# Integrin-dependent phagocytosis – spreading from microadhesion to new concepts

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## Summary

**By linking actin dynamics to extracellular components, integrins are involved in a wide range of cellular processes that are associated with or require cytoskeletal remodelling and cell-shape changes. One such function is integrin-dependent phagocytosis, a process that several integrins are capable of mediating and that allows the binding and clearance of particles. Integrin-dependent phagocytosis is involved in a wide range of physiological processes, from the clearance of microorganisms and apoptotic-cell removal to extracellular-matrix remodelling. Integrin signalling is also exploited by microbial pathogens for entry into host cells. Far from being a particular property of specific integrins and specialised cells, integrin-dependent uptake is emerging as a general, intrinsic ability of most integrins that is associated with their capacity to signal to the actin cytoskeleton. Integrin-mediated phagocytosis can therefore be used as a robust model in which to study integrin regulation and signalling.**

Key words: Integrin, Adhesion, Phagocytosis, Microbial pathogens, Apoptotic cells

## Introduction

Integrins are heterodimeric receptors (comprising one α- and one β-subunit) that are expressed at the surface of most metazoan cells (Fig. 1). To date, 18 α- and eight β-subunits have been described in human cells. They combine to form 24 different heterodimers that can bind a great variety of ligands (Hynes, 2002). Because of their diversity, integrins mediate a wide range of cellular functions, all of which are related to adhesion. For example, integrins are required for the adhesion and spreading of cells onto extracellular matrix (ECM) components such as fibronectin or collagen. Integrins can also mediate cell-cell adhesion, particularly during the formation of the immunological synapse and the transendothelial migration of leukocytes (Barreiro et al., 2007).

Two main mechanisms regulate integrin function (Fig. 1). First, the affinity of integrins for their ligands is greatly enhanced by an 'inside-out' signalling pathway that induces conformational changes in integrin extracellular domains such that they display a high affinity for their ligands (Luo et al., 2007). The binding of the N-terminal head domain of the cytoskeletal protein talin to an NPX[Y/F] motif in integrin-β tails is necessary and sufficient for such inside-out integrin activation. The NPX[Y/F] motif is phylogenetically conserved and only absent in human β4 and β8 integrins and in the β1B- and β1C-integrin variants, none of which are regulated by inside-out signalling (Tadokoro et al., 2003). Second, ligand binding to activated integrins in turn triggers an 'outside-in' signalling pathway that recruits a huge network of proteins (Zaidel-Bar et al., 2007) and leads to the local reorganisation of the actin cytoskeleton. Regulators of outside-in signalling include signalling molecules [e.g. the FAK (focal adhesion kinase)–c-Src complex and Rho GTP-binding proteins] and adapters (e.g. Cas, Crk and paxillin) that assemble within dynamic adhesive structures (Geiger et al., 2001; Zamir and Geiger, 2001) (Fig. 1). The clustering of integrins constitutes a third regulatory mechanism, which is often referred to as the controlling of integrin avidity (Carman and Springer, 2003). Because avidity changes are associated with increased ligand binding, it is not completely clear whether they are associated with inside-out or outside-in signalling. Finally, integrin trafficking and recycling is another mechanism that regulates cell adhesion and migration (Caswell and Norman, 2006).

Integrin signalling is mostly considered in the context of cell adhesion and migration. However, as reviewed here, many integrins can also mediate phagocytosis (Table 1). Phagocytosis is a process that allows a cell to successively bind, internalise and finally degrade particles over 0.5 μm in diameter (Aderem and Underhill, 1999). In vertebrates, phagocytosis is fundamental both for the removal of pathogens as part of innate and adaptive immunity, and for the clearance of apoptotic cells (ACs) that are generated during development and cell turnover in tissues (Greenberg and Grinstein, 2002; Maderna and Godson, 2003). To capture and phagocytose targets, mammalian professional phagocytes [e.g. macrophages, neutrophils and dendritic cells (DCs)] have a range of phagocytosis-dedicated surface receptors, such as Fcγ receptors [FcγRs; which bind the Fc region on immunoglobulin G (IgG)], scavenger receptors and also integrins (Aderem and Underhill, 1999; Underhill and Ozinsky, 2002). In contrast to macropinocytosis – an ingestion process that involves the formation of a large membrane extension away from the agonist-binding site (Conner and Schmid, 2003) – phagocytosis is a processive mechanism that is mediated by the successive ligation of receptors in a zipper-like process: during phagocyte uptake, receptors bind particles and signal a three-dimensional microadhesive process that wraps membrane in an actin-driven manner around the particle, leading to full engulfment (Griffin, Jr et al., 1975). Particle recognition and uptake can involve one of several types of receptor, particularly when very large or complex phagocytic targets are recognised, e.g. microorganisms or ACs (Underhill and Ozinsky, 2002). In these situations,

