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The Role of Epithelial Integrin Receptors in Recognition of Pulmonary Pathogens

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Integrins · Lung · Epithelial cells · Innate Immunity · Pattern recognition receptors · Host-pathogen interactions

Abstract
Integrins are a large family of heterodimeric transmembrane cell adhesion receptors. During the last decade, it has become clear that integrins significantly participate in various host-pathogen interactions involving pathogenic bacteria, fungi, and viruses. Many bacteria possess adhesins that can bind either directly or indirectly to integrins. However, there appears to be an emerging role for integrins beyond simply adhesion molecules. Given the conserved nature of integrin structure and function, and the diversity of the pathogens which use integrins, it appears that they may act as pattern recognition receptors important for the innate immune response. Several clinically significant bacterial pathogens target lung epithelial integrins, and this review will focus on exploring various structures and mechanisms involved in these interactions.

Introduction

Integrins are a large family of αβ heterodimeric transmembrane receptors that interact with components of the extracellular matrix (ECM) and some cell-surface receptors. In humans there are 18 α and 8 β subunits which form 24 different heterodimers [reviewed by 1]. Large extracellular domains of integrins mediate interactions with extracellular ligands, while the cytoplasmic domains mediate communications with the cytoskeleton and signaling molecules [reviewed by 2]. Based on the crystal structure resolution of the αvβ3 integrin extracellular domain, ligand recognition is mainly mediated by a cationic binding site on the β subunit adjacent to the exposed α subunit [3, 4]. In addition, half of the 18 α subunits contain a 200-amino acid inserted (I) domain which contributes to ligand recognition and specificity [2, 5].

During the last decade, the role of integrins in interactions of various cells with their microenvironment has become a focus of intensive research. Recent studies on monocytes, neutrophils, platelets, fibroblasts, endothelial, and pulmonary as well as intestinal epithelial cells (EC) demonstrated integrin involvement in regulation of virtually all vital cellular functions, including cell survival, proliferation, differentiation, migration, and cytokine production [reviewed by 1, 6, 7]. Upon binding their extracellular ligands, integrins transmit outside-in signals that regulate various cellular functions. In addition, integrins are able to provide inside-out signaling regulating the affinity of integrin binding to its ligand, and such signaling can be induced by cellular activation with chemokines or cytokines. Hence, integrins act as bidirectional signaling molecules [1]. Several signaling pathways activated by integrin engagement were identified, such as mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3-K) pathways [8, 9]. Integrins

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are involved in focal adhesion complexes comprising over 20 signaling and adaptor proteins, regulating actin cytoskeleton rearrangement and cell motility [10]. Binding of an integrin receptor to its ligand results in large-scale conformational changes such as separation of the cytoplasmic domains of the α and β subunits which causes cytoskeletal rearrangements and activation of downstream signaling [11]. According to the current concept, integrins act as specific sensors for dynamic changes in the microenvironment that occur during tissue development, inflammation, and tumorigenesis, and modulate cellular responses to these changes [6, 7, 9].

Integrin receptors of leukocytes are vital in both innate and adaptive immune responses. In particular, β2 integrins, such as LFA-1 (αLβ2) and Mac-1 (αMβ2), are essential for the activation of lymphocytes and for leukocyte migration during inflammatory responses. Congenital deficiency in β2 integrins (i.e. the leukocyte adhesion deficiency) is characterized by recurrent, severe bacterial infections that are eventually fatal [12]. Recent studies have emphasized the importance of leukocyte integrins in the cross talk with immunoreceptors, including T cell receptor and Fc receptors, for immune responses [13]. However, the role of epithelial integrins in innate immune and inflammatory responses in mucosal tissues, such as pulmonary epithelium, remains poorly understood.

Integrin Receptors in the Lung

EC are currently recognized as primary elements generating inflammatory signals to activate other cells in the lung [14]. Pulmonary EC express an array of innate immune receptors, such as Toll-like receptors (TLR), as well as cytokine, growth factor and histamine receptors, involved in the regulation of dynamic interactions of the epithelium with the environment. Integrin receptors are significantly represented in pulmonary epithelium. Eight different integrin heterodimers are expressed in airway EC, i.e. α2β1, α3β1, α6β4, α9β1, α5β1, αvβ5, αvβ6, αvβ8 [7]. These heterodimers recognize a range of ECM proteins: collagen I, tenascin C, laminins 5, 10, 11, osteopontin, fibronectin (Fn), vitronectin (Vn), and others [7]. It is known that lung epithelial integrins are critical for maintaining epithelial integrity, repair of damaged cells, and regulation of cell differentiation and proliferation [7, 15]. The expression of integrin receptors in the respiratory epithelium is tightly regulated, and rapid increase in α5β1 integrin level in response to injury has been demonstrated [16]. Accordingly, integrin receptor ligands such as Fn, Vn, tenascin C, and osteopontin are rapidly induced at sites of epithelial damage or injury [7].

