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MINIREVIEW

The $\alpha_M\beta_2$ integrin and its role in neutrophil function

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ABSTRACT

Neutrophils are the first cell type to arrive at the injury sites and play a critical role in host defense, by virtue of its ability to adhere and transmigrate through endothelium, to phagocytose foreign pathogens, and to produce free oxygen radicals and proteolytic enzymes. Yet, inappropriate neutrophil activation causes tissue damage and various inflammatory diseases. These physiological and pathological functions of neutrophils depend on the engagement of certain surface receptors, especially $\alpha_M\beta_2$, the major β_2 integrin receptor present on neutrophil surface. Understanding of the molecular mechanisms underlying ligand binding by $\alpha_M\beta_2$, as well as the roles of $\alpha_M\beta$-ligand interactions in neutrophil functions will enable us to regulate more precisely neutrophil activities: that is, to promote their host defense functions, and at the same time to minimize their deleterious effects on normal cells.

Key words: *Leukocyte, integrin, receptor, adhesion.*

INTRODUCTION

Neutrophils play key roles in the host defense network against pathogens by virtue of their abilities to phagocytose microorganisms and to produce oxygen free radicals

ABREVIATIONS: EC, endothelial cell; Fg, fibrinogen; ICAM, intracellular adhesion molecule; leukocyte Adhesion Deficiency; mAb, monoclonal antibody.

and proteolytic enzymes. Extravasation of neutrophils from the blood stream proceeds through three coordinated steps: rolling and tethering, firm adhesion, and transmigration[1]. The first step depends on the selectin molecules expressed on both neutrophils and endothelial cells (EC)[2],[3]. The second step is mediated through interactions of the β_2 integrins[4],$\alpha_L\beta_2$ and $\alpha_M\beta_2$, present on the neutrophils and their counter receptors, ICAM-1 and ICAM-2, on the EC. Neutrophil-EC interaction can also be mediated by fibrinogen (Fg)[5]. ICAMs bind directly to $\alpha_L\beta_2$ [6]and aMb2[7], whereas Fg bridges neutrophils and EC by binding to $\alpha_M\beta_2$ and ICAM-1[5]. Neutrophils from patients with Leukocyte Adhesion Deficiency (LAD) fail to adhere and transmigrate through EC, resulting in life-threatening bacterial and fungal infections[8]. The role of $\alpha_M\beta_2$ in neutrophil adhesion and transmigration has been well demonstrated in animal models using function blocking mAbs[9-11], $\alpha_M\beta_2$ inhibitors[12], and $\alpha_M\beta_2$ -deficient mice[13],[14].

Neutrophils and their associated diseases

Despite the essential role of neutrophils in host defense, inappropriate neutrophil activation has detrimental consequences[15]. The superoxide radicals and proteolytic enzymes produced by activated neutrophils cause ischemia/reperfusion injury and tissue damage[16],[17]. In addition, activated neutrophils produce a multitude of cytokines [18],[19] which initiate and sustain the chronic inflammatory process, leading to the development of various autoimmune diseases[16],[20]. Consistent with these deleterious effects, blockade of $\alpha_M\beta_2$ -mediated ligand recognition by neutrophils using mAbs or inhibitors decreases ischemia/reperfusion injury[21],[22], reduces myocardial infarction size, myocardial necrosis[23], and liver cell injuries[24], and diminishes neointimal thickening and restenosis after angioplasty[25]. The $\alpha_M\beta_2$ blocking mAbs are also effective in the treatment of gram-negative sepsis and hemorrhagic shock[26]. Although therapies using these function-blocking antibodies are very promising, nonselective blockade of all leukocyte functions, such as neutrophil activation, transmigration, and phagocytosis, also leads to severe complications, such as bacteriall and fungal infections[27].

The $\alpha_M\beta_2$ integrin recognizes multiple ligands

$\alpha_M\beta_2$, a heterodimeric surface receptor, belongs to the β_2 integrin subfamily. These "leukocyte" integrins are composed of a common β_2 subunit noncovalently linked to one of four distinct yet highly homologous a subunits, *αL, αM, αX, and αD*[28], [29]. $\alpha_M\beta_2$ is expressed by neutrophils, monocytes and NK cells, and recognizes a multitude of very different protein and nonprotein ligands. These multiple interactions provide a molecular basis for the versatile roles of neutrophils and monocytes in host defense. Protein ligands for $\alpha_M\beta_2$ include extracellular matrix proteins such as fibronectin, laminin, collagen and vitronectin[30]; counter-receptors of the immunoglobulin super-

family such as ICAM-1[31] and ICAM-2[32]; blood coagulation proteins such as fibrinogen[33], factor X[34], and kininogen[35]; and the complement pathway product, C3bi[36]; as well as haptoglobin[37],denatured albumin[38], KLH[39], myoloperoxidase [40] and elastase[41]; non-protein ligands for $\alpha_M\beta_2$ include LPS[42], zymosan, β-glycans[43], heparin[44],[45] and oligodeoxynucleotide[46]. In addition, a variety of microorganisms produce $\alpha_M\beta_2$ ligands (e.g. NIF[47], WI-1[48] and gp63[49]) as a means of subverting or bypassing host defense mechanisms [50]. Unlike certain integrins in the $\beta_1$and 3 sub-families, where a single receptor interacts with many different proteins through the common RGD sequence[51], the $\alpha_M\beta_2$ ligands share few, if any, similarities or conserved sequences. The molecular structure of the $\alpha_M\beta_2$ receptor that enables it to interact with many unrelated ligands is not yet clear, nor are the physiological functions of these interactions.