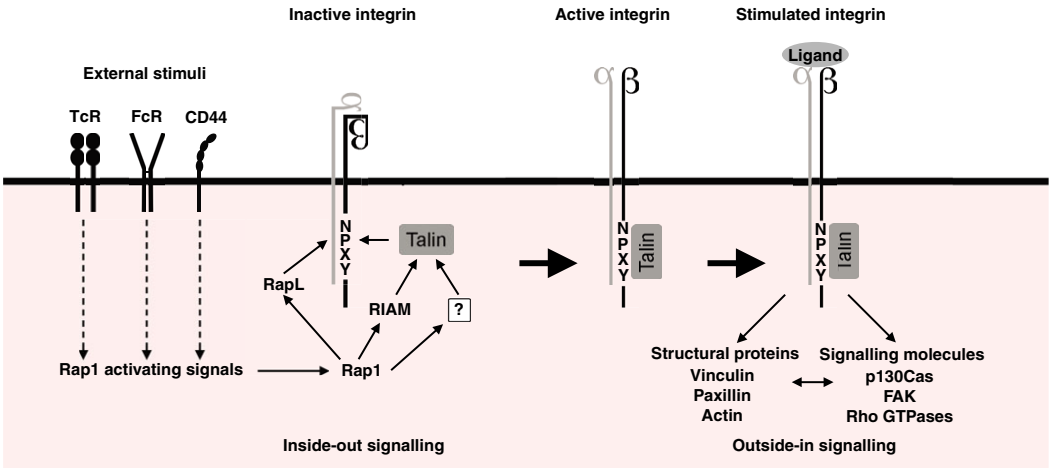


**Fig. 1.** Signalling pathways that control integrin function. The extracellular domains of inactive integrins are folded and display a conformation that has a low affinity for ligands. The inside-out signalling pathway activates integrins by inducing conformational changes that open the extracellular domains into a high-affinity ligand-binding state. Inside-out signalling is triggered intracellularly in response to external stimuli. Shown are surface receptors that are known to activate β2 integrins. The small GTPase Rap1 is a potent mediator of integrin inside-out signalling and activation. Rap1 is activated downstream of surface receptors. At least two downstream effectors of Rap1 have been shown to be involved in the activation of integrin function: RapL acts on the α-subunit of the αLβ2 integrin and RIAM is proposed to stimulate the binding of talin to the membrane-proximal NPX[Y/F] motif of the β-subunits. The binding of talin to the cytoplasmic tail of β-subunits is sufficient for integrin activation and characterises the active state of integrins. The binding of ligand to the extracellular domains of activated integrins stimulates outside-in signalling pathways, i.e. the activation and/or recruitment of multiple structural (e.g. vinculin, paxillin and actin) and signalling (e.g. p130Cas, FAK and Rho GTPases) proteins, leading to the remodelling of the actin cytoskeleton and the formation of adhesion structures.

distinct receptors can be devoted to particle recognition ('tethering' receptors) and stimulation of particle uptake ('tickling' receptors) (Zullig and Hengartner, 2004). Interestingly, zipper-like phagocytosis can also take place in non-professional phagocytes, either naturally or following transfection of phagocytic receptors (Caron and Hall, 1998; Underhill and Ozinsky, 2002).

Similar to cell adhesion and migration, phagocytosis crucially depends on remodelling of the actin cytoskeleton and on membrane dynamics (DeMali and Burridge, 2003; Niedergang and Chavrier, 2004). Moreover, the signalling pathways that underlie these processes have several regulatory proteins in common, including Rho-family proteins (Niedergang and Chavrier, 2005; Ridley et al., 2003). Interestingly, distinct Rho GTPases regulate actin polymerisation during phagocytosis and actually define several modes of phagocytosis that are phenotypically different. Indeed, during FcγR-dependent (also referred as type I) uptake, activation of Rac1 and Cdc42 is thought to drive the formation of local pseudopods and membrane ruffles that engulf the particles. By contrast, actin polymerisation during integrin-αMβ2-dependent (referred as type II) phagocytosis is regulated by RhoA activity, and particles that are bound to αMβ2 sink into cells without generating major protrusions (see below and Fig. 2) (Allen and Aderem, 1996; Caron and Hall, 1998).

On the basis of the criteria defined above for phagocytosis (particle size, zipper-like receptor tickling, and actin-dependent and Rho-GTPase-dependent uptake), we review here the role of integrins in phagocytosis, both in professional phagocytes and also in cells that are not classically considered phagocytic. We highlight the similarities between the signalling basis of integrin function during adhesion and phagocytic uptake. Not only is phagocytosis the predominant function of certain integrins, it should be seen as an intrinsic ability of most integrins. By extension, integrin-mediated phagocytosis can be proposed as a good model to better understand the regulation of integrin function.

## Phagocytic integrins and immunity – integrin αMβ2 as a paradigm

Because phagocytosis allows rapid ingestion of microscopic and submicroscopic particles, it has been evolutionarily selected as an important mechanism in maintaining homeostasis and fighting pathogens (Underhill and Ozinsky, 2002). Although several integrins have now been identified as mediators of phagocytosis (Table 1), integrin αMβ2 was the first phagocytic integrin to be characterised. αMβ2, a bona fide phagocytic receptor, also known as complement receptor 3 (CR3), CD11b/CD18 or Mac-1, has an essential role in the uptake of complement-opsonised microorganisms and ACs (Aderem and Underhill, 1999; Underhill and Ozinsky, 2002) (Fig. 1). The central role of β2 integrins in anti-microbial defence is illustrated by the fact that patients with leukocyte adhesion deficiency type I (LADI) syndrome, who lack a functional β2 integrin, are particularly prone to bacterial infections (Bunting et al., 2002; Hogg et al., 2002).

### The small GTPase Rap1 controls inside-out signalling to the αMβ2 integrin

Most phagocytic receptors that are expressed by professional phagocytes (e.g. FcγR) can constitutively perform phagocytosis. By contrast, phagocytosis that occurs through the αMβ2 integrin requires extracellular stimulatory signals such as chemokines, cytokines (e.g. tumour necrosis factor-α) and bacterial products (e.g. lipopolysaccharide) (Aderem and Underhill, 1999). The phagocytic potential of αMβ2 can also be experimentally induced by phorbol esters (Wright and Meyer, 1986). The discovery that Rap1, a Ras-family small GTP-binding protein, is activated by these stimuli, and that accumulation of GTP-bound Rap1 is necessary and sufficient to activate αMβ2 binding and phagocytic properties in macrophages, has provided a molecular basis for these observations. Furthermore, the demonstration of a link between Rap1 activity and αMβ2-integrin function during phagocytosis has advanced the

**Table 1. Integrin chains that mediate phagocytosis**

| Integrin | Ligand | Cell type | Function | References |
|---|---|---|---|---|
| **Physiological phagocytosis in mammals** | | | | |
| αMβ2 | C3bi, fibrinogen | Phagocytes | Innate immunity | (Beller et al., 1982) |
| αVβ3, αVβ5 | MFG-E8 | Phagocytes | Clearance of apoptotic cells | (Hanayama et al., 2002; Savill et al., 1990) |
| αVβ5 | MFG-E8 | RPE | Circadian rhythm of RPE | (Finnemann et al., 1997; Nandrot et al., 2007) |
| α2β1 | Collagen | Fibroblasts | ECM remodelling | (Arora et al., 2000; Lee et al., 1996) |
| α6β1* | fAβ peptide | Phagocytes | Removal of fAβ deposits | (Koenigsknecht and Landreth, 2004) |
| **Pathological phagocytosis-like process in mammals** | | | | |
| β1 | *Yersinia* spp. invasin | Epithelial cells | *Yersinia* spp. entry | (Tran Van Nhieu and Isberg, 1993) |
| α5β1 | Fibronectin | Many† | *S. aureus* entry | (Fowler et al., 2000; Sinha et al., 1999) |
| α5β1, αVβ3? | Glycoproteins | Many† | Herpes virus entry | (Akula et al., 2002; Garrigues et al., 2008) |
| αVβ3, αVβ5 | Capside protein | Epithelial cells | Adenovirus entry | (Li et al., 1998; Wickham et al., 1993) |
| **Physiological phagocytosis in insects** | | | | |
| *A. gambia* | | | | |
| BIMP2 | Unknown | Haemocyte | Immunity | (Moita et al., 2006; Moita et al., 2005) |
| *D. melanogaster* | | | | |
| $h\beta_3$-like | Unknown | Haemocyte | Immunity | (Foukas et al., 1998) |