Despite the significant advances in the understanding the functions of pulmonary integrins, signaling pathways regulated by these receptors in the lung are still incompletely characterized. Lung epithelial integrins are known to provide co-stimulatory signals towards growth factor receptors, regulating cell survival and proliferation [reviewed by 7]. However, the co-stimulatory functions of pulmonary integrins appear to be wider and involve the cross talk with other receptors. We have recently found that β1 integrins in human bronchial EC provide co-stimulatory signals that increase TNF-induced proinflammatory responses [17]. Interestingly, integrin-mediated responses in these cells involved activation of the nonreceptor protein tyrosine kinase (PTK) Syk recently discovered in the respiratory epithelium [17].

The Role of Integrin Receptors in Recognition of Pathogenic Microorganisms

Several significant human pathogens are known to utilize integrins and exploit integrin-mediated signaling to invade various types of host cells. Such mechanisms can be advantageous to the microorganisms, because the invasion of host cells often confers protection against the immune response, and may facilitate microbial growth and spreading to other cells. On the other hand, the resulting integrin-mediated signaling is potentially important for innate immune and inflammatory responses to the pathogen. As a variety of pathogens (bacteria, viruses, and fungi) bind integrins and elicit integrin-mediated signaling, it seems likely that integrins may serve as pathogen recognition receptors.

Several pathogenic bacteria are able to bind integrin receptors directly, via some specific adhesins. These are typically not respiratory pathogens but ones that rather invade other mucosal tissues such as the gastrointestinal epithelium (*Yersinia enterocolitica, Y. pseudotuberculosis* [18–21] and *Helicobacter pylori* [22]), or urethral epithelium (*Neisseria gonorrhoeae* [23]). The best-studied example of bacteria directly binding and exploiting integrin-mediated signaling mechanisms is the enteric pathogen *Y. pseudotuberculosis* [reviewed by 24]. These bacteria possess an outer membrane protein (OMP) invasin that binds to the β1 subunit of five integrin heterodimers (α3β1, α4β1, α5β1, α6β1 and αvβ1) expressed on microfold (M) cells in Peyer's patches of the small intestine [18].

Table 1. Respiratory bacterial pathogens that exploit integrins or their ECM ligands during infection

Bacteria	Bacterial structures interacting with integrins	Integrins involved	Results of bacterial interactions with integrins	Ref. No.
S. aureus	FnBP A or B	α5β1	Adhesion/invasion Activation of FAK and Src signaling ILK-dependent internalization	39–41 46–48 49
S. pyogenes	M1 protein PrtF1/SfbI	α5β1	Adhesion/invasion of lung EC ILK activation Paxillin phosphorylation-dependent internalization	49, 57, 60
	Scl1	α2β1	Adhesion/invasion of lung EC	64
Mycobacterium species	FAP Antigen 85B	α5, αv, β1, β3	Adhesion to and invasion of lung EC	66, 73
P. aeruginosa	Putative 50 kDa OMP	α5β1 or αvβ5	Adhesion to and invasion of lung EC	76, 78, 79
B. pertussis	FHA	α5β1 (lung EC)	Activation of lung EC inflammatory response	83, 87, 88
		αMβ2 (alveolar macrophages)	Invasion of alveolar macrophages and induction of inflammation	81, 86
H. influenzae	Hap	α5β1, potentially α3β1, αvβ6	Involved with TLR-4 and platelet-activating factor receptor-dependent uptake by M cells	92, 93
S. pneumoniae	PavA FnBP	α5β1?	Unclear, associated with adherence and invasion of EC	93, 94

Binding of invasin to integrin receptors leads to formation of focal adhesion complexes and subsequent activation of intracellular signaling [20]. The resulting activation of the guanosine triphosphatase (GTPase) Rac1 causes cytoskeletal rearrangement, mediating bacterial internalization [reviewed by 25].

Some bacteria have the ability to bind integrins both directly and indirectly through an ECM ligand. For example, *Borrelia burgdorferi*, the causative agent of Lyme disease, possesses an Fn-binding protein (FnBP) BBK32 [26]. In addition, *B. burgdorferi* has an α3β1 integrin-binding protein BBB07 which directly activates proinflammatory responses in human chondrocytes [27, 28], as well as an αvβ3-binding OMP P66 which mediates bacterial adhesion to host cells [29].

However, the majority of integrin-binding microorganisms interact with integrins indirectly using ECM-binding proteins as a molecular bridge to engage these receptors. In these cases, integrin receptors recognize the common arginine-glycine-aspartate (RGD) sequence that is present in ECM proteins, such as Fn or Vn [30]. The resulting integrin-mediated signaling does not seem to depend on the type of the interactions, as both direct and indirect binding to integrins lead to tyrosine kinase phosphorylation, recruitment of adaptor molecules, and cytoskeletal rearrangement required for bacterial engulfment, as well as induction of proinflammatory cellular responses. However, the number of known microbial ECM-binding adhesins greatly outweighs those that bind integrins directly.

Several clinically significant bacterial pathogens target lung epithelial integrins, and this review will focus on exploring various structures and mechanisms involved in these interactions (summarized in table 1).

