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Minireview

Rho proteins, PI 3-kinases, and monocyte/macrophage motility

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Abstract Rho proteins and phosphatidylinositide 3-kinases (PI 3-kinases) have been widely implicated in regulating cell motility both in cultured cells and in animal models. Monocytes are recruited from the bloodstream in response to inflammatory signals, and migrate across the endothelial barrier into the tissues, where they differentiate into macrophages and phagocytose bacteria and cells. Studies of monocytes and macrophages have revealed that different Rho family members and PI 3-kinases are not functionally redundant but play unique and distinct roles in motile responses.

Key words: Endothelial cell; Actin cytoskeleton; Monocyte; Macrophage; Rho GTPase; Phosphatidylinositide 3-kinase; Motility

1. Introduction

Neutrophils and macrophages are the body's first line of defence against invading micro-organisms. Macrophages not only phagocytose micro-organisms, infected cells and dying cells, but release a battery of cytokines that are key to initiating and maintaining inflammatory and immune responses. However, macrophages can also play a negative role in chronic inflammatory diseases such as rheumatoid arthritis, multiple sclerosis and atherosclerosis. Macrophages are therefore a prime target for therapies aimed at treating chronic inflammatory diseases. In order to understand how macrophage-mediated tissue damage can be effectively inhibited, it is essential to elucidate the mechanisms whereby they are recruited to and activated in tissues. This review addresses the roles of specific signalling proteins in the motile responses of monocytes and macrophages, in their recruitment both out of blood vessels and into tissues, and in their subsequent activity as phagocytes (Fig. 1).

2. Rho proteins

The Rho protein family is part of the Ras superfamily of small GTPases. There are at least 20 genes encoding Rho family members in mammals, including Rho (A, B, C), Rac (1, 2, 3), Cdc42, TC10, TCL, RhoD, RhoG, RhoE/Rnd3, Rnd1, Rnd2, Chp (1, 2), RhoBTB (1, 2), Rif and TTF. In addition, splice variants of Rac1 and Cdc42 have been described. Many of these proteins have been studied for their effects on the actin cytoskeleton, and in some cases on cell–cell adhesion, vesicle trafficking, cell cycle progression and transcriptional activation [1]. However, nearly all studies on cell migration have concentrated on the roles of Rho, Rac and Cdc42, and very little is known about how other Rho family members influence cell migration. Rho, Rac and Cdc42 were the first members of the family to be characterised for their roles in regulating the actin cytoskeleton: Rho stimulates actomyosin-based contractility and induces actin organisation into stress fibres in many but not all cell types; Rac induces the extension of lamellipodia, involving actin polymerisation at the plasma membrane; while Cdc42 induces the extension of finger-like plasma membrane protrusions known as filopodia, which again are driven outward through actin polymerisation.

Most Rho proteins bind and hydrolyse GTP, and change conformation depending on whether they are bound to GTP or GDP (Fig. 2). When bound to GTP, they are 'active', in that they can then interact with and activate their downstream signalling partners. Hydrolysis of GTP to leave them bound to GDP releases them from their signalling partners, thereby terminating the response. They can be reactivated by guanine nucleotide exchange factors (GEFs), which induce release of bound nucleotide (generally GDP) and thereby allow GTP to bind. GTP hydrolysis is catalysed by GTPase activating proteins (GAPs), which thereby act to downregulate the response to Rho proteins. Rho proteins are normally post-translationally modified by prenylation at the carboxy-terminus, and this is important for their association with membranes. A further level of regulation is provided for some Rho proteins by guanine nucleotide dissociation inhibitors, which form an inactive complex with the Rho protein in the cytoplasm, masking the prenyl group and preventing it from interacting with the plasma membrane.

A number of point mutants have been useful in defining the functions of Rho family proteins (Fig. 1). First, constitutively active versions of the proteins can be generated by mutating residues that play essential roles in the catalysis of GTP hydrolysis. These mutations lock the proteins in their GTP-bound conformation, as they are unable to hydrolyse GTP to GDP. By introducing these into cells, it is possible to find out the consequences of not being able to turn the proteins off. Second, dominant negative mutations act to prevent

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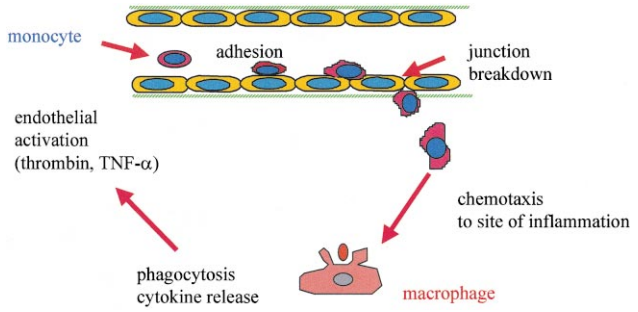