Several integrins have been involved in phagocytosis in metazoa. For each integrin, the ligand involved, the cell type that mediates uptake and the relevant biological function are indicated. The term phagocyte refers to cells that are classically associated with phagocytic uptake, i.e. neutrophils, macrophages, DCs and microglial cells in mammals. See text for details.

*Necessary but not sufficient; †epithelial, endothelial, fibroblast.

understanding of integrin inside-out signalling in general (Caron et al., 2000). Other macrophage receptors have been shown to activate αMβ2-mediated phagocytosis through Rap1 activation (Vachon et al., 2007). Similarly, Rap1 activity controls the activation of many different integrins, such as α3β1, α4β1, α5β1, αIIbβ3 and αLβ2 (Bertoni et al., 2002; de Bruyn et al., 2002; de Bruyn et al., 2003; Enserink et al., 2004; Katagiri et al., 2002; McLeod et al., 2004; Reedquist et al., 2000; Sebzda et al., 2002).

How active Rap1 signals to integrins in general, and in particular to αMβ2, remains unclear, although several downstream effectors of Rap1 have been proposed to mediate its pro-adhesive effects (Fig. 1). RapL (regulator for cell adhesion and polarisation enriched in lymphoid tissues) interacts with GTP-bound Rap1 and is necessary for the ability of Rap1 to polarise the distribution of active αLβ2 integrins in lymphoid cells (Katagiri et al., 2003). Because specific mutations in αL abrogate both RapL co-immunoprecipitation with αL and Rap1- and RapL-dependent αLβ2 polarisation, RapL was proposed to mediate Rap1 activity on the αL subunit, as suggested in Fig. 1 (Katagiri et al., 2003). In agreement with the exclusive expression of αL in leukocytes, RapL knock-out mice only show abnormalities in lymphoid organs, a result that also correlates with RapL expression in lymphoid tissues. However, no phagocytic or general adhesion defects were observed in these mice (Katagiri et al., 2004). Considering that Rap1 is a general activator of integrins that controls inside-out signalling in many cell types, it is reasonable to assume that RapL is not the universal Rap1 effector that regulates integrin activation. Additionally, as alluded to above, direct binding of the talin head domain to the cytoplasmic tail of many integrin-β subunits has been described as a powerful way of activating integrins, including β2 integrins (Lim et al., 2007; Tadokoro et al., 2003). Importantly, Rap1-induced regulation of the platelet integrin αIIbβ3 was recently shown to depend on another Rap1 effector, RIAM (Rap-interacting adhesion molecule), an adapter protein previously shown to stimulate αLβ2- and α4β1-dependent adhesion of Jurkat cells (Han et al., 2006; Lafuente et al., 2004). RIAM colocalises and can be co-immunoprecipitated with talin, and RIAM silencing reduces Rap1-dependent activation of αIIbβ3 integrin (Han et al., 2006). It has thus been proposed that RIAM links Rap1 to integrin activation by promoting the recruitment of talin (Fig. 1). However, silencing of RIAM in macrophages has no effect on αMβ2-dependent binding and phagocytosis of complement (C3bi)-opsonised red blood cells, suggesting that RIAM is not involved in the inside-out activation of αMβ2. Moreover, unlike talin, RIAM overexpression in macrophages or αMβ2-transfected cells is not sufficient to increase particle binding (our unpublished data). Despite the universal role of Rap1 signalling in activating different integrin heterodimers, it is conceivable that different downstream effectors of Rap1 could mediate inside-out signalling to distinct integrins (Fig. 1). In any case, the mechanisms that link Rap1 to integrin activation and their dependency on talin still remain to be fully elucidated for αMβ2 and other integrins.

### RhoA mediates outside-in signalling downstream of αMβ2

A significant advance in the understanding of phagocytosis was the discovery that, contrary to FcγR (the main receptor for IgG-opsonised particles) and most other phagocytic receptors, the αMβ2 integrin requires RhoA, but not Rac1 or Cdc42, activity to drive actin polymerisation and particle uptake (Caron and Hall, 1998) (Fig. 2). Early electron-microscopy studies showed that αMβ2-mediated phagocytosis is not associated with the formation of membrane protrusions (Allen and Aderem, 1996), an event that correlates with activation of Rac1 and Cdc42. By contrast, following αMβ2 ligation, RhoA is specifically activated and recruited to the site of phagocytosis (Wiedemann et al., 2006). Accordingly, inhibition of RhoA (but not of Rac1 or Cdc42) using either dominant-negative mutants or C3 transferase (a specific Rho inhibitor) inhibits αMβ2-dependent phagocytosis both in macrophage cell lines and in αMβ2-transfected fibroblasts (Caron and Hall, 1998; Colucci-Guyon et al., 2005; Wiedemann et al., 2006). The implication of the presence of RhoA in the pathway that links αMβ2 to actin dynamics is also highlighted by the fact that phagocytosis requires two specific RhoA effectors – the serine/threonine kinase Rock and the formin-related elongating factor mDia (known as DIAPH in humans) – in both cell models (Colucci-Guyon et al., 2005; Olazabal et al., 2002). Rock activity

