INTEGRINS AS PATTERN RECOGNITION RECEPTORS

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ABSTRACT

Integrins have long been recognized as cell adhesive molecules anchoring cells to the extracellular matrix. However, recent evidence is emerging that certain integrins can recognize bacterial products and initiate immune responses independently from other pattern recognition receptors, such as Toll-like receptors. Recognition is likely initiated when ligands bind integrins, leading to separation of the cytosolic domains, allowing access of src-family kinases to tyrosine phosphorylation sites. This provides anchoring points for multiple adaptors and kinases, such as Pyk2 and FAK, which in turn provide scaffolding for multiple adaptor and signaling proteins. In addition to actin cytoskeletal rearrangement, recognition by integrins also activates signaling cascades, such as the NFκB and MAP kinase pathways. These pathways can ultimately result in the stimulation of many different cellular responses, such as increased expression of the inflammatory cytokine IL-8.

Keywords: pattern recognition receptors, integrins, signaling, innate immunity

Pattern recognition receptors are a diverse group of receptors that recognize molecular patterns associated with pathogens (PAMPs). These include Toll-like receptors (TLRs), nuclear oligomerization domain proteins (NODs), the macrophage mannose receptor (MR) and other C-type lectin-like receptors, several scavenger receptors (SR), peptidoglycan recognition proteins (PGRPs), peptidoglycan-lytic enzymes (lysozyme and amidase), cytoplasmic RNA helicase, and several integrins. Until recently, integrins were considered passive sticky points for cellular adhesion to the extracellular matrix, but current research indicates a much more dynamic role for integrins: adhesion can be quickly turned off and on to allow for cell movement. Furthermore, integrin-mediated cell contact has been shown to trigger a surprisingly extensive signaling network, not only regulating actin cytoskeleton reassembly but also cellular survival, proliferation, and release of inflammatory mediators (Cheng et al., 2001; Pacifici et al., 1992; Rubel et al., 2003). Simultaneously, evidence has emerged that some integrins recognize degraded proteins and bacterial proteins, in addition to host proteins. In at least some cases, this leads to immune system activation, similar to the activation generated by more widely recognized pattern recognition receptors such as TLRs and NODs. This review will present examples of pattern-recognizing integrins, a short summary of integrin structure and signaling, and how signaling could lead to observed immunological responses.

Integrins recognizing PAMPs

Integrins have traditionally been understood to be involved in cell adhesion, but in addition, some integrins recognize degraded proteins and bacterial products (Table 1). For example, Vorup-Jensen and colleagues ascertained pattern recognition function for $\alpha_x\beta_2$ (CD11c/CD18, CR4) and $\alpha_M\beta_2$ (Mac-1, CD11b/CD18, CR3) integrin receptors by demonstrating recognition of degraded proteins with exposed acidic amino acid residues (Vorup-Jensen et al., 2005). The authors considered this significant, since bacterial infections and other pathologic process liberate numerous misfolded, degraded proteins such as bacterial subtilisin-digested fibrinogen (subtilisins are serine proteases secreted by members of the genus *Bacillus*). These degraded proteins need to be efficiently recognized and removed by systems such as the $\alpha_x\beta_2$/urokinase-type plasminogen activator complex, as they represent obstacles to cell migration during wound-healing. Additionally, they also represent danger signals that warrant activation of the immune system to fight off invading pathogens (Gallucci and Matzinger, 2001; Murphy and Gavrilovic, 1999; Petty et al., 2002).

In addition, $\alpha_L\beta_2$ (LFA-1, CD11a/CD18) and activated $\alpha_M\beta_2$ recognize *Porphyromonas gingivalis* FimA peptides leading to leukocyte activation (Hajishengallis et al., 2005; Ogawa et al., 2002). Both $\alpha_M\beta_2$ and $\alpha_x\beta_2$ appear to initiate TLR/CD14-independent LPS responses as block/knockout of β_2 significantly reduces LPS-responses in CD14- CHO cells (Flaherty et al., 1997; Ingalls et al., 1997; Ingalls and Golenbock, 1995; Medvedev et al., 1998).