Fig. 1. Motile responses of monocytes/macrophages. Monocytes in the bloodstream adhere to activated endothelial cells, and then transmigrate across junctions between endothelial cells. Once outside blood vessels, they differentiate into macrophages and migrate towards a site of infection or inflammation, and then extend their plasma membrane to engulf the particle by phagocytosis.

the activation of their endogenous counterparts, and thus tell us what the consequence of not being able to turn the proteins on is. How these dominant negative proteins act is not known in most cases, although at least with Rac and Cdc42 they have been shown to have very low affinity for nucleotide, especially GTP, and thus may act primarily by binding to exchange factors and preventing them acting on endogenous Rac or Cdc42 respectively. Third, a battery of other point mutations have been useful in dissecting out the roles of different domains of each protein in mediating distinct downstream responses in cells. For example, mutation of amino acid 40 in Rac prevents binding in vitro to one of its downstream signalling partners, PAK [2,3]. In cells, this mutant can still induce lamellipodium extension [3].

3. PI 3-kinases

Phosphatidylinositide 3-kinases (PI 3-kinases) are involved in regulating a number of cellular responses, including cell growth, survival and migration [4]. PI 3-kinases are a family of enzymes that add a phosphate to the 3′ position of the inositide ring of phosphatidylinositides (for review see [4]). They are divided into three classes, based on sequence similarity and biochemical properties. Class I PI 3-kinases are best established for their roles in signal transduction, and act to generate phosphatidylinositol 3,4,5-triphosphate at the plasma membrane in response to extracellular stimuli. Class Ia PI 3-kinases consist of a catalytic subunit, p110 (α, β or δ), and an adapter subunit, generally p85 (α or β). The p85 subunit binds to phosphorylated tyrosines on signalling proteins and transmembrane receptors, leading to the recruitment and activation of the p110 subunits at the plasma membrane. There is only one class Ib PI 3-kinase, which consists of a catalytic subunit, p110γ, and probably an associated p101 subunit [5]. PI 3-kinase γ is recruited and activated by G protein-coupled receptors.

A number of reagents have been used to study the function of PI 3-kinases. First, LY294002 and wortmannin are two small molecule inhibitors of PI 3-kinases and act relatively specifically to inhibit nearly all PI 3-kinase isoforms [5]. In addition, introduction of mutated tyrosine kinase receptors that no longer bind the p85 adapters have been used to implicate class 1a PI 3-kinases in specific responses induced by growth factor receptors. An alternative approach is to express a dominant negative form of p85.

Class I PI 3-kinases can act upstream of Rac but so far there is no good evidence that they act upstream of any other Rho family members. One mechanism whereby they could activate Rac is through phosphatidylinositol trisphosphate-induced activation of Rac GEFs such as Vav, Sos and Tiam1 [6,7]. However, it is also possible for Rac to be activated independently of PI 3-kinase activity [8].

4. Monocyte endothelial adhesion and diapedesis

Monocyte transmigration across endothelial cells is the first step required for the recruitment of monocytes into tissues. Monocytes transmigrate across quiescent endothelial cells at very low rates, but the rate of transmigration is massively increased following endothelial cell activation. A variety of factors stimulate endothelial cells and enhance transmigration, including the pro-inflammatory cytokines tumour necrosis factor-α and interleukin-1, which stimulate leukocyte transmigration primarily by inducing the expression of adhesion receptors (including E-selectin, ICAM-1 and VCAM-1) and chemokines on the apical surface of endothelial cells [9].

Monocyte adhesion and transmigration involves a two-way signalling process between monocytes and endothelial cells. E-selectin can mediate weak adhesion of leukocytes to endothelial cells, allowing leukocytes to slow down and 'roll' on the endothelial surface. Chemokines can then activate leukocyte integrins, which in turn adhere to ICAM-1 and VCAM-1, leading to firm adhesion of leukocytes to the endothelial surface. Recent data indicate that endothelial cells are themselves activated through engagement of monocyte binding receptors, including E-selectin, ICAM-1 and VCAM-1. Antibody-induced clustering of any of these receptors can induce Rho-mediated stress fibre formation in endothelial cells, as well as tyrosine phosphorylation of a number of proteins [10–12].