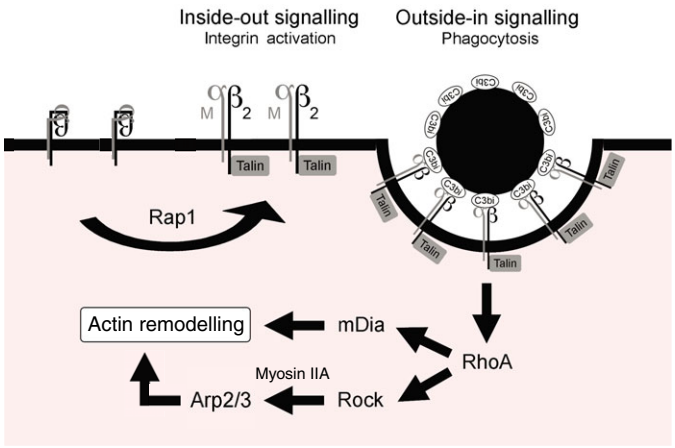


**Fig. 2.** αMβ2-dependent phagocytosis is mediated by RhoA. αMβ2 integrin binds ligands poorly in resting phagocytes. External stimuli induce an inside-out signalling pathway that activates the small GTP-binding protein Rap1, which results in the induction of structural changes in αMβ2 and increased affinity for its ligand, the C3bi complement fragment that coats phagocytic preys. The capture of a C3bi-opsonised particle by αMβ2 integrins generates forces that pull the particle inside the cell along a Rho-dependent actin-polymerisation pathway that involves Rock and mDia (outside-in signalling). See text for details.

mediates the local recruitment of the actin-branching protein Arp2/3 and the activation of myosin IIA underneath bound particles (May et al., 2000; Olazabal et al., 2002). mDia is required for actin polymerisation and cooperates with the Rock-Arp2/3 pathway to pull the particles inside cells (Colucci-Guyon et al., 2005) (Fig. 2). Finally, mutations in two distinct regions of the β2-integrin cytoplasmic tail abrogate RhoA recruitment and activation as well as actin polymerisation and phagocytosis, showing the importance of the β2 subunit in the signalling pathway of uptake (Wiedemann et al., 2006).

Multiple lines of evidence therefore support the model of a Rho-dependent, Rac1-independent mechanism for αMβ2-mediated phagocytosis. However, a recent article has challenged this view by proposing that both Rho and Rac activities are required for efficient uptake through αMβ2 (Hall et al., 2006). Bone-marrow-derived macrophages that originate from either *vav1*$^{-}$ *vav3*$^{-}$ or *rac1*$^{-}$ *rac2*$^{-}$ double-knockout mice are impaired in their ability to phagocytose αMβ2 ligands. Because Vav1 and Vav3 are two closely related members of the Vav family of guanine nucleotide-exchange factors for Rho-family proteins (RhoGEFs), and therefore activate Rho-family proteins, these data suggest that Vav1 and Vav2 regulate Rac activity downstream of αMβ2 ligation. This discrepancy could easily be explained by the existence of different pathways that regulate αMβ2-dependent uptake in primary and immortalised cells. However, it is worth noticing that, in all macrophage types that have been examined so far, αMβ2-mediated uptake can clearly occur independently of tyrosine-kinase signalling (Allen and Aderem, 1996; Lutz and Correll, 2003). Because Vav activity is mainly regulated by the tyrosine-kinase-dependent release of an inhibitory intramolecular interaction in all cell systems hitherto used (Aghazadeh et al., 2000), a Vav-Rac pathway that is downstream of αMβ2 implies the existence of a novel, tyrosine-phosphorylation-independent mechanism of Vav activation. Alternatively, in primary macrophages, Vav and Rac could signal downstream of a receptor that cooperates with αMβ2 in particle uptake or be involved in the activation of αMβ2 by inside-out signalling.

Importantly, integrin-mediated phagocytosis also contributes to the immune response in insects. Although invertebrates lack αMβ2, phagocytosis of *Escherichia coli* by *Drosophila melanogaster* haemocytes is reduced in the presence of RGD (Asp-Gly-Arg)-containing peptides, which are well-known competitors for integrin ligands, or after pre-incubation with anti-human β3-integrin antibodies. These data clearly indicate a role for a β3-like integrin in anti-microbial immunity in the fly (Foukas et al., 1998). The specific *Drosophila* integrin that is involved in bacterial uptake is still unknown. It is, however, likely to be the orthologue of a phagocytic integrin that has been recently identified in other insects. Indeed, genetic screening in the mosquito *Anopheles gambia* has isolated a β integrin (BINT2), the knockout of which reduces phagocytosis of *E. coli* by 70% (Moita et al., 2006; Moita et al., 2005). The fact that integrins are used both in mammals and insects to remove pathogens suggests that these receptors have been selected early in evolution as mediators of phagocytosis. It is therefore possible that, in mammals, certain integrins have become specialised to allow efficient phagocytosis. αMβ2 is clearly the paradigmatic phagocytic integrin. In this Commentary, however, we will see that phagocytic ability is in fact common to many mammalian integrins.

## Integrin-dependent phagocytosis and tissue remodelling

Phagocytosis is not restricted to the clearance of pathogens by professional phagocytes. It is also a mechanism that allows the removal of ACs and components of the ECM, such as the collagen fibrils that are generated during tissue remodelling (Lee et al., 1996; Ravichandran and Lorenz, 2007; ten Cate, 1972). Interestingly, several integrins are involved in these two processes.

### αVβ3, αVβ5 and apoptotic-cell phagocytosis

ACs that arise during development, normal tissue turnover, inflammation and repair are quickly cleared to avoid secondary necrosis and the release of toxic materials into the environment. Moreover, AC phagocytosis is generally associated with the inhibition of inflammation (Henson, 2005). The integrins that are involved in AC phagocytosis are αVβ3 and αVβ5, which are best known for their potential roles in angiogenesis and tumour-cell metastasis (Reynolds et al., 2002; Taverna et al., 2004). Pre-treatment with RGD peptides or with αVβ3-blocking antibodies severely inhibits the ability of human macrophages to phagocytose apoptotic neutrophils (Savill et al., 1990). By contrast, DCs – phagocytes that also express αVβ3 – use αVβ5 integrin to internalise ACs (Akakura et al., 2004; Albert et al., 1998). Nonetheless, in all cases, integrin-dependent AC uptake is indirect and is mediated by MFG-E8 (milk fat globule-EGF factor 8), a protein that is secreted by macrophages and DCs. MFG-E8 binds phosphatidylserine, an ‘eat-me’ signal that is exposed on the cell surface shortly after induction of the apoptotic programme (Akakura et al., 2004; Hanayama et al., 2002). The crucial role of MFG-E8 in mediating the recognition of ACs by phagocytes is illustrated by the fact that AC uptake is strongly impaired in MFG-E8-knockout mice (Hanayama et al., 2004). Interestingly, αVβ5-mediated MFG-E8-dependent phagocytosis is not restricted to professional phagocytes: HEK293 epithelial cells can also phagocytose ACs in an αVβ5-dependent manner (Albert et al., 2000), as can retinal pigment epithelial cells during circadian-synchronised phagocytosis of photoreceptors, a daily process that is crucial for vision (Finnemann et al., 1997; Nandrot et al., 2007; Nandrot et al., 2004).