Behera and coworkers recently demonstrated binding of *Borrellia burgdorferi* to $\alpha_3\beta_1$, which results in TLR-independent induction of proinflammatory cytokines, chemokines, and end-effector molecules such as matrix metalloproteinases in primary human chondrocyte cells (Behera et al., 2006). Similarly, *Streptococcus mutans* proteins I/IIf are recognized by $\alpha_5\beta_1$ in epithelial cells leading to IL-8 release (Al-Okla et al., 1999). Integrin subunits α_5, α_V,

β_1, and β_3 can recognize fibronectin-binding proteins produced by *Mycobacterium avium* and other bacteria (Secott et al., 2004). Furthermore, $\alpha_5\beta_1$ is considered a pattern recognition receptor by Tyrer and colleagues (Tyrer et al., 2006), although they only report that *Mycobacterium* binding to this receptor leads to bacterial invasion, while immunological responses have not been monitored.

Integrin structure

Recognition of exposed acidic amino acid residues and the negatively charged moieties of degraded host proteins is most likely linked to the unique structure of integrin receptors, which allow existence of two states: open and closed (Li et al., 1998). However, intermediate states have been explored recently for $\alpha_L\beta_2$ (Salas et al., 2006). In the open or active state, integrins bind substrates with high affinity whereas at the closed or inactive state they bind substrates with low affinity. Integrin receptors are heterodimers of an a chain and a β chain, which are transmembrane glycoproteins characterized by a 950-1100 amino acid residue extracellular domain containing a globular head region (N-terminal), a long cysteine-rich stem, a transmembrane region, and a short cytosolic tail (15-77 amino acids) capable of binding different cell matrix proteins and signaling proteins (Akiyama, 1996; Diamond and Springer, 1994; Nermut, 1988). The a

Table 1. A summary of pattern recognition by integrins.
This table has been adapted from Diamond and Springer (1994), with bacterial ligands added.

The Integrin superfamily as pattern recognition receptors				
Integrin	Pattern recognition	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
$\alpha_3\beta_1$	***Borrelia burgdorferi***	Laminin, Collagen I, fibronectin, epiligrin	Domain 1 & 4 (VCAM-1), EILDV (CS-1, fibronectin)	Broad
$\alpha_4\beta_1$		VCAM-1, fibronectin	RGD	B & T-cells, Macrophages, neural crest cells
$\alpha_5\beta_1$	***Streptococcus mutans*** proteins I/IIf, ***Mycobacterium avium*** fibronectin binding prot.	Fibronectin		Broad
$\alpha_6\beta_1$		Laminin		Broad
$\alpha_7\beta_1$		Laminin		Muscle
$\alpha_8\beta_1$		fibronectin, osteopontin		endothelial cells, kidney
$\alpha_V\beta_1$	***Mycobacterium avium*** fibronectin binding protein	Vitronectrin, Fibronectin	RGD (Fibronectin)	epithelial cells
$\alpha_L\beta_2$	***Porphyromonas gingivalis*** FimA	ICAM-1, ICAM-2, ICAM-3	Domain 1 (ICAM-1)	Leukocytes
$\alpha_M\beta_2$	***Porphyromonas gingivalis*** FimA, LPS (?), degraded protein	iC3b, ICAM-1, fibronectin, Factor X, Fibrinogen	Domain 3 (ICAM-1), 30 kD plasmin fragment (Fibrinogen)	Granulocytes, macrophages, NK cells, CD8+ T-cells
$\alpha_X\beta_2$	degraded protein, LPS (?)	Fibrinogen, iC3b	GPRP (Fibrinogen)	Macrophages, granulocytes, activated B cells, DC subset
$\alpha_{IIb}\beta_3$		Fibrinogen, fibronectin, vWF, vitronectin, thrombospondin	RGD, KQADGV(fibrinogen)	Platelets
$\alpha_V\beta_3$	***Mycobacterium avium*** fibronectin binding protein	Vitronectin, Fibrinogen, vWF, thrombospondin, fibronectin, osteopontin, collagen	RGD	Endothelial cells, 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	airway epithelial cells
$\alpha_4\beta_7$		VCAM-1, fibronectin, MadCAM-1	EILDV (fibronectin)	Activated B and T lymphocytes, macrophages, intraepithelial lymphocytes
$\alpha_E\beta_7$		E-Cadherin	?	Activated B and T lymphocytes, macrophages, intraepithelial lymphocytes
$\alpha_V\beta_8$	**Foot and mouth disease virus**	Vitronectin, Fibrin	RGD (Vitronectin)	Astrocytes, Schwann cells, airway epithelial cells

