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Immune Pathology Associated with Altered Actin Cytoskeleton Regulation

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Abstract

The actin cytoskeleton plays a crucial role in a variety of important cellular processes required for normal immune function, including locomotion, intercellular interactions, endocytosis, cytokinesis, signal transduction and maintenance of cell morphology. Recent studies have uncovered not only many of the components and mechanisms that regulate the cortical actin cytoskeleton, but have also revealed significant immunopathologic consequences associated with genetic alteration of actin cytoskeletal regulatory genes. These advances have provided new insights into the role of cortical actin cytoskeletal regulation in a number of immune cell functions, and have identified cytoskeletal regulatory proteins critical for normal immune system activity and susceptibility to autoimmunity.

INTRODUCTION

The cytoskeleton is especially important for cells of the immune system as many of their essential actions, including migration, phagocytosis, activation, secretion, and cell-cell interaction, are dependent on cytoskeletal mobilization. Furthermore, although many of the cytoskeletal processes are common to diverse cell types, their regulators, particularly those modulating the cortical (beneath the plasma membrane) actin cytoskeleton, are often specific for the immune system or hematopoietic lineages. Recent discoveries have revealed new insights into the regulation and function of the actin cytoskeleton in cellular processes of immune cells and, importantly, alterations in several actin regulatory proteins have resulted in immune deficiency, autoimmunity, and autoinflammatory disease. This review will provide an overview of cytoskeletal processes critical for immune cell function focusing on regulators of the cortical actin cytoskeletal system that have been associated with immune pathology.

CYTOSKELETON

The cytoskeleton comprises a filamentous network that serves to maintain cell shape, and to regulate and implement important dynamic cellular functions. It provides essential scaffolding for the localization and activation/inhibition of diverse cytoplasmic signaling molecules, including protein and lipid kinases, phospholipases, and GTPases, as well as anchors for motor proteins, such as kinesin, dynein, and myosin, that are necessary for intracellular transport and cell division (1,2). Importantly, the ability of cells to sense and respond to extrinsic and intrinsic stimuli relies to a great extent on the ability of the cytoskeleton to undergo remodeling.

Three filamentous biological macromolecules, filamentous (F)-actin, microtubules, and intermediate filaments, comprise the basic structural elements of the cytoskeleton. Actin filaments, composed of two intertwined actin chain polymers, play major roles in maintaining cell morphology, assembling membrane protrusions, endocytosis, cytokinesis, and forming cell-to-cell or cell-to-matrix junctions. Microtubules are hollow cylindrical structures consisting of polymers of α and β tubulin that are organized by microtubule organizing centers (MTOCs), such as centrosomes and basal bodies. They play key roles in the intracellular transport of organelles such as mitochondria and in the formation of the mitotic spindle, and are structural components of flagella and cilia. The family of intermediate filaments includes about 70 different proteins subclassified into six types, of which vimentin is the most common. Intermediate filaments are the more stable constituents of the cytoskeleton and serve to maintain cellular shape.

ACTIN FILAMENT ASSEMBLY AND DISASSEMBLY

In vertebrates, there are three major isoforms of actin, α -, β -, and γ -actin, encoded by separate genes. Alpha actin is an essential component of the contractile mechanism in muscle cells while β and γ isoforms are ubiquitously expressed as parts of the cytoskeleton, intracellular transport system, or nuclear transcription process.

Actin filaments are formed by the unidirectional assembly of soluble monomeric G (globular)-actin subunits and consist of pointed and opposite barbed ends, corresponding to the degrading or extending parts of filaments (Figure 1). These polymers, which can either form linear bundles or a dendritic network, are initiated by 5 known types of actin nucleators (2). Linear actin filament formation is catalyzed by four types of nucleators, mainly the formin family, and in more limited cell types, Cobl, Lmod, and Spire (Figure 1, right panel). In mammals, the 15 member formin family, expressed in diverse cell types, commonly contains formin homology (FH) domains critical for actin binding and filament progression (3–8). In addition to nucleation, formins also promote elongation of actin filaments by moving forward with the growing barbed end thereby continuing to facilitate the addition of new actin monomers. Cobl is primarily expressed in the brain (9), Lmod in cardiac and skeletal muscle (10), and the two isoforms of Spire are expressed in the nervous system with Spire-2 also present in the digestive tract, liver, and testes (11). In contrast, branched-chain F-actin is nucleated solely by the 7 subunit Arp2/3 complex, which initiates the addition of actin polymers at a 70° angle to existing mother filaments (12–14) (Figure 1, left panel). Following nucleation, open