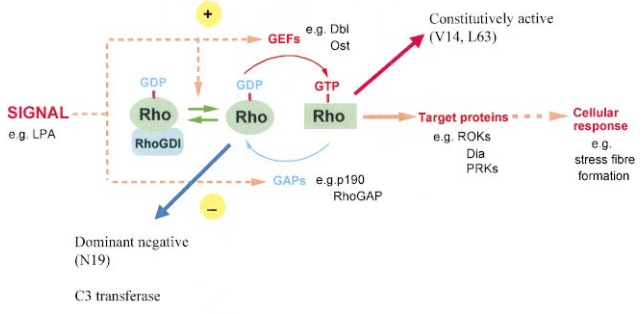

Fig. 2. Regulation of Rho proteins. Rho cycles between an active GTP-bound conformation and an inactive GDP-bound conformation. In resting cells, most Rho is bound in an inactive complex in the cytoplasm with GDIs (guanine nucleotide dissociation inhibitors). Incoming signals from extracellular factors such as lysophosphatidic acid (LPA) release Rho from GDIs and activate guanine nucleotide exchange factors (GEFs) to stimulate exchange of GDP for GTP on Rho. Rho-GTP can then interact with downstream targets, such as the serine/threonine kinases ROK and PRK, and the adapter protein Dia. Rho is inactivated by GTP hydrolysis, catalysed by GTPase activating proteins (GAPs). Mutants of Rho proteins that are used to analyse protein function include constitutively active mutants that are impaired in GTP hydrolysis (mutation of amino acids 14 in Rho from Gly to Val, and 63 from Glu to Leu), and dominant negative mutants (mutation of amino acid 19 in Rho from Thr to Asn). A number of bacterial toxins, such as the *Clostridium botulinum* exoenzyme C3 transferase, are also used to inhibit or activate Rho proteins.

Rho activation in turn is important for stabilising the interaction between monocytes and endothelial cells [10]. In contrast, inhibition of Rac or Cdc42 has no discernible effect on monocyte adhesion.

5. Macrophage chemotaxis

Following tissue damage or invasion of micro-organisms, tissue macrophages are rapidly recruited to the site of damage. In addition, once monocytes have crossed the endothelial barrier, they differentiate into macrophages and migrate from the blood vessels to sites of inflammation. Macrophage chemotaxis can be induced by small peptides known as chemokines produced during inflammation, as well as by the cytokine colony-stimulating factor-1 (CSF-1), which is both a survival factor and a chemoattractant for macrophages.

CSF-1 stimulates the tyrosine kinase receptor c-fms to activate a variety of signalling pathways [13,14]. Like other tyrosine kinase receptors, c-fms can recruit class 1a PI 3-kinases through their p85 adapter proteins [13]. Studies on a mouse macrophage cell line, Bac1.2F5, have shown that both PI 3-kinases and Rho GTPases are required for CSF-1-induced migration [15,16]. Inhibition of the p110β and p110δ isoforms of PI 3-kinase by injecting isoform-specific antibodies impairs lamellipodium extension and macrophage migration (Fig. 2), whereas antibodies to the p110α isoform inhibit cell proliferation but not migration [16]. The relative involvement of different p110 isoforms in migration appears to be cell type-dependent. For example, in porcine aortic endothelial cells, which express p110α and β but not p110δ, p110α is required for plasmid-derived growth factor-induced migration whereas p110β is required for insulin-induced migration [17].

In CSF-1-induced migration, both Rho and Rac are required for migration, but play distinct roles (Fig. 3A) [15,18]. Rac is required for lamellipodium extension and formation of new adhesions to the extracellular matrix. Rho stimulates actomyosin-mediated contractility [19], and inactivation of Rho reduces intracellular tension so that macrophages extend very long processes with lamellipodia at their tips. The cell body remains stationary and is unable to follow the leading lamella, hence cell migration is prevented. In contrast to Rac and Rho, Cdc42 is not required for cell migration but is required for sensing the chemotactic gradient of CSF-1. When Cdc42 is inhibited cells migrate randomly despite the presence of a chemotactic gradient [15]. Cdc42 is also required for polarity generation in other systems. For example, in *Caenorhabditis elegans* it is involved in generating cell polarity in early embryos [20,21]. In addition, Cdc42 is required for T cells to polarise towards antigen-presenting cells [22].