Structural basis of $\alpha m \beta 2$-ligand interactions

At least five structural domains exist in $\alpha_M\beta_2$: the I-domain, the cation-binding repeats, and the lectin binding domain in the a subunit, and the putative I-domain and the protease resistant cysteine-rich region in the b subunit. The I-domain is a region of ~ 200 amino acids and is found only in certain integrin a subunits. The crystal structure of the αMI-domain was solved[52] and was shown to contain seven helices and six b sheets connected by short surface loops. Five residues located within several of these loops form a novel cation binding site, termed the MIDAS motif[52]. Recently, structures of other I-domains were determined. a-helices/b-sheets folds similar to those of the αMI-domain have been observed[53-55]. The role of the I-domains in the ligand binding has been well established. Diamond, et al[56], find that binding of C3bi and ICAM-1 to $\alpha_M\beta_2$ is blocked by mAbs to the aMI-domain, suggesting a spatial proximity between these ligand-binding sites. We[57] and others[58], [59] showed that the recombinant aMI-domain interacts with NIF, ICAM-1, C3bi, and Fg. The importance of the I-domain in ligand binding has also been demonstrated in other integrins ($\alpha_L\beta_2$,$\alpha_X\beta_2$, $\alpha_2\beta_1$and $\alpha_1\beta_1$)[60-63]. Given the similarity of the I-domain structures, it is not well understood how I-domains can recognize a multitude of very different proteins. Previous studies have shown that five amino acids (Asp^{140}, Ser^{142}, Ser^{144}, Asp^{242}, and Thr^{209}), conserved within essentially all I-domains, are critical to ligand-binding activity of all I-domain-containing integrins[52],[64-68], regardless of the nature of their ligands. Thus, the specificity of each integrin, which is vital to its individual in vivo roles, can not be derived from these conserved residues. To determine the molecular basis for integrin $\alpha_M\beta_2$ to interact with multiple ligands, we have used the Homologue- Scanning Mutagenesis approach[69] and systematically probed the entire outer hydrated surface of the I-domain of aM. We found that overlapping but non-identical regions within the I-domain are involved in recognition of different ligands by the $\alpha_M\beta_2$ receptor[70]. We have further mapped the ligand binding pocket for NIF to a narrow region composed of

Pro^{147}-Arg^{152}, Pro^{201}-Lys^{217}, and Asp^{248}- Arg^{261} of αm[71].

In addition to the I-domain, the cation binding repeats in integrin a subunits also have been implicated in ligand binding. D' Souza, et al, first implicated the second cation-binding repeat of aIIb in $\alpha_{IIb}\beta_3$ binding of the Fg-γ chain[72]. The cation-binding repeats of $\alpha_M\beta_2$ are also important in ligand binding. Altieri, et al, showed that a mAb (OKM1) recognizing an epitope in the cation-binding repeat[56], completely blocked Fg binding to $\alpha_M\beta_2$[73]. Other antibodies mapped to the cation-binding repeats have potent inhibitory effects on ICAM-1 binding to $\alpha_M\beta_2$ [56]. In contrast, antibodies recognizing the region between the cation-binding repeats and the transmembrane region are poor inhibitors of $\alpha_M\beta_2$ functions, and are presumed not to be directly involved in ligand interaction[56]. The involvement of the cation-binding repeats in ligand recognition was also illustrated in $\alpha_L\beta_2$ and $\alpha_2\beta_1$using recombinnt fragments[61],[74]. Springer has recently proposed, based on computational analysis, that the region surrounding the cation-binding repeats folds into a β-propeller-like structure[75]. Though very appealing, this model is yet to be confirmed directly with experimental data. To this end, several recent studies have provided encouraging data consistent with this model [76],[77]. The ultimate test of this model will rely on structural studies using either X-ray crystallography or two-dimensional NMR.

In addition to the a subunit, binding of some ligands requires cooperation from the bsubunit as well. Similar to aIIbβ_3, the homologous D^{134}XSXS sequence within β_2 is also important for $\alpha_M\beta_2$ binding to C3bi, Fg, and ICAM-1. Mutations of these oxygenated residues into Ala abolished $\alpha_M\beta_2$ binding to these three ligands[70],[78],[79]. Thus, $\alpha_M\beta_2$ -ligand interaction involves discrete regions within the I-domain and the β-propeller of the a subunit, as well as the β_2 subunit. Further studies are required to ascertain the exact roles of these different regions of $\alpha_M\beta_2$ in ligand binding.

Conclusion

$\alpha_M\beta_2$ is involved intimately in every aspects of neutrophil functions, by virtue of its ability to recognize multiple different protein and non-protein ligands. The molecular basis that confers $\alpha_M\beta_2$ with such extraordinary capability is, in part, an overlapping but non-identical ligand binding pocket, and involves the cooperation between the I-domain and the β-propeller region, as well as the β-subunit. Understanding of the molecular basis of $\alpha_M\beta_2$-ligand interactions will enable us to precisely control neutrophil functions, that is, to avoid the deleterious effects of neutrophil activation while keeping intact its host defense function.

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