Staphylococcus aureus

S. aureus is an important pathogen causing nosocomial pneumonia as well as an initial lung infection in cystic fibrosis (CF) patients frequently followed by *Pseudomonas aeruginosa* [31]. The exploitation of ECM products and integrins by *S. aureus* during the infectious process was recently discussed in an excellent review by Hauck and Ohlsen [32].

The ability of *S. aureus* to bind Fn is mediated by the FnBP that exists as two variants, A and B, encoded on two closely related genes [33]. *S. aureus* FnBP adheres strongly to the N-terminal of Fn [34], specifically to the five type I-module amino terminal repeat motif of Fn [35], exploiting the modular structure of Fn by forming a tandem β-zipper attachment site [36].

S. aureus exploit the adhesion to Fn by using the latter as a molecular bridge between bacteria and host integrins, allowing the bacteria adhere to the cell surface and also to invade the host cells by becoming internalized [reviewed by 37, 38]. The use of Fn to bind to α5β1 integrins on host cells was originally proposed by Sinha et al. [39] based on the ability of β1 antibody to inhibit *S. aureus* invasion of human embryonic kidney cells, and these findings were supported by similar studies using HeLa EC and endothelial cells [40, 41].

S. aureus α-toxin is secreted by the bacterium during later stages of infection, and can interact with β1 integrins resulting in decreased adhesion and invasion of the pathogen. The interaction of α-toxin with β1 integrin inhibits bacterial adhesion via the Fn bridge, thus eliminating cell signaling activation that would cause the internalization. This is an elegant example of a bacterial interference with the adhesion/invasion cell machinery during the later stages of infection, when it may be advantageous for the pathogen to seek out new infectious targets [42]. Although the molecular mechanisms of interactions between *S. aureus* α-toxin and integrin receptors remain undefined, a recent study suggests a possibility of a direct binding of the α-toxin to α5β1 integrin in lung EC [43]. Such interactions can be potentially involved in the pathogenesis of staphylococcal pneumonia as the α-toxin-induced death of EC was found to be partially mediated by α5β1 integrin [43].

A number of studies suggest that the internalization of *S. aureus* depends on cellular events initiated by integrin receptors following bacterial adhesion [39, 44]. PTKs, which can be found in integrin-associated signaling complexes, are activated by the engagement of β1 integrins and involved in *S. aureus* internalization [45]. More specifically, it is the signaling via the Src family of PTKs that is necessary, since Src inhibitors and certain Src-deficient cell lines show decreased *S. aureus* uptake [46, 47]. Focal adhesion kinase (FAK) is another PTK whose inhibition results in decreased *S. aureus* invasion, suggesting a role of FAK as a signaling intermediate between integrins and Src [48]. The serine-threonine protein kinase integrin-linked kinase (ILK) is attached to the actin cytoskeleton, and is also necessary for *S. aureus* uptake, emphasizing the importance of actin remodeling for the internalization of bacteria [49]. In addition, *S. aureus* binding to integrins via FnBP can activate actin remodeling that results in increased bacterial motility on the cell surface preceding the internalization [50]. Hence, binding of *S. aureus* to α5β1 integrin via FnBP activates signaling pathways that can mediate host responses to the pathogen.

Although interactions of *S. aureus* with airway EC are incompletely understood, *S. aureus* adhesion to these cells has been shown to be significantly dependent on the presence of a functional FnBP, suggesting the involvement of integrins in this process [51]. However, the role of integrins in the internalization of *S. aureus* by pulmonary EC has not been directly addressed.

Streptococcus pyogenes

S. pyogenes is an important pathogen that primarily infects the skin and epithelium of the upper respiratory tract. *S. pyogenes* can cause severe pneumonia, as well as wound infections, septicemia, and endocarditis [52]. It has been known since the 1980s that *S. pyogenes* is capable of binding Fn, and that the attachment to ECM proteins may be important in invasion of the epithelium [53, 54]. This is now understood to be due to numerous FnBPs present on the surface of *S. pyogenes*, particularly M1 protein and PrtF1/SfbI [55]. Cue et al. [56] found that *S. pyogenes* binding of Fn via M1 protein was critical for invasion of EC, and that this process was abrogated by antibodies to α5β1 integrin. Using integrin inhibitors, it was demonstrated that the invasion of EC by streptococci was mediated by formation of integrin α5β1-Fn-M1 protein complexes [57]. However, M1–/SfbI+ strains of *S. pyogenes* are also capable of invading EC in an integrin-mediated fashion suggesting the redundancy in the mechanisms of bacterial pathogenesis [58, 59]. More recent studies have demonstrated that blocking of ILK, a key molecule in integrin-mediated signaling, abolished *S. pyogenes* uptake [49]. Downstream of ILK, phosphorylation of the adaptor protein paxillin has been shown to be crucial in M1+ *S. pyogenes* internalization [60]. These findings provide clear evidence that *S. pyogenes* utilize integrins as receptors during EC invasion.