The signalling pathways that mediate AC uptake have been partially characterised. Interestingly, they differ from those that are mediated by the αMβ2 integrin (compare Fig. 2 and Fig. 3). Whereas αMβ2-dependent phagocytosis is mediated by RhoA activity, integrin-dependent AC uptake is dependent on Rac1. Indeed, a dominant-negative mutant of Rac1 inhibits AC phagocytosis; in parallel, the levels of active Rac1 increase during AC challenge of macrophages and DCs (Kerksiek et al., 2005; Leverrier and Ridley, 2001; Nakaya et al., 2006). By contrast, a dominant-negative mutant of RhoA enhances AC phagocytosis in macrophages (Leverrier and Ridley, 2001; Tosello-Trampont et al., 2003). The implication of Rac1 in AC uptake is also suggested by the fact that, similar to the Rac1-dependent FcγR-mediated phagocytosis of IgG-coated beads, AC engulfment requires membrane ruffling (Hoffmann et al., 2001; Ogden et al., 2001).

Significantly, the role of Rac1 during AC uptake is phylogenetically conserved and was initially unveiled in *Caenorhabditis elegans*. In this genetically tractable model system, a series of mutants deficient in the engulfment of dying cells and cell corpses (*ced* mutants) was identified. CED proteins fall into two partially redundant pathways (CED-1–CED-6–CED-7 and CED-2–CED-5–CED-12), which converge into CED-10, the *C. elegans* orthologue of mammalian Rac1. In the worm, local activation of CED-10 is ensured by CED-5 and CED-12, orthologues of mammalian Dock180 and Elmo, respectively. CED-5 and CED-12 are themselves thought to be recruited to the vicinity of bound ACs by CED-2, the CrkII orthologue (Brugnera et al., 2002; Ellis et al., 1991; Gumienny et al., 2001; Hedgecock et al., 1983; Kinchen et al., 2005; Lu et al., 2004; Reddien and Horvitz, 2000; Wu et al., 2001; Zhou et al., 2001a).

Remarkably, the AC-uptake pathway that has been identified in *C. elegans* appears to be conserved in mammals. In the HEK293T epithelial cell line, αVβ5-dependent phagocytosis of ACs is inhibited by dominant-negative mutants of CrkII and Rac1, and ligation of αVβ5 by vitronectin stimulates the formation of a complex between p130Cas, CrkII and Dock180 (Albert et al., 2000). However, recruitment of a CrkII-Dock180 complex and activation of Rac1 could be dependent on receptors that cooperate with integrins in the recognition and/or uptake of apoptotic bodies. Indeed, macrophages display multiple recognition receptors for ACs (reviewed by Ravichandran and Lorenz, 2007). One such receptor is Mer, a tyrosine-kinase receptor. Similar to integrins, Mer binds phosphatidylserine indirectly, through the serum protein GAS6 (Nagata et al., 1996). Macrophages from *Mer*-knockout mice are strongly inhibited in AC phagocytosis both in vitro and in vivo (Cohen et al., 2002; Scott et al., 2001). Interestingly, Mer stimulates the formation of a p130Cas-CrkII-Dock180 ternary complex in a αVβ5-dependent manner (Wu et al., 2005). Mammalian integrins therefore probably contribute to AC uptake together with co-receptors. The importance of CrkII in the AC-uptake pathway was recently challenged, because Dock180-Elmo can activate Rac1 independently of CrkII both in mammalian cells and in *C. elegans*, possibly via another Rho-family protein, RhoG (deBakker et al., 2004; Tosello-Trampont et al., 2007). Another possibility is that Dock180-Elmo is directly recruited to some receptors, such as BAI1 (brain-specific angiogenesis inhibitor 1), a seven-transmembrane protein that binds phosphatidylserine directly and mediates Rac1 activation by recruiting the Dock180-Elmo complex (Park et al., 2007).

Others phagocytic receptors, such as CD36 (Fadok et al., 1998; Greenberg et al., 2006; Lucas et al., 2006) and CD14 (Devitt et al., 1998), are engaged in the binding of ACs and contribute to AC phagocytosis both in vitro and in vivo. Whether they induce or modulate signalling downstream of αVβ5 or αVβ3 remains unknown. In particular, whether the activation of αVβ3 and αVβ5 by inside-out signalling is necessary for integrin-dependent phagocytosis of ACs and whether co-receptors control this pathway remain unclear. Surprisingly, it has recently been shown that, whereas the NPXY motif of β5 integrin is required for the adhesion of cells on vitronectin, it is not necessary for αVβ5-dependent phagocytosis (Singh et al., 2007). Unlike β1-β3, β5 does not harbour the conventional talin-head-domain binding site, which is characterised by a tryptophan residue that is located seven to eight amino acids before the NPXY motif. It is therefore possible that αVβ5 is activated in a talin-independent manner. Alternatively, integrin-dependent AC phagocytosis could be regulated at the level of integrin availability at the plasma membrane, as recently shown for αVβ5-dependent phagocytosis of photoreceptor outer segments by retinal pigment epithelial (RPE) cells. In these cells, CD81, which belongs to the tetraspanin family (membrane proteins that regulate the activity of surface receptors, including integrins), stimulates αVβ5-dependent phagocytosis by increasing the level of αVβ5 expression at the cell surface (Chang and Finnemann, 2007).