chain globular head consists of seven repeats of approximately 60 amino acids, forming a seven-bladed β-propeller motif, containing four antiparallel β-sheets each (Springer, 1997; Takagi and Springer, 2002). Many integrins have an intervening, double-folded I-domain, between β-sheet 2 and 3, which consists of a hydrophobic β-sheet surrounded by hydrophilic α-helices (Huang et al., 2000). This hydrophobic β-sheet contains the metal-ion dependent adhesion site (MIDAS) (Takada et al., 1997), formed by five amino acid residues that coordinate a divalent metal ion (such as Mg^{2+}, Ca^{2+}; Mn^{2+} insertion leads to permanent activation) directly, or indirectly by the use of water molecules. Integrin ligands with acidic/negatively charged residues form the sixth coordinating partner for the divalent cation in the MIDAS. For many ligands such as fibronectin, the MIDAS-binding motif contains two critical and synergistic amino acid sequences: an Arg-Gly-Asp (RGD) sequence, and a Pro-His-Ser-Arg-Asn (PHSRN) sequence. The globular region of β-chains is less defined, but consists of an approximately 250 residue conserved region, which contains a predicted I domain, with a MIDAS-like motif (DXSXS). Mutations in this region have been shown to lead to leukocyte adhesion deficiency. Conformational changes within the I domain, such as movement of the C-terminal α-helices, can expose or block access to the MIDAS site, producing the open, closed, and intermediate states of integrins (Hobb et al., 2002; Salas et al., 2006; Takada et al., 1997). The cysteine-rich stalk for the α domain consists of a two layer β-sandwich structure, while the cysteine-rich stalk of the β domain is composed of the regions preceding and succeeding the β I-domain. These regions also contain a PSI domain, a region of homology with plexins and semaphorins. The four distinct regions of the cysteine-rich β-stalk appear to regulate ligand binding (Lu et al., 2001).

Cytoplasmic signaling pathways

While the interaction of integrins with ligands (*i.e.*, fibrinogen, β_2 integrins) regulate a wide array of cellular responses such as apoptosis, spreading, degranulation, cytokine release, and metalloproteinase release, the cytoplasmic signaling of these processes has not been well defined (Fernandez et al., 2005; Forsyth et al., 2002; Loike et al., 1995; Rubel et al., 2001; Rubel et al., 2002; Rubel et al., 2003; Vacca et al., 2001; Yan et al., 2004). However, it has been shown that ligand binding to integrins causes an extracellular conformational change, leading to separation of the cytoplasmic domains of the α and β chains (Figure 1), which operates in both inside-out and outside-in signaling (Kim et al., 2003). These two types of signaling are important as not only can ligand binding to integrins induce events inside the cell, but intracellular events can lead to activation of the integrin receptor, allowing higher affinity for substrates.

Beyond linking the extracellular matrix to the actin cytoskeleton, control of cell shape and motility (reviewed in Blystone, 2004; Liu et al., 2000), integrins also initiate signaling cascades leading to other cell responses (Figure 1). In most cases these cascades are believed to be initiated when ligand binding induces a conformational change separating the cytoplasmic tails, allowing recruitment of src homology tyrosine kinases and focal adhesion kinases to the β-chain (Lowell et al., 1996; Stupack and Cheresh, 2002). Any contribution of the α-chains to signaling has not yet been elucidated. It has been suggested that the role of the α chain might be to anchor chaperones or provide specificity to signaling (Hemler, 1998). For the β_3, β_1, β_7, and potentially β_5 chains, src kinases have been demonstrated to phosphorylate the highly conserved NPXY motif within the cytoplasmic tails and adaptor molecules. Concurrently, SH2-domain containing adaptor molecules such as Pyk2 (a focal adhesion kinase related molecule), bind the β subunit of the receptor (Pfaff and Jurdic, 2001). Pyk2 serves as a scaffold for PI3 kinase and Vav binding (Gismondi et al., 2003; Koziak et al., 2001). PI3 kinase phosphorylates phosphatidylinositol 3,4-bisphosphate (PIP2) to phosphatidylinositol 3,4,5-trisphosphate (PIP3). PIP3, as a specialized lipid, recruits serine/threonine-kinase Akt and phosphoinositol-dependent kinase 1 (PDK1) to the cell membrane, where PDK1 activates Akt by phosphorylation. This leads to inactivation of pro-apoptotic proteins (i.e., Bad, caspase 9), induction of anti-apoptotic proteins from NFκB-controlled genes by activation of IKB kinases, activation of endothelial nitrogen oxide synthetase (eNos) and translation regulators such as m-TOR (reviewed in Paez and Sellers, 2003). In addition, the small Rho-family GTPase rac1, serine-threonine kinase raf, and integrin linked kinase (ILK) are also targeted, affecting actin remodeling, MAPK pathways, and integrin signaling respectively. Recruitment of Vav, a guanine-nucleotide exchange factor, allows activation of GTPases like rac1 or rhoA. GTPase activation can activate cyclin dependent kinase cdc42, which is critical for WASP-protein induced activation of actin nucleation factors Arp2/3. In addition to actin remodeling, this process is also associated with higher