It has been suggested that streptococci may induce upregulation of integrins in EC to allow for increased bacterial adhesion and internalization. Indeed, during infection of lung EC with *S. pyogenes*, gene transcription of α5 integrin and Fn greatly increased and was followed by an increase in both α5 integrin and Fn protein expression by EC [61]. Moreover, *S. pyogenes* are able to induce active transforming growth factor (TGF)-β_1 production in human tonsil fibroblasts, and TGF-β_1 in turn upregulates the expression of both α5 integrin and Fn [62]. As a result of an increased α5β1 integrin expression, a subsequent increase in streptococcal invasion occurred, this time in an FnBP-dependent manner [62]. The latter study also

suggested that *S. pyogenes*-infected fibroblasts can represent chronic sources of TGF-β_1 in vivo, causing upregulation of integrins in the surrounding epithelium. Interestingly, there appears to be a reciprocal interaction between TGF-β and integrin receptors, as αvβ6 integrin can bind TGF-β latency-associated peptide and activate TGF-β [63]. This study suggested that the complex interplay involving TGF-β and integrin αvβ6 in lung EC initiated by microbial compounds is critical in lung innate immune defense [63].

In addition to the well-known process of streptococcal internalization mediated by the FnBP, there is a possibility of direct interactions of these pathogens with integrin receptors. A recent study demonstrated that *S. pyogenes* can adhere to and become internalized by human pharyngeal EC via a direct interaction between the collagen-like bacterial protein Scl1 and the epithelial collagen receptor, α2β1 integrin [64]. The authors suggested that this novel molecular mechanism can contribute to the bacterial pathogenesis as it enhances streptococcal intracellular survival and reemergence from infected cells [64].

Mycobacteria

Pathogenic mycobacteria, including the cause of the most significant infectious disease worldwide *Mycobacterium tuberculosis*, possess remarkable abilities to evade the immune system of the infected host. Interaction of *M. tuberculosis* with alveolar macrophages allowing these bacteria to survive and even replicate within the phagocytic cells is a hallmark of pulmonary tuberculosis, and has been studied extensively [65]. However, the mycobacteria also invade EC in the respiratory mucosa, and this may represent the site of primary uptake of bacteria during the infection process [66]. Although the molecular mechanisms behind mycobacterial invasion of pulmonary EC remain largely undefined, some data indicate that integrin receptors as well as their ECM protein ligands can be significantly involved. Fn was first implicated in *M. bovis* adherence to bladder epithelium [67, 68], and later it appeared that both attachment and internalization via Fn binding were highly conserved in mycobacteria [69]. Middleton et al. [70] demonstrated that *M. tuberculosis* adheres to ECM components at least in part via an Fn attachment protein (FAP) and antigen 85B protein, the latter also being involved in Fn binding [71]. Similarly, *M. avium* adheres to Fn via FAP in areas of epithelial damage [72].

As the ECM proteins are natural ligands for integrins, such bacteria-ECM interactions may serve to bridge mycobacteria to integrin receptors. Indeed, a study by Bermudez and Goodman [66] demonstrated that *M. tuberculosis* invasion of A549 type II alveolar pneumocytes was greatly inhibited by treating cells with anti-αv or anti-β1 integrin antibodies, and nearly abolished when treating them with both. Secott et al. [73] showed similar inhibition of *M. paratuberculosis* adhesion to and invasion of bovine intestinal EC following treatment with blocking peptides or neutralizing antibodies to α5, αv, β1, and β3 integrins. These studies indicate that various ECM components can serve as a molecular bridge between mycobacteria and integrins, and that multiple integrins can potentially mediate mycobacterial invasion of epithelium. Interestingly, a recent study implicated β1 integrins, along with TLR-2 and ADAM9, in macrophage fusion during formation of tuberculous granulomas, representing a critical event in the pathogenesis of pulmonary tuberculosis, although the precise mechanisms of integrin involvement in this process remain unknown [74].

Pseudomonas aeruginosa

The opportunistic Gram-negative pathogen *P. aeruginosa* causes acute life-threatening infections in immunocompromised patients. It is also the leading cause of ventilator-associated pneumonia in intensive care units and of burn wound infections with high mortality rates. *P. aeruginosa* is the major cause of chronic pulmonary infection in CF patients [75]. Both integrin receptors and their ligands have been implicated in adhesion and internalization of *P. aeruginosa* in the lung epithelia. A number of studies demonstrated the ability of *P. aeruginosa* to bind Fn [76, 77] and Vn [78], the α5β1 and αvβ5 integrin ligands, respectively. Some papers suggest that αvβ5 and α5β1 integrins can also directly mediate *P. aeruginosa* adherence to and invasion of respiratory EC [76, 78, 79]. The molecular mechanisms of such interactions have not yet been defined, although a 50-kDa OMP of *P. aeruginosa* was found associated with α5β1 integrins in respiratory EC [79].