Therefore, the picture that emerges for AC phagocytosis is one of receptor cooperation. Such a multiple-recognition system could have been selected to ensure efficient uptake. Receptor cooperation also exists for AC uptake in *C. elegans*, although the real picture remains incomplete. Whether integrins also mediate AC recognition and phagocytosis in nematodes remains unclear. In *C. elegans*, the

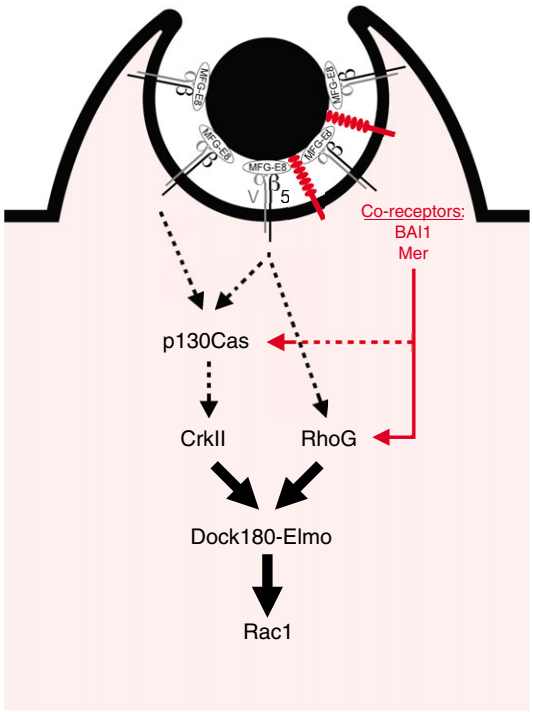


**Fig. 3.** αVβ5-dependent phagocytosis of apoptotic cells is mediated by Rac1. MFG-E8-opsonised ACs are captured by αVβ5 integrins in DCs. The membrane protrusions that engulf the AC are triggered by the activation of Rac1 by the bimolecular Dock180-Elmo complex. A cascade that involves the adaptor proteins p130Cas and CrkII and a pathway that involves RhoG can lead to the recruitment and activation of Dock180-Elmo. Both pathways could be dependent on the engagement of co-receptors (such as BAI1 or Mer) by ACs.

integrin repertoire is limited to two, because two α-subunits (encoded by the *ina-1* and *pat-2* genes) and one β-subunit (encoded by *pat-3*) are expressed (Cox and Hardin, 2004). Unsurprisingly, integrin mutants have numerous developmental defects, which may have masked a possible role in phagocytosis (Baum and Garriga, 1997; Lee et al., 2001; Williams and Waterston, 1994). The receptor(s) upstream of the CED-2–CED-5–CED-12 cascade is still missing and could be an integrin. By contrast, CED-1 is a transmembrane protein that controls AC uptake by acting upstream of CED-6 and CED-7; CED-1 clusters around AC corpses in a CED-6-dependent manner (Zhou et al., 2001b). Noticeably, the cytoplasmic tail of CED-1 displays an NPXY motif that is reminiscent of the one found in integrins. Whether this domain contributes to the function of CED-1 remains to be elucidated.

Obviously, the main challenge in this field will entail teasing out the receptors that are solely involved in AC recognition from those that also significantly contribute to internalisation.

### Integrin-dependent uptake and degradation of extracellular-matrix components

Degradation of collagen by fibroblasts is a fundamental process in the growth and development of connective tissues, the maintenance of tissue homoeostasis and during wound healing (Everts et al., 1996). Two pathways mediate collagen degradation: an extracellular pathway involves the secretion of metalloproteases that degrade collagen into fibrils (Birkedal-Hansen et al., 1993), whereas the so-called intracellular pathway in fact reflects a phagocytic event (Everts et al., 1996). The engulfment of collagen fibrils is mediated by α2β1 integrins (Knowles et al., 1991; Lee et al., 1996). This actin-driven uptake process is comparable to type I phagocytosis, with protrusive uptake and involvement of Rac1 (Arora et al., 2004; Arora et al., 2000; Melcher and Chan, 1981; Serrander et al., 2000). Surprisingly, whereas α2β1 is widely expressed, phagocytosis of collagen is a particular feature of fibroblasts. This could be due to the fibroblast-specific expression of co-receptors and/or signalling components that are essential for the phagocytic response. Overexpression or misregulation of these molecules could participate in the invasion of tissues through the ECM by tumour cells (Gaggioli et al., 2007).

So far, no other ECM protein (e.g. fibrinogen or fibronectin) has received attention in this context. Nevertheless, because beads that are coated with fibronectin are efficiently internalised by fibroblasts and RPE cells (Tsai et al., 1999; Zhao et al., 1999), and because some pathogens use fibronectin to trigger their uptake (see below), the phagocytic-like removal of other large ECM aggregates is conceivable. Overall, these findings suggest that whether integrins mediate adhesion or phagocytosis depends on the size of the ECM surface that is presented and on the ability of the cell to wrap around the ECM-coated material. Interestingly, the β-amyloid plaques that are formed by the aggregation of β-amyloid peptides in the brain of patients who have Alzheimer disease are extracellular nanostructures that could be internalised by phagocytosis. Indeed, microglial cells – bona fide phagocytes of the central nervous system – can phagocytose microaggregates of β-amyloid peptide in vitro (Paresce et al., 1996). Moreover, microglial cells are recruited to β-amyloid plaques in patients who have Alzheimer disease, which suggests that a lack of recognition and/or phagocytosis of β-amyloid aggregates might contribute to disease. Interestingly, the recognition of fibrillar β-amyloid peptides involves a complex between the integrin α6β1, the integrin-associated protein CD47 and the scavenger receptor CD36 (Bamberger et al., 2003). Phagocytosis of fibrillar β-amyloid peptides involves Vav and Rac1 activities (Koenigsknecht and Landreth, 2004; Wilkinson et al., 2006).

## Integrins that turn phagocytic in pathogenic conditions

Integrins are often targeted by bacterial and viral pathogens, which use them to establish intimate contact with, and often gain entry into, host cells (Hauck et al., 2006; Nemerow and Cheresh, 2002; Stewart and Nemerow, 2007).