Little is known about the roles of Rho GTPases in chemokine-induced macrophage chemotaxis, although studies in T cells have shown that Rho, Rac, Cdc42 and and the Cdc42 target WASp are required for chemotaxis of T cells towards the chemokine SDF-1 [23,24]. Chemokines act through G protein-coupled receptors, and in mice lacking the class 1b p110γ PI 3-kinase macrophage migration towards a variety of chemokines is impaired [25–28].

6. Phagocytosis

The final goal of macrophages is to phagocytose invading micro-organisms, virally infected cells, dying (apoptotic) cells and other debris at sites of infection and inflammation. At the same time, macrophages produce a range of cytokines and chemokines that modulate the recruitment and activation of further macrophages and other leukocytes and lymphocytes. Macrophages generally phagocytose through the recognition of molecules on the surface of objects, including immunoglobulins, the complement component C3b, and phosphatidylserine on apoptotic cells. Phagocytosis is dependent on actin polymerisation, but different Rho proteins are implicated in the uptake of different types of particles. Phagocytosis of immunoglobulin-coated particles and apoptotic cells requires Rac and Cdc42 but not Rho (Fig. 3B) [29–31], whereas uptake of C3b-coated particles requires Rho but not Rac or Cdc42 [31]. PI 3-kinases have been implicated in the phagocytic uptake of immunoglobulin-coated particles, C3b-coated particles, and apoptotic cells [29,32,33]. Which isoforms of PI 3-kinase mediate these responses is not known, although it appears that p110γ is unlikely to be involved from studies on p110γ-null mice [25,26]. Interestingly, however, in contrast to chemotaxis, where PI 3-kinases appear to act upstream of Rac, PI 3-kinases act at a later stage to Rac during phagocytosis of immunoglobulin-coated particles and apoptotic cells. PI 3-kinases are not required for actin-driven membrane protrusion around particles, but appear to act at a later stage to inhibit membrane fusion [29,33].

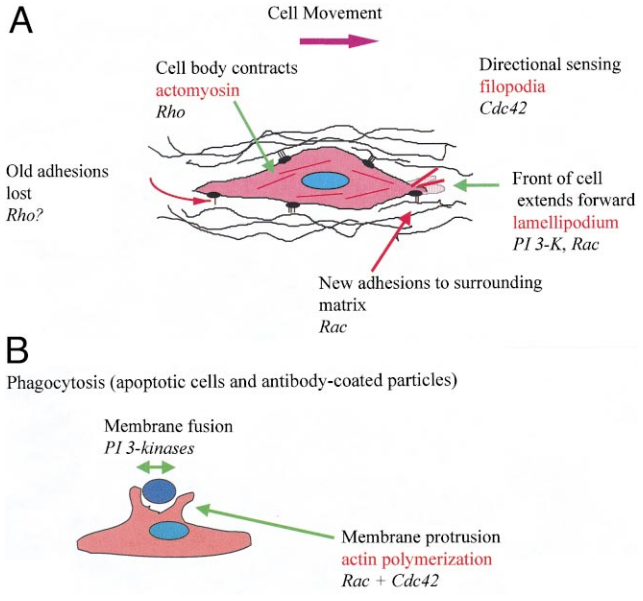

Fig. 3. Model for the regulation of macrophage functions by PI 3-kinases and Rho proteins. (A) Migrating cells extend lamellipodia at their leading edge, and this is dependent on PI 3-kinases and Rac-driven actin polymerisation. They also form new adhesions to the extracellular matrix in lamellipodia, and this requires Rac activity. The cell body follows the front of the cell through Rho-induced actomyosin contraction. Detachment of the tail is probably also dependent on this Rho-driven contraction. Directional sensing (chemotaxis) requires Cdc42, which induces the extension of filopodia. (B) Macrophages phagocytose apoptotic cells and antibody-coated particles by extending their plasma membrane around the particle. This is dependent on Rac and Cdc42, and requires actin polymerization. PI 3-kinases act at a later stage and may promote membrane fusion and final engulfment of the particle.

7. Conclusions

Both PI 3-kinases and Rho proteins play important roles at multiple steps in the recruitment and activation of macrophages, and these proteins are therefore potentially good candidates for therapies aimed at treating chronic inflammatory diseases. Of the isoforms studied, each makes a different contribution to motile responses. Inhibiting Rho, for example, reduces both monocyte transmigration and macrophage chemotaxis, although it does not reduce phagocytosis of immunoglobulin-coated particles or apoptotic cells. In the future, it will be interesting to find out how other members of the Rho family affect cell migration, as well as to determine how isoform-specific functions of PI 3-kinases are mediated.

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