In the process of epithelial injury and repair, the expression of α5β1 receptors is increased with their redistribution from basolateral to apical sides, and respiratory EC synthesize large amounts of Fn potentially providing a basis for an enhanced adherence of *P. aeruginosa* [79]. Adherence of *P. aeruginosa* to laminin, another compo-

nent of the ECM and the α3β1 integrin ligand, unmasked following epithelial injury, was also implicated in bacterial colonization of injured tissues [80].

Our recent observations have demonstrated that *P. aeruginosa* infection caused a rapid upregulation of integrins α5, αv, β1, and β4 in A549 type II pneumocytes [Gravelle et al., unpubl. data]. Interestingly, this effect required live bacteria possessing intact pili and lipopolysaccharide (LPS), because heat-killed, pili-deficient, or outer-core oligosaccharide-deficient *P. aeruginosa* mutants did not alter the expression of integrins [Gravelle et al., unpubl. data]. These findings imply that pulmonary epithelial integrins can be involved in recognition of specific microbial products of *P. aeruginosa* and hence be important in innate immune responses to this pathogen.

Bordetella pertussis

The Gram-negative coccobacillus *B. pertussis* is the causative agent of whooping cough. The bacteria possess a number of virulence factors that are capable of exploiting integrin receptors of both pulmonary EC and monocytes/macrophages in the process of microbial pathogenesis. The major *B. pertussis* adhesin, filamentous hemagglutinin (FHA), contains an RGD sequence which allows the bacterium to invade alveolar macrophages by binding to αMβ2 integrin [81, 82], as well as airway EC by binding to α5β1 integrin [83].

The interactions of FHA with αMβ2 integrin are essential for *B. pertussis* internalization into macrophages and intracellular survival [82]. *B. pertussis* binding to αMβ2 integrin activates cell signaling pathways which lead to upregulation of β3-containing integrins and the integrin-associated protein CD47, which in turn upregulates αMβ2 [84]. The bacterium is thus able to exploit integrins using a positive feedback loop, resulting in increased survival and persistence at the site of infection. In addition, *B. pertussis* produces a repeat in toxin (RTX) adenylate cyclase toxin called CyaA, which further exploits αMβ2 integrins in macrophages by binding them and subsequently converting cellular ATP to cAMP, suppressing the bactericidal activities of these cells [reviewed by 85]. Recent studies demonstrated that via interaction with αMβ2 integrins, the adenylate cyclase toxin also induces cyclooxygenase 2 (COX-2) in macrophages. The latter protein can then significantly contribute to the inflammatory responses caused by *B. pertussis* [86].

B. pertussis is also able to invade host EC through the interactions of FHA with α5β1 integrins [83]. Such interactions appeared to be important not only for bacterial invasion, but also for inflammatory responses. Indeed, in vitro engagement of α5β1 integrins by FHA caused RGD-dependent activation of nuclear factor kappa B (NF-κB) and, as a result, up-regulation of intercellular adhesion molecule-1 (ICAM-1) expression in lung EC [87, 88].

Haemophilus influenzae

H. influenzae are Gram-negative commensal bacteria commonly found in the upper respiratory tract but they also can cause respiratory diseases such as pneumonia, as well as invasive systemic infections [89]. The major virulence factor of *H. influenzae* is the polysaccharide capsule. Encapsulated strains of *H. influenzae* are designated as types a, b, c, d, e, and f according to their capsular antigens, type b being the most important clinically and causing severe invasive diseases, i.e. meningitis, epiglottitis, and septicemia. *H. influenzae* that lack capsular polysaccharides are referred to as nontypeable and are less virulent. Many clinical isolates of nontypeable *H. influenzae* are able to bind ECM proteins [90]. For example Hap, an ubiquitous nonpilus adhesin of *H. influenzae,* specifically binds to Fn, laminin and collagen IV, and such interactions mediate bacterial adhesion to the ECM [91]. These data suggest that *H. influenzae* can indirectly bind integrin receptors representing the natural ligands for these ECM proteins in the respiratory epithelium, i.e. α5β1, α3β1, αvβ6 [7]. However, integrin involvement in adherence of *H. influenzae* to the respiratory epithelium has not been directly explored. Nevertheless, the uptake of nontypeable *H. influenzae* by M cells in the intestinal epithelium was mediated by α5β1 integrin along with TLR-4 and platelet-activating factor receptor, as demonstrated by the blocking of translocation of bacteria into M cells in the presence of specific receptor inhibitors [92].

Streptococcus pneumoniae

The leading cause of community-acquired pneumonia, *S. pneumoniae* (pneumococcus), possesses an FnBP protein PavA essential for the virulence [93]. PavA is structurally homologous to the FnBP of other pathogenic bacteria such as *S. pyogenes* and *S. gordonii* [94]. It is possible that adhesion to and invasion of lung EC that is

critical in the pathogenesis of pneumococcal pneumonia can be mediated by Fn-α5β1 integrin interactions, as in case of other infections. However, the direct role of PavA in pathogen-host interactions and inflammatory responses caused by *S. pneumoniae* remains to be determined.