### β1 integrins and bacterial invasion

Enteropathogenic *Yersinia* species present the best-known case of the hijacking of integrin function for bacterial invasion. These Gram-negative bacteria express an adhesin (a surface protein that mediates their interaction with host cells) called invasin, the binding of which to α5β1 integrins is sufficient to trigger bacterial entry into epithelial host cells (Tran Van Nhieu and Isberg, 1993; Wong and Isberg, 2005). As shown by the use of invasin-coated beads to challenge cells that express either the β1A or β1B splice variant of the β1 subunit, the ability of invasin to induce phagocytosis relies on signalling from the β1 subunit. β1A (the common β1 subunit) and its β1B variant only differ in the C-terminal region of their cytoplasmic tail. In particular, the β1B variant does not display the NPXY motif that is necessary for binding to talin (Czuchra et al., 2006). Whereas invasin-coated beads bind similarly to β1A- and β1B-containing integrins, only β1A supports phagocytosis (Gustavsson et al., 2002). Thus, as seen for β2 during αMβ2-dependent phagocytosis (Wiedemann et al., 2006), the cytoplasmic tail of the β1 subunit is essential for invasin-induced uptake. Consistent with previous studies showing that β1 integrins signal to Rac1 during cell spreading (Hirsch et al., 2002; Pankov et al., 2005; Stewart and Nemerow, 2007; Suzuki-Inoue et al., 2001), invasin-triggered phagocytosis requires Rac1 activity (Alrutz et al., 2001; McGee et al., 2001). The similarities between the classical and phagocytic behaviours of β1 integrins is further illustrated by the fact that, when β1A-expressing cells spread onto an invasin-coated surface, β1A integrins colocalise with vinculin, paxillin and FAK into structures that are essentially identical to the focal adhesions that form when cells spread onto fibronectin; comparatively, β1B-expressing cells spread poorly (Gustavsson et al., 2002; Gustavsson et al., 2004). Thus, invasin-mediated phagocytosis corresponds to the embezzlement of the β1 signalling pathways that are classically used for cell adhesion and spreading.

This notion is further validated by the example of pathogens that hijack the physiological ligands of β1 to enter host cells. Another *Yersinia* spp. adhesin, YadA, also targets the β1 integrin to promote bacterial entry. Unlike invasin, which binds to β1 directly, YadA-induced α5β1-dependent uptake requires the formation of a fibronectin bridge between YadA and the integrin. Accordingly, *Yersinia enterocolitica* YadA, which binds fibronectin less efficiently than *Yersinia pseudotuberculosis* YadA, fails to promote bacterial entry into HepG2 cells (Heise and Dersch, 2006). A similar uptake mechanism has also been described for *Staphylococcus aureus* (Dziewanowska et al., 1999; Fowler et al., 2000; Sinha et al., 2000; Sinha et al., 1999) and has been linked to the activation of Src- and FAK-dependent signalling pathways (Agerer et al., 2005; Agerer et al., 2003). It has also been proposed that the binding of another invasive bacterium, *Shigella flexneri*, to host cells uses β1 integrins (Watarai et al., 1996). It is now clear, however, that the signalling pathways that control the uptake of *Shigella* species are largely independent of β1 signalling (Nhieu et al., 2005). The exact role

of β1 integrins in the initial binding of *Shigella* species to host cells remains to be elucidated.

β1 integrins are therefore targeted by several bacterial pathogens to allow their entry into host cells through a phagocytic zipper-like process. Remarkably, pathogen-induced uptake involves conventional integrin signalling. In other words, the signalling pathways that are induced by β1 integrins during cell adhesion also underlie adhesin-dependent phagocytic processes. Consequently, the study of adhesin-promoted phagocytosis should help to better understand β1 signalling and regulation. For example, the fact that an invasin mutant that is unable to dimerise β1 integrins fails to activate Rac1 clearly suggests that outside-in signalling from β1 is dependent on integrin clustering (Dersch and Isberg, 1999; Dersch and Isberg, 2000). Strikingly, no other type of integrin is targeted by bacteria as often as is β1. The fact that β1 signalling is particularly prone to hijacking by invasive bacteria might be because bacteria easily find themselves coated with fibronectin within tissues; it might also be linked to the relative abundance of β1 on the cell surface.

### Virus internalisation – a phagocytic-like process?

Integrins are also frequently targeted by viruses in the process of host-cell entry (Stewart and Nemerow, 2007). Whether viruses thereby specifically use the ability of integrins to signal a phagocytic-like process remains unclear, particularly because viruses are below the size of particle that is commonly admitted for actin-driven phagocytic events (0.5 μm in diameter). Virus entry is more classically associated with membrane fusion, or with clathrin- or caveolae-dependent endocytosis (Smith and Helenius, 2004).

Nevertheless, specific virus families use a process that is reminiscent of phagocytosis to invade host cells. Herpes simplex virus type I (HSV-1) enters cells through a protrusive phagocytic-like mechanism that requires Rho GTPase activity (Clement et al., 2006). Similarly, invasion of Chinese hamster ovary (CHO) cells by equine herpes virus type 1 (EHV-1) is independent of clathrin and caveolin but dependent on Rock activity, which suggests a role for acto-myosin contraction in EHV-1 internalisation (Frampton, Jr et al., 2007). Whether integrins are involved in EHV-1 and HSV-1 entry remains unclear. However, envelope glycoprotein H (gH) of HSV-1 and of human cytomegalovirus (HCMV; another herpes virus) binds the αVβ3 integrin (Parry et al., 2005). Interestingly, the binding of HCMV gH to αVβ3 is RGD independent and virus entry therefore requires cooperation between integrins and the epidermal growth factor (EGF) receptor (Wang et al., 2005); this process is reminiscent of the multi-receptor situation discussed above for AC uptake. In addition, the glycoprotein B (gB) from HCMV displays a disintegrin-like domain (conserved in HSV-1 and EHV-1 gBs) that promotes β1-dependent HCMV internalisation. Notably, soluble gB from HCMV is sufficient to trigger cytoskeletal rearrangements, and stress-fibre and filopodia formation in a β1-dependent manner (Feire et al., 2004). By contrast, gB from Kaposi's sarcoma-associated herpes virus (KSHV/HHV-8) contains an RGD motif, which, together with FAK activity, is required for virus entry, strongly suggesting an integrin-mediated entry process (Akula et al., 2002; Krishnan et al., 2006). Although KSHV gB was initially thought to interact with α3β1, it appears more likely that it binds αVβ3 (Akula et al., 2002; Garrigues et al., 2008).