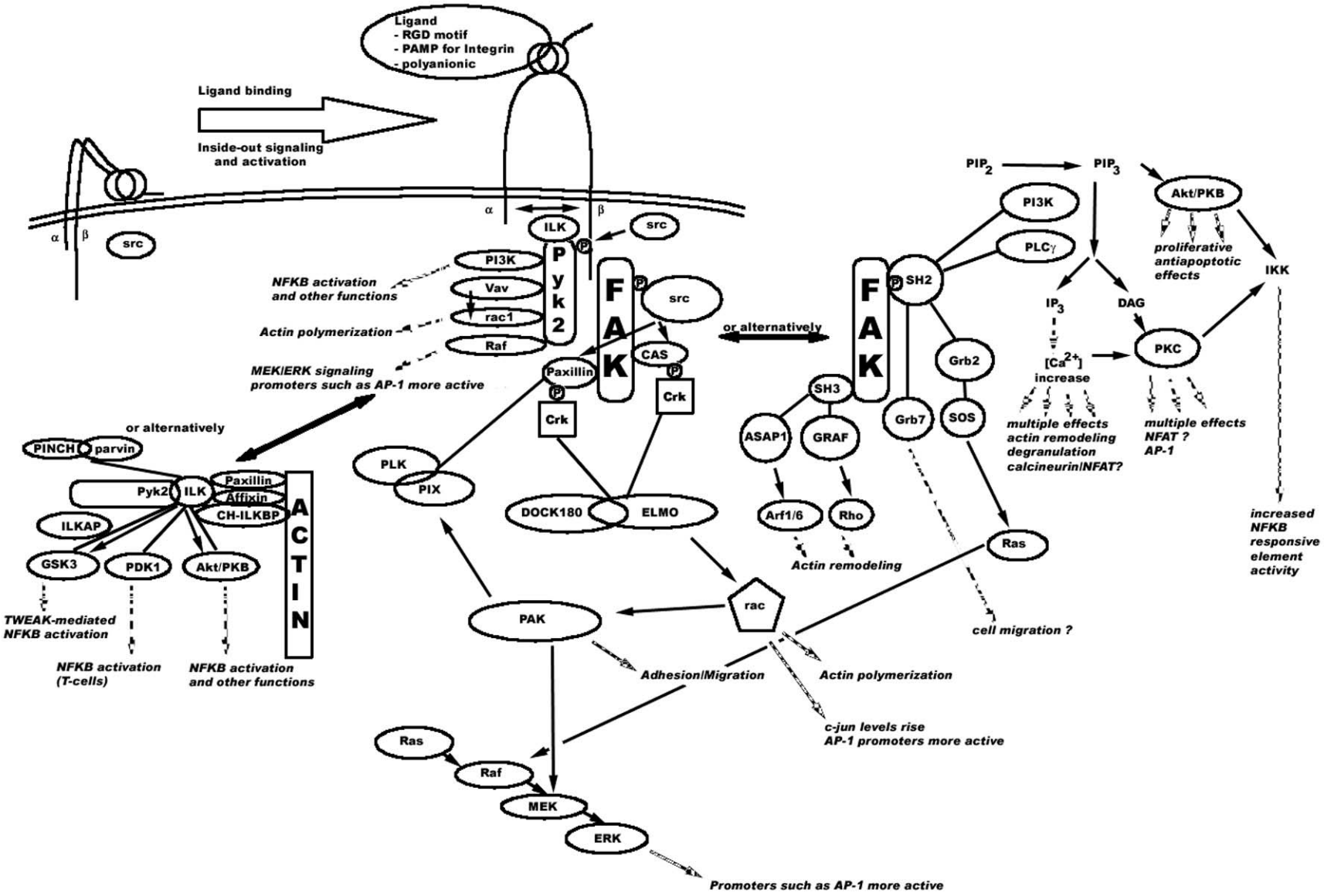

Figure 1. Integrin-mediated signaling. This diagram is based on the findings of Kim, Carman and Springer (2003), the reviews of Blystone (2004), Wu and Dedhar (2001), and Parsons (2003).

levels of the transcription factor c-jun, which leads to higher levels of transcription from AP-1 controlled genes.

