Actin Cytoskeleton and Related Proteins Role During Immune Cells Migration, Polarity and Activation: An Overview

Claudio Vieira da Silva

Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Brazil

ABSTRACT

Immune cells migration, polarity and activation are essential during host immune response. Upon recognition of specific peptides presented by Human Leukocyte Antigen (HLA) molecules on Antigen Presenting Cells (APC), lymphocytes execute subset-related functions such as killing, help and regulation. These cells travel through the organism in a succession of steps, including entry into tissues, interstitial migration, APC scanning, synapse formation and tissue exit. Such ability is possible because of a plastic motility behavior, which is highly controlled in time and space. The scope of this review is to discuss recent data pointing to the key role of regulators of actin cytoskeleton remodeling in tuning migration, polarity and activation of immune cells during host immune response. We believe that the more complete understanding of actin and related proteins recruitment during these processes the better to obtain novel targets for establishing strategies to control immune responses.

Keywords: Actin Cytoskeleton, Immune Cells, Activation, Migration, Immune Response

1. INTRODUCTION

The actin cytoskeleton is a dynamic structure that plays a fundamental role in diverse processes in all eukaryotic cells. Actin-dependent cellular processes are typically associated with membrane dynamics and the coordinated polymerization of actin filaments against cellular membranes provides the force for these processes. Probably the most thoroughly characterized of such processes are cell migration and endocytosis.

During cell migration, precisely coordinated polymerization of actin filaments against the plasma membrane induces the formation of plasma membrane protrusions and consequent advancement of the leading edge of the cell. Similar membrane protrusions, in the absence of retraction of the trailing edge of the cell, drive cell elongation during morphogenetic processes such as neuronal outgrowth (Chhabra and Higgs, 2007).

During endocytosis, actin polymerization provides the force for generating membrane invaginations and for the scission of the endocytic vesicles from the plasma membrane (Kaksonen et al., 2006).

The structure and dynamics of the actin cytoskeleton in cells are regulated by a vast number of actin-binding proteins. These include proteins that promote nucleation of new actin filaments (Arp2/3 complex, formins, Cobl, Spire, leiomodin) and proteins that enhance actin filament severing and depolymerization (e.g., ADF/cofilins, gelsolin) (Chesarone and Goode, 2009; Ono, 2007).

Also, proteins that regulate actin filament polymerization either by interacting with actin monomers (e.g., profilin, twinfilin, β-thymosins) or with filament barbed ends (e.g., capping protein, Eps8, Ena/VASP) play crucial roles in actin dynamics in cells. Finally, actin dependent cellular processes typically rely on the organization of actin filaments to desired three-dimensional structures through a large number of actin filament bundling and cross-linking proteins (e.g., α-actinin, fascin) (Cooper and Sept, 2008; Disanza et al., 2004; Drees and Gertler, 2008; Le Clainche and Carlier, 2008; Paavilainen et al., 2004).

Fundamental studies by the Carlier laboratory revealed that the minimal set of actin-binding proteins required for actin-based motility in vitro consists of ADF/cofilin, profilin, activated Arp2/3 complex and capping protein (Loisel *et al.*, 1999). However, most cellular processes driven by actin filament treadmilling depend on a significantly larger number of actin-associated proteins and their regulators. The activities of actin-binding proteins are controlled through various signaling pathways to ensure proper spatial and temporal regulation of actin dynamics in cells.

The most thoroughly characterized regulators of actin binding proteins are the Rho-family small GTPases. In mammals, these include, e.g., RhoA that induces the formation of contractile stress fibers, Rac1 that drives the formation of lamellipodial actin filament network at the leading edge of motile cells, as well as Cdc42 and Rif that induce the formation of thin actin-rich filopodial protrusions at cell periphery (Hall, 1998; Heasman and Ridley, 2008; Pellegrin and Mellor, 2005).

1.1. Actin Cytoskeleton Dynamics During Immune Cell Migration and Polarity

Cell migration plays a key role for the function of the immune system: Maturation of cells of the innate and adaptive immune system takes place in distinct, anatomical compartments and immunological effector functions have to be operative wherever the integrity of the organism is challenged. Thus, immune cells are constantly "en route" to complete their maturation and to exert their effector functions. Migration of cells is orchestrated by a complex interplay of interactions of cell-surface receptors with their respective cognate ligands (Von Andrian and Mempel, 2003).

Endothelial cells limit the emigration rate of leukocytes. Being located between blood and tissues, they permit or deny the passage. The exact mechanism of this process called diapedesis is not solved yet. Leukocytes can principally traverse either between cells (paracellularly) or directly through an individual endothelial cell (transcellularly). The transcellular way has recently gained experimental support, but it is not clear how the endothelial cytoskeleton manages to open and close a transmigratory channel. During this process, the endothelial cell turned out to become softer in a confined region strictly underneath the leukocyte that rearranges the endothelial cytoskeleton to form transmigratory channels (Isac *et al.*, 2011).