Several adenoviruses might also fall into the group of phagocytosed viruses. Human adenoviruses types 2 and 5 (Ad2 and Ad5) use a specific membrane receptor for cell attachment (Bergelson et al., 1997; Tomko et al., 1997); however, their uptake is subsequently facilitated by the interaction with αVβ3 and αVβ5 integrins of five RGD motifs in a capsid viral protein (Mathias et al., 1994; Wickham et al., 1994; Wickham et al., 1993). Interestingly, the entry of Ad2 relies on actin-cytoskeleton remodelling and on the activity of Rac1 and Cdc42, which is reminiscent of type I phagocytosis (Li et al., 1998; Patterson and Russell, 1983). Similarly, the uptake of adenovirus type 3 (Ad3) was recently shown to be dependent on the αV integrin and on Rac1- and Pak-1-dependent actin remodelling (Amstutz et al., 2008). Finally, α2β1, αXβ2, α4β1 and α4β7 integrins promote the entry of rotaviruses (Coulson et al., 1997; Graham et al., 2005; Graham et al., 2003; Graham et al., 2006; Guerrero et al., 2000; Hewish et al., 2000), although it remains to be seen whether this follows a phagocytic-like process.

Overall, several viral and bacterial pathogens use integrin signalling to enter host cells in an actin-dependent process. Deciphering the molecular pathways that are used for local, transient, integrin-triggered actin and membrane remodelling should aid in the design of drugs that will help fight infection in vitro and in vivo.

## Conclusions and perspectives

In addition to their well-known role in mediating adhesion and motility, integrins clearly mediate a wide range of phagocytic processes of physio-pathological relevance. These range from the uptake of big particles such as ACs to the capture of smaller prey, such as bacteria and collagen fibrils. The high phagocytic ability of αMβ2, the main receptor for opsonic complement fragments in professional phagocytes, has been recognised for over 20 years. As we have shown, it is now becoming clear that other integrin heterodimers can perform phagocytosis, including in non-immune cells. Phagocytosis is therefore not a property of specialised integrins or even of specific cells, but rather the natural ability of numerous integrin-family members. This is true of several β1-, β3- and β5-based integrins, including integrins that usually mediate binding to ECM components such as vitronectin (binds αVβ3 and αVβ5), fibronectin (binds α5β1) or collagen (binds α2β1). This strongly suggests that the ability of integrins to mediate phagocytosis is an extension of their capacity to mediate adhesion. The reason why all integrins have not been linked to phagocytosis is unclear. Because phagocytosis is an actin-driven process, it is possible that β6-, β7- and β8-based integrins, which are known to induce actin remodelling, might also support the phagocytic uptake of suitable ligands. By contrast, β4 integrins, which are linked to the intermediate filament but not the actin network, seem unlikely to display phagocytic potential (Hynes, 2002).

The idea that integrin-mediated phagocytosis relies on integrin signalling is particularly strengthened by the example of integrin-dependent bacterial invasion, in which the interaction of a microorganism with α5β1, either directly or through a fibronectin bridge, is sufficient for phagocytic internalisation. This also shows that integrin-dependent phagocytosis and adhesion rely on the same regulatory and signalling mechanisms. This is why spreading on a ligand-coated surface has been compared to the frustrated phagocytosis of an infinitely large object (Cannon and Swanson, 1992). Indeed, whereas cells commonly spread onto a collagen-coated surface, collagen fibrils, once degraded, are readily phagocytosed by fibroblasts (Everts et al., 1996; Knowles et al., 1991; Lee et al., 1996); the same is true for fibronectin-coated surfaces versus fibronectin-coated particles (Tsai et al., 1999; Zhao

et al., 1999). A clear difference between the two processes is, however, that phagocytosis requires a transient, sequential and three-dimensional interaction of integrins with their ligand.

Investigating integrin-dependent phagocytosis has shown that integrins cannot work in isolation and always require the activity of co-receptors to be fully efficient. αMβ2-dependent phagocytosis in macrophages is dependent on additional signals to activate integrin-binding abilities via the small G protein Rap1. Interestingly, lipopolysaccharide that is present on the cell surface of most bacteria is a potent activator of Rap1, providing a clear example of cooperation between a pathogen-recognition receptor and an integrin receptor to mediate particle recognition and uptake. Similarly, αVβ3- and αVβ5-dependent phagocytosis of ACs illustrate how integrin signalling cooperates with co-receptors to trigger a full outside-in signalling pathway. Elucidation of the exact role of all receptors that contribute to integrin-dependent phagocytosis is needed in most cases to disentangle the different signalling pathways (inside-out versus outside-in) that are involved.

In the context of integrin-dependent uptake, signalling to the actin cytoskeleton involves either RhoA (for αMβ2-mediated phagocytosis of C3bi-opsonised particles) or Rac1 (and sometimes also Cdc42) activity. This dichotomy reflects the current understanding of phagocytosis in general and confirms both that particle engulfment can follow different actin-based mechanisms and that the mechanisms of integrin-dependent internalisation are similar to the general modes of phagocytosis. Interestingly, outside-in signalling relies exclusively on the β-chain of phagocytic integrins – at least this is true of β2, which activates RhoA, and of β1, which controls Rac activation (Gustavsson et al., 2002; Gustavsson et al., 2004; Wiedemann et al., 2006). Defining the precise mechanisms of preferential Rho-protein usage downstream of integrin ligation is a fascinating challenge.

Whereas the general role of integrins in phagocytosis is only emerging, integrins are clearly essential mediators of cell adhesion and migration. Understanding the molecular basis of their function in these complex processes is particularly challenging because several layers of regulation (e.g. surface expression, affinity, avidity and recycling) and multiple signalling pathways (e.g. inside-out and outside-in) are intertwined. In this regard, the model system of integrin-dependent phagocytosis offers a range of advantages when studying integrin regulation and function. First, the apparent conservation of pathways that regulate integrin activation and signalling makes it a suitable alternative system. Second, the easy distinction between bound and phagocytosed particles constitutes a straightforward and reliable way to assess integrin activation and signalling. Third, phagocytosis involves three-dimensional remodelling at the cell surface, as does adhesion and motility in vivo. Fourth, the adhesive stimulus (i.e. the phagocytic bait) can be controlled in terms of size, shape and ligand(s) density. Finally, phagocytosis is spatially and temporally well defined. This contrasts with migrating cells, in which signalling to and from integrins is not homogenous in the entire cell (Ridley et al., 2003). We therefore believe that integrin-dependent phagocytosis provides an exciting study system in which to better understand integrin function and regulation.

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