Other Microbes

Integrins are also implicated in the pathogenesis of some fungal and viral pulmonary infections. The fungus *Pneumocystis carinii*, a major cause of acute pneumonia in AIDS patients, uses an FnBP to adhere to αv and α5 integrins [95, 96]. Furthermore, *P. carinii* is able to induce upregulation of integrins, possibly enhancing its own adherence to lung EC [97]. Interestingly, some pathogenic fungi, such as *Pneumocystis* species and *Candida albicans*, possess molecules with integrin-like features that mediate fungal adhesion to Fn [98, 99]. A novel *Pneumocystis* molecule PCINT1 with significant structural features of an integrin-like adhesion receptor has been recently characterized [99]. The results of the latter study suggest an important role of this molecule in pathogen-host lung EC interactions during *Pneumocystis* pneumonia [99].

A number of viruses that infect the respiratory epithelium have been shown to use integrin receptors for both cell entry and induction of intracellular signaling important for disease pathogenesis. Some examples include members of adenovirus, herpesvirus, hantavirus, picornavirus, Reoviridae families [reviewed by 100]. Such viruses directly bind to a variety of integrins present in the respiratory epithelium, e.g. α2β1, α3β1, α5β1, αvβ5, αvβ6, and use them as receptors to attach to the cells and enter them. The mechanisms of virus interactions with integrins and their significance for viral pathogenesis have been recently discussed in a comprehensive review [100]. Several viruses, e.g. Coxsackieviruses, foot-and-mouth disease viruses, human parechoviruses, and echoviruses possess a functional RGD motif in one of their capsid proteins that allow viruses to bind integrins, i.e. αvβ3 or αvβ6 [101]. Interactions of viruses with integrin receptors are proven to be important in the pathogenesis of a variety of conditions ranging from acute upper respiratory tract infections and foot-and-mouth disease to highly lethal hantavirus pulmonary syndrome [100]. Recent data implicate that the severe acute respiratory syndrome-related coronavirus possesses the ability of binding to integrin I domains [102]. However, it is still unclear whether integrin-mediated interactions are involved in coronavirus entry into lung EC.

Integrins as Innate Recognition Receptors

According to the current concept, the recognition of pathogen-associated molecules is critical for innate immunity. Among the pattern recognition receptors (PRRs), TLRs are key molecules that sense the invasion of pathogens based on their typical molecular structures, such as LPS, peptidoglycans, flagella, single-stranded or double-stranded RNA, CpG DNA, etc. [103]. Such structures are unique to microorganisms in contrast to metazoans, and therefore allow for discrimination between self and non-self that is essential for immune defense [103]. Upon their activation, TLRs induce signal transduction leading to inflammatory responses and eventually to elimination of the invader. However, microorganisms are capable of binding various receptors of host cells resulting in complex cellular responses. It has now been recognized that there exists a huge diversity in innate immune receptors, in addition to the best-studied TLRs. The importance of non-TLR PRRs, such as Nod-like receptors, C-type lectins, scavenger receptors, or protease-activated receptors, as integral components of innate immune recognition, has been recently discussed in several excellent reviews [104–106]. The role of integrins as PRRs is still unclear, although leukocyte integrins serving as complement receptors, e.g. Mac-1, are recognized as PRRs for certain pathogens [107]. The role of epithelial integrins in innate immunity is even less understood, despite the fact that these receptors are highly expressed in mucosal surfaces, such as airway EC.

We propose that lung epithelial integrins may act as PRRs based on both their significance in many host-pathogen interactions and on common characteristics with other PRRs, such as TLRs (table 2). Like TLR, integrin receptors are germline-encoded and highly conserved in the evolution, present in all metazoans including invertebrates, e.g. ascidians, nematode worms and *Drosophila* [108]. As outlined above, integrins are able to bind a wide variety of microorganisms, including both Gram-positive and Gram-negative bacteria, viruses, and fungi. Moreover, it has been demonstrated that integrin receptors are able to sense diverse pathogen-associated molecular structures, although many of the specific ligands involved in such pathogen-host interactions are still unknown. Interestingly, a variety of microorgan-

