-out exposes epitopes), the genu (which is masked in the bent form) and the EGF-like repeats (which are partly covered in the bent form). Although most activating mAbs recognise the β-subunit, activating anti-α-subunit mAbs do exist, with examples that affect conformation of the αA domain or detect leg separation. Thus, the choice of stimulatory mAbs for research purposes should be informed by the mechanism of action of the mAb, and this is generally determined by the location of its epitopes. The acquisition of a high-affinity state is best achieved using mAbs that stabilise an extended conformation or that stabilise the high-affinity form of the βA domain.

Inhibitory mAbs

It is generally assumed that inhibitory mAbs sterically interfere with ligand binding and therefore act as competitive inhibitors. Surprisingly, of those anti-integrin mAbs on which detailed analyses have been performed, most act as allosteric inhibitors (Mould et al., 1996). In this case, mAbs appear to prevent the conformational changes that are needed for ligand binding to occur, and they might therefore stabilise the unoccupied state of the receptor. Although many inhibitory mAbs are allosteric inhibitors, their epitopes are usually very close to ligand contact sites. Similarly to stimulatory mAbs, some of these epitopes are regulated by ligand and cation binding and are termed LABS (ligand-attenuated binding sites). There are also a few examples of mAbs that recognise the high-affinity conformation of integrins, but their binding blocks ligand

engagement (e.g. 12F1, which recognises α2; and CBRM1/5, which binds αM).

Non-functional mAbs

The use of the most appropriate non-functional integrin mAb is of paramount importance for the correct interpretation of data relating to the study of integrin regulation and function. These reagents therefore constitute the best type of control and, where available, a mAb directed against the same subunit and domain of the integrin of interest should be used. There are relatively few non-functional mAbs reported, and the choice of reagents can therefore be limited. The reason for this paucity is probably the conformationally dynamic nature of integrins, with stabilisation of many conformations, even distal from the main sites of ligand binding, having a resultant effect on integrin function.

Therapeutics

Integrin-based cell adhesion contributes to the pathogenesis of a wide spectrum of human disorders. mAbs that block integrin functions have been shown to be of clinical benefit for the treatment of thrombotic and autoimmune disorders, with total sales of anti-integrin antibodies for therapeutic use exceeding $1 billion in 2008 (La Merie, 2009). Anti-integrin mAbs that are currently in clinical use are ReoPro (abciximab) and Tysabri (natalizumab). ReoPro is a humanised version of a function-blocking mAb (7E3) that recognises the β3 subunit and blocks the platelet fibrinogen receptor αIIbβ3; it is used in the treatment of unstable angina and as an adjuvant to percutaneous coronary interventions. The epitope of 7E3 includes residues on the top face of the β3 subunit A-domain, close to the MIDAS site (Artoni et al., 2004). Tysabri is an inhibitory mAb that recognises the α4 subunit and is used in the treatment of relapsing forms of multiple sclerosis. Blockade of T-cell α4β1 integrin prevents these cells from entering the central nervous system, thereby slowing the destruction of nerve sheaths. The epitope of Tysabri lies in the β-propeller domain of the α4 subunit (Huryn et al., 2004). A number of other function-blocking antibodies are currently at preclinical stages of development for the treatment of cancer and fibrosis. In the future, it is likely that antibodies that stimulate integrin function will also find clinical uses, such as enhancing repair in the central nervous system (Andrews et al., 2009).

Perspectives

Integrin mAbs have contributed immensely to our understanding of integrin activation-state regulation, integrin biology and integrin-based therapeutics. Nonetheless, much is still to be learned, for example regarding what differentiates conformation-specific signals. The development of new mAbs that can discriminate between these signals would undoubtedly aid progress in this area and might also reveal new avenues for therapeutic intervention. It is noteworthy that mAbs that recognise certain integrin subunits or heterodimer activation states (e.g. ligand-mimetic β1 mAbs or activation-specific α-subunit mAbs) are at present missing or under-represented in the arsenal of available mAbs, and their production would shed further light on many areas of integrin biology. Moving forward, it is apparent that global systems-based analyses of the complexity of integrin signalling is both required and informative, and the use of integrin mAbs in such studies might help to elucidate activation-state-specific signalling complexes and pathways.

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