ILK (reviewed in Wu and Dedhar, 2001), is a binding site for further adaptor proteins such as paxillin, affixin and CH-ILKBP. These adaptors connect the integrin/ILK complex to the actin cytoskeleton. ILK also binds other signaling proteins such as GS3K, PDK1, and PKB (GSK-3β and PKB are phosphorylated by ILK).

β2 integrins, however, have no tyrosines in their short cytoplasmic tail, necessitating other signaling pathways. Vavs are involved in controlling β2-dependent leukocyte functions such as phagocytosis (reviewed in Gakidis et al., 2004). This likely controls actin rearrangements and may also lead to higher levels of c-jun, possibly increasing transcription from AP-1 controlled genes. In addition, src kinases (fgr, lyn, hck) appear to be involved in recruiting a 110 kDa tyrosine-phosphorylated protein complex containing Pyk2 or focal adhesion kinase (FAK, reviewed in Parsons, 2003) to the β2 chain (Totani et al., 2006). FAK has multiple tyrosine residues that can be autophosphorylated and serve as binding points for SH2-domain-containing signaling proteins. For example, phosphorylation of Y397 allows binding of Src family kinases which can efficiently phosphorylate other tyrosines on FAK, as well as other molecules bound to FAK-tyrosines, such as cas and paxillin (Owen et al., 1999; Schaller et al., 1999). Both phosphorylated cas and paxillin may serve as binding points for SH2/SH3 adaptor Crk, which could further recruit rac activating complexes DOCK180/ELMO, associated with actin rearrangement and membrane ruffling (Lu and Ravichandran, 2006; Wheeler et al., 2006). Interestingly, paxillin and rac may recruit and phosphorylate p21-activated kinase (PAK), in turn influencing MAPK pathways through MEK phosphorylation (del Pozo et al., 2000).

Additionally, phosphorylation of FAK Y397 also allows other SH2-domain containing proteins, such as the 85 kDa subunit of PI3 kinase (Akagi et al., 2002), phospholipase C g (PLCg) and adaptor protein Grb7. Furthermore, phosphorylation of Y397 and Y925 recruits the Grb2-SOS complex. This potentially allows initiation of multiple signaling cascades, such as the PI3K/PTEN/Akt proliferative/anti-apoptotic response pathway, and the NFκB activation pathway (reviewed in Paez and Sellers, 2003). The PLCγ pathway results in elevated IP3, DAG, and intracellular calcium levels. Elevated calcium levels activate calcium-dependent phosphatases, such as calcineurin, which dephosphorylates and activates transcription factor NFAT in T-cells. Elevated calcium and DAG levels also activate many PKC isoforms (reviewed in Patterson et al., 2005), leading to NFκB and JNK/AP-1 transcription factor activation in T-cells (Carpenter and Ji, 1999), (Isakov and Altman, 2002). For other cells, downstream targets of other PKC isoforms are less known, but seem to involve at least activation of AP-1 in some cells (Fung et al., 1997). Grb7 downstream signaling is still unresolved, but is thought to regulate cell migration (reviewed in Han et al., 2001; Shen and Guan, 2004). Finally, Grb2-SOS complex activates specific MAPK pathways in different cells ((Blaukat et al., 1999); MAPK transcription factor modulation reviewed in Treisman, 1996).

Furthermore, FAK's proline-rich site II motif binds two SH3-domain containing regulators of small GTPases that regulate cytoskeleton reorganization: GRAF, a GAP (GTPase activating protein) for Rho (Taylor et al., 1999), and ASAP1, a GAP for Arf1 and Arf6 (Brown et al., 1998; KruljacLetunic et al., 2005).