Table 2. Characteristics of integrin receptors shared with other PRRs, i.e. TLRs

Characteristics	Integrins	TLRs
Genetic encoding	Germline-encoded receptors [120]	Germline-encoded receptors [103, 105, 122]
Evolutionarily conserved	Present in nematode worms, insects, ascidians and all vertebrates [108]	Present in nematode worms, insects and all vertebrates [123]
Recognition of broad classes of pathogens	Gram+ bacteria [39, 57, 66] Gram– bacteria [79, 83, 121] Fungi [96] Viruses [100]	Gram+ bacteria, Gram– bacteria, fungi, viruses, parasites [122]
Recognition of pathogen-associated molecules	FnBP [39] Invasin [18] FHA [81, 83]	Bacterial lipopolysaccharide, lipoprotein, lipoarabinomannan, peptidoglycan, flagellin, *Pseudomonas* exotoxin S, bacterial and viral DNA, viral RNA, fungal zymosan, parasitic phospholipids [122]
Inflammatory responses	NF-κB activation: *B. pertussis* [88] Adhesion molecule expression: *B. pertussis* [87] Proinflammatory cytokine production: *Y. enterocolitica* [21]	Activation of transcription factors NF-κB, AP-1, IRF3, IRF7 [122] Adhesion molecule expression [122] Proinflammatory cytokine and chemokine production [122]
Upregulation upon receptor engagement	*St. pyogenes* [62] *P. aeruginosa* [Gravelle et al., unpubl. data] *P. carinii* [97]	LPS and dsRNA cause upregulation of TLR2 [109, 110, 124, 125]
Cross talk with other PRRs	With TLRs [112–114]	Cross talk between TLR2 and TLR4, TLR3 and TLR9 [126–128] With NOD1 and NOD2 [129, 130] With dectin-1 [131, 132] With integrins [112–114]

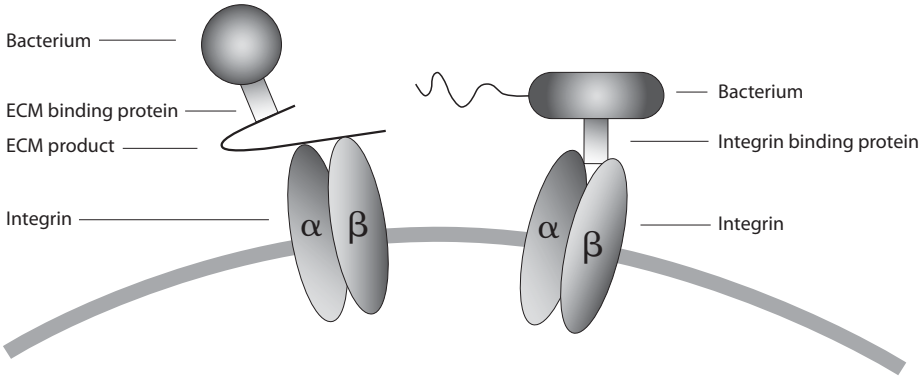
AP-1 = Activator protein 1; dsRNA = double-stranded RNA; IRF = interferon regulatory factor; NOD = nucleotide-binding oligomerization domain.

isms, i.e. *S. aureus*, *S. pyogenes*, mycobacteria, *S. pneumoniae*, possess various Fn-binding proteins allowing interactions with integrin heterodimers using Fn as a molecular bridge. Remarkably, the subsequent cellular responses are different from those elicited by Fn alone. Some other pathogens bind directly to integrins, forgoing the use of ECM proteins. For example, *Y. pseudotuberculosis* invasin interacts directly with β1 integrins, as does *B. pertussis* FHA [18, 81, 83]. Interestingly, FHA binds to integrins via an RGD domain like the interaction between integrin receptors and their natural ECM ligands (fig. 1).

As in case of other PRRs, integrin engagement by pathogenic microorganisms results in activation of cellular responses important for innate immunity and inflammation. The hallmark of such responses is the activation of the transcription factor NF-κB followed by transcriptional regulation of proinflammatory cytokine, chemokine, and adhesion molecule (ICAM-1) expression. Indeed, integrin receptor engagement by some pathogenic bacteria, such as *B. pertussis* and *Y. pseudotuberculosis* caused NF-κB-mediated proinflammatory cellular responses [21, 88] (fig. 2).

It is known that PRRs, i.e. TLRs, can be upregulated upon their engagement enhancing host responses to infection [109–111]. Similarly, integrins can be upregulated during infection as demonstrated in models of *S. pyogenes* [62], *P. carinii* [97] and *P. aeruginosa* infections [Gravelle et al., unpubl. data].

It is recognized that TLRs as well as other PRRs are engaged in an integrated signaling cross talk [105]. Similarly, some recent studies identified integrins as important components of signaling complexes involved in cellular responses to pathogen-associated molecular pat-



Microbes using ECM products to bind integrins

Microbe	ECM binding protein	ECM product	Integrin
Staphylococcus aureus	FnBP	Fn	α5β1
Streptococcus pyogenes	M1 protein, PrtF1/Sfbl	Fn	α5β1
Pseudomonas aeruginosa	Putative OMP	Fn, Vn	α5β1, αvβ5
Streptococcus pneumoniae	PavA	Fn	α5β1
Haemophilus influenzae	unknown	Fn	α5β1
Mycobacterium	FAP, Antigen 85B	Vn? Fn?	αv, β1
Pneumocystis carinii	FnBP	Fn	αv, β5

Microbes binding directly to integrins

Microbe	Integrin binding protein	Integrin
Bordetella pertussis	FHA	α5β1
Streptococcus pyogenes	Scl1 (proposed)	α2β1
Viruses (i.e: coxsackievirus)	RGD containing capsid proteins	α5β1, α3β1, α5β1, αvβ5, αvβ6

Fig. 1. The most important bacterial interactions with epithelial integrin receptors. Microorganisms can interact with lung epithelial integrins either directly via integrin-binding proteins or indirectly by using an extracellular martix protein, e.g. Fn or Vn, as a molecular bridge to engage these receptors.

terns. Indeed, the activation of NF-κB and MAPK cascade induced in macrophages and intestinal EC lines by LPS stimulation required simultaneous engagement of integrin receptors providing essential co-stimulatory signals [112–114]. Although integrins appear to be critical for responses of some cell types to TLR agonists, the molecular interactions between integrins and signaling intermediates elicited by other PRRs are largely undefined.

Conclusion

Integrin receptors are complex molecules that mediate both physiological and pathological processes, e.g. inflammation and tumorigenesis. During the last decade, it has become clear that integrins significantly participate in various host-pathogen interactions involving pathogenic bacteria, fungi, and viruses. Many bacteria possess adhesins that can bind either directly or indirectly to integrins. However, there appears to be an emerging role for

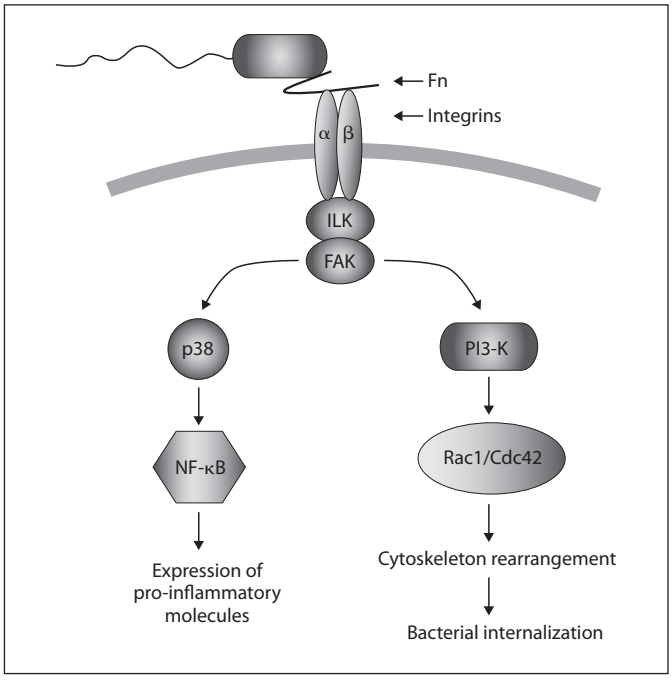


Fig. 2. Intracellular signaling pathways activated upon bacterial engagement of integrin receptors. Bacteria such as *S. aureus* or *S. pyogenes* bind Fn using it as a molecular bridge to bind integrins. Integrin receptor engagement causes activation of ILK and FAK. The resulting signaling cascade leads to inflammatory cellular responses, i.e. via phosphorylation of p38 MAPK and a subsequent activation and nuclear translocation of NF-κB followed by gene expression of various proinflammatory molecules. Integrin-mediated signaling also leads to cytoskeletal rearrangement and bacterial internalization via activation of PI3-K and small GTPases Rac1 and Cdc42. Some bacteria are also capable to bind integrins directly, which results in similar signaling events (not shown).

integrins beyond simply adhesion molecules. Given the extremely conserved nature of integrin structure and function, and the diversity of the pathogens which use integrins, it appears that they may act as PRRs, involved in bacterial recognition and initiation of the innate immune response.

However, the role of integrin receptors in host defense still remains poorly understood. Although a number of studies identified integrins as receptors used by pathogenic bacteria for their internalization by host cells, the significance of this process for innate immunity is not clearly defined. Internalization of bacteria may represent an important step in host defense. Indeed, internalized bacteria may be cleared due to inflammatory signaling initiated by intracellular Nod-like receptors and endosome-located TLRs, or as a result of apoptosis of infected cells. Furthermore, internalization may be critical in activation of adaptive immunity because infected cells, including mucosal EC, are able to present microbial antigens to lymphocytes [115, 116]. However, although the results from some studies suggest the role of integrins as innate recognition receptors important for mucosal immune defense, there remain many questions to be answered.

Understanding the role of epithelial integrins in the pathogenesis of pulmonary infections may be important for developing new therapies targeting critical mechanisms of the pathogenesis of conditions such as acute bacterial pneumonia, chronic *P. aeruginosa* infection in CF patients, or fungal lung disease in immunocompromised patients. Of interest, aerosolized integrin inhibitors have been demonstrated to inhibit pulmonary inflammatory responses by blocking leukocyte infiltration into the lung using in vivo models of allergic asthma [117–119]. Although these studies did not investigate infectious processes, they demonstrated the feasibility of employing integrin inhibitors in vivo to suppress lung inflammation. Would it be possible to use integrin inhibitors to interfere with bacteria-host interactions to alleviate potentially detrimental integrin-mediated cellular responses? Although more studies into the mechanisms of pathogen-integrin interactions are required before this question can be answered, the inhibition of integrins may represent a promising new tool to combat pulmonary infections.

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