Along leukocyte migration, different F-actin-related proteins are activated. In this sense, Protease-Activated Receptor-2 (PAR-2) promotes activation of the actin filament severing protein cofilin, which is crucial for the reorganization of the actin cytoskeleton and chemotaxis. PAR-2 promotes the formation of a complex containing β-arrestins, cofilin and chronophin (CIN) in primary leukocytes and cultured cells. Both association of cofilin with CIN and cell migration are inhibited in leukocytes from β-arrestin-2$^{-/-}$mice. In response to PAR-2 activation, β-arrestins scaffold cofilin with its upstream activator CIN, to facilitate the localized generation of free actin barbed ends, leading to membrane protrusion (Zoudilova *et al.*, 2010). Hence, providing evidences for an important role of of β-arrestins in chemotaxis of PAR-2-stimulated immune.

Moreover, authors have shown that The Wiskott-Aldrich Syndrome Protein (WASP) plays an important role in the locomotion of lymphocytes, DC and granulocytes *in vitro* and *in vivo* and thus, reveal a crucial role of WASP in physiological trafficking of various hematopoietic cell lineages (Snapper *et al.*, 2005). Likewise, Cybr, a scaffold protein highly expressed in the hematopoietic/immune system, binds to cytohesin-1, a guanine nucleotide exchange factor for the ARF GTPases, which affects actin cytoskeleton remodeling during cell migration. In this context, Cybr was found to play a role in leukocyte trafficking in response to proinflammatory cytokines in stress conditions (Coppola *et al.*, 2006).

The large GTPase Guanylate-Binding Protein-1 (GBP-1) was found as a novel member within the family of actin remodeling proteins, specifically mediating IFN-γ-dependent defense strategies (Ostler *et al.*, 2013).

Concerning B cells migration, authors have shown that SWAP-70, a Rac-interacting protein involved in actin rearrangement (Pearce *et al.*, 2006) and ezrin, a member of the membrane-cytoskeleton cross-linking ezrin-radixin-moesin proteins (Parameswaran *et al.*, 2011) play important role during B cells chemotaxis. In addition, Rap-1 and -2 GTPases play an important role in mediating adhesion and cytoskeletal reorganization during SDF-1a-dependent B cell migration (McLeod *et al.*, 2002; 2004; Durand *et al.*, 2006; Chen *et al.*, 2008).

Establishment of cell polarity is crucial for the migration of immature and mature leukocytes across vascular endothelial barriers and provides the basis for Hematopoietic Stem and Progenitor Cell (HSC/HPC) homing. Directional cues for the migration of leukocytes to the sites of inflammation are provided by the

chemokine family members (Kitano *et al.*, 2008) and are promptly executed via intrinsic signaling pathways downstream of chemokine receptors. Similarly, chemokines have been implicated in controlling HSC/HPC homing (Luster, 1998). In both contexts, cells switch from spherical to polarized phenotypes in response to polarization cues. These morphological changes are accompanied by an orchestrated compartmentalization of certain cell surface associated molecules (Lapidot *et al.*, 2005; Serrador *et al.*, 1998; Dustin and Chan, 2000). Additionally, cytoskeletal reorganization (Giebel *et al.*, 2004; Serrador *et al.*, 1999), lateral compartmentalization of functional membrane microdomains (Merwe *et al.*, 2000; Gomez-Mouton *et al.*, 2001) and redistribution of some cellular proteins have been observed (Del Pozo *et al.*, 1998).

Actin cytoskeletal rearrangement during directional migration is a highly conserved and well documented process in amoeboid cells (Gerisch *et al.*, 1993; Chung *et al.*, 2001; Parent *et al.*, 1998) and leukocytes display the characteristic leading and trailing edges. While the leading edge is marked by a concentration of F-actin (Del Pozo *et al.*, 1998; Gerisch *et al.*, 1993) and chemokine receptors (Gomez-Mouton *et al.*, 2001; Parent *et al.*, 1998; Gomez-Mouton *et al.*, 2004), the trailing edge, termed uropod, is marked by the accumulation of several adhesion molecules (Serrador *et al.*, 1998; Giebel *et al.*, 2004; Del Pozo *et al.*, 1997), the hyaluron receptor (Ariel *et al.*, 2000; Del Pozo *et al.*, 1995), sialoglycoproteins (Serrador *et al.*, 1998; Gubina *et al.*, 2002; Savage *et al.*, 2002) and the ERM family of proteins (Serrador *et al.*, 1999). Lipids also show different polarization patterns during lymphocyte migration (Giebel *et al.*, 2004; Gomez-Mouton *et al.*, 2001).