Finally, there seems to be considerable crosstalk between integrin-mediated signaling and other signaling cascades, such as from Toll-like receptors. For instance, FAK is interlinked with the MyD88 pathway (Toll, see (Zeisel et al., 2005)) or cytohesin/PI3K with CD14 and TLR2 (Sendide et al., 2005). Additionally, it has been reported that integrin receptors recruiting Toll/Interleukin 1 receptor signaling intermediates such as TRAF6, ultimately lead to NFκB activation (Shi et al., 2001). Furthermore, integrins may regulate signaling from other pathways. In the case of $\alpha_M\beta_2$ (CR3) signaling through the PI3K pathway brings about a delayed wave of degradation and inactivation of IRAKs in the CD14/TLR4 signaling pathway (Noubir et al., 2004).

Effects of integrin-mediated PAMP recognition and signaling

One of the primary effects of integrin receptor pattern recognition and signaling is reorganization of the cytoskeleton, which could lead to phagocytosis of an integrin-bound pathogen. This would lead to internalization of bacteria (Tyrer et al., 2006), possibly allowing antigen processing and presentation on MHC II complexes in antigen-presenting cells. In the case of granulocytes, pattern recognition by integrins contributes to actin reorganization necessary for degranulation and release of inflammatory mediators (Sivalenka and Jessberger, 2004; Walzog et al., 1994). Similarly, production of superoxide anions in granulocytes is in part regulated by integrin signaling (Jakus et al., 2004; Lowell et al., 1996; Suzuki et al., 2003).

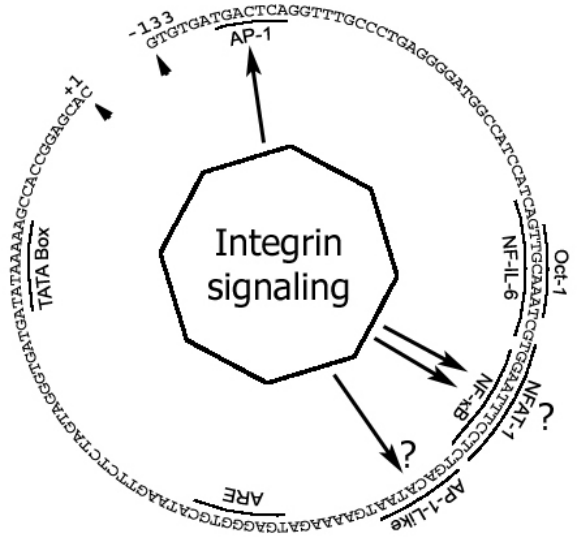

Figure 2. Integrin-mediated signaling and putative responsive elements within the IL-8 promoter. Modified from Roebuck, K.A., (1999).

In addition to cytoskeleton-mediated effects, integrins recognizing PAMPs induce release of cytokines and chemokines. For instance, *Streptococcus mutans* proteins I/IIf are recognized by $\alpha_5\beta_1$ in epithelial cells leading to IL-8 release (Al-Okla et al., 1999). This may be possible since IL-8 gene expression is controlled by derepression of the gene promoter (Figure 2), transcriptional activation of the gene by NFκB and JNK pathways, and stabilization of the mRNA by the p38 mitogen-activated protein kinase pathway (Hoffmann et al., 2002). As outlined earlier in this review, NFκB transcription factors can be activated in numerous ways during integrin signaling, which might allow binding of IL-8 specific NFκB transcription factors next to NRF, converting it to a coactivator, and binding of CBP/p300, which allows chromatin remodeling and derepression of the IL-8 gene. In addition to NFκB binding sites, IL-8 gene expression requires AP-1 element binding. This might be provided by activation of JNK signaling induced by the integrins as outlined above. Finally, p38 MAPK could be activated by rho GTPase rac (Murga et al., 2002), a downstream target of integrin signaling. Therefore, pattern recognition of *S.mutans* protein I/IIf by integrins could meet all requirements needed for IL-8 gene expression, leading to IL-8 release.

Summary

Recent evidence suggests that β integrins are capable of recognizing distinct molecular patterns of human pathogens. This leads to conformational changes within the integrin receptor, which allows for recruitment of signaling mediators to the β-chain cytoplasmic tail. In addition to triggering cytoskeletal rearrangements, these signaling mediators can activate NFκB and MAP kinase pathways which upregulate cell survival and the expression of many cytokines. Since integrins are expressed abundantly on leukocytes and epithelial cells, integrins may be in prime position to recognize invading pathogens and initiate an effective immune response, together with more specialized pattern recognition receptors.

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