Along similar lines, insights into the importance of lipid rafts or membrane microdomains in the process of chemokine-induced polarization have been provided by recent studies (Gubina *et al.*, 2002; Millan *et al.*, 2002; Manes *et al.*, 2001). However, none of the molecules implicated in either chemokine-induced polarization or raft residency show asymmetric localization under resting conditions that could impart pre-polarization cues. A recent study has shown that the lipid microdomain resident proteins, flotillin-1 and -2 (Salzer and Prohaska, 2001; Babuke and Tikkanen, 2007), confer intrinsic polarity to leukocytes by their asymmetric localization (Rajendran *et al.*, 2003). Authors have also shown that flotillin-1 and -2 accumulate at uropods and co-localize with CD43, CD44 and moesin and presented evidence for the distinct spatial and temporal localization of flotillin platforms with respect to the actin cytoskeleton upon chemokine-induced migration. Moreover, flotillins were accumulated at the central supramolecular Activation Cluster (c-SMAC) during immunological synapse formation concomitant with membrane ordering in these regions. Based on these results, authors proposed that a subset of lipid microdomains provide pre-polarization cues for hematopoietic cell polarization (Rajendran *et al.*, 2009).

1.2. Actin Cytoskeleton Dynamics During Immune Cells Activation

Immune cells activation is a process triggered by different pathogens and compounds (Gomez-Flores *et al.*, 2005). On the other hand, drugs, such as methamphetamine inhibits β-chemokines and co-stimulatory molecules by dendritic cells (Nair *et al.*, 2007). However, it is not known whether this activity relies on impairing the activation of actin cytoskeleton related proteins.

The molecular basis for the adaptable motility behavior of T lymphocytes is only starting to be unraveled and this subject was recently reviewed (Lafouresse *et al.*, 2013; Beemiller and Krummel, 2013).

Actin polymerization plays a critical role in activated T lymphocytes both in regulating T Cell Receptor (TCR)-induced Immunological Synapse (IS) formation and signaling. Using gene targeting, authors demonstrated that the hematopoietic specific, actin- and Arp2/3 complex-binding protein coronin-1A contributes to both processes. Coronin-1A links cytoskeleton plasticity with the functioning of TCR signaling components (Mugnier *et al.*, 2008). T cells express drebrin, a neuronal actin-binding protein, that co-localizes with the chemokine receptor CXCR4 and F-actin at the peripheral supramolecular activation cluster in the immune synapse. Drebrin interacts with the cytoplasmic tail of CXCR4 and both proteins redistribute to the immune synapse with similar kinetics (Perez-Martinez *et al.*, 2010).

RLIP76, a multifunctional transporter protein , is necessary for activation, membrane trafficking and functional maturation of Dendritic Cells (DC) (Borvak *et al.*, 2010). Further exploration of the role of RLIP76 in DC biology is warranted and may provide promising means in DC-based immunotherapies.

Formation of IS between T cells and Antigen Presenting Cell (APC-DC, macrophages and B cells) requires multiple rearrangements in the actin cytoskeleton and selective receptor accumulation in Supramolecular Activation Clusters (SMAC). The inner cluster (central SMAC) contains the TCR/CD3 complex.

The outer cluster (peripheral SMAC) contains the integrin LFA-1 and Talin. Sustained LFA-1 clustering in the IS is a consequence of the combined activities of the actin-bundling protein L-Plastin (LPL) and calmodulin (Wabnitz *et al*., 2010).

Natural Killer (NK) cells discriminate between healthy and unhealthy target cells through a balance of activating and inhibitory signals at direct immune synapses. Authors have brought together the vast literature on this subject, the number of different ways in which the cytoskeleton is important becomes evident. The dynamics of filamentous actin are critical in (i) creating the nanometer-scale organization of NK cell receptors, (ii) establishing cellular polarity, (iii) coordinating immune receptor and integrin-mediated signaling and (iv) directing secretion of lytic granules and cytokines (Lagrue *et al*., 2013).

1.3. Future Research

Here, we reviewed different aspects of actin and actin related proteins during immune cells activation. Although many progresses in this research field was achieved, it is important to highlight the need of novel studies devoted to understand the kinetics of actin and actin related proteins recruitment during the different steps comprising immune cell differentiation, migration, phagocytosis and activation. We strong believe that a more detailed comprehension of these processes may help researchers from different field to develop approaches to interfere and regulate immune response against different targets.

2. CONCLUSION

Actin is essential for the survival of most cells. The actin cytoskeleton is a dynamic structure that plays a fundamental role in diverse processes in all eukaryotic cells. Actin-dependent cellular processes are typically associated with membrane dynamics and the coordinated polymerization of actin filaments against cellular membranes provides the force for these processes. A demanding task of medicine is to understand and control the immune system. Central players in the cellular immune response are the leukocytes that leave the blood stream for host defense. In this context, the understanding the physiological role of these cells and their products along as the actin rearrangements and actin associated proteins role during immune activation are crucial to establish immune based methods to control immune response. The more complete understanding of actin and related proteins recruitment during these processes the better to obtain novel targets for establishing strategies to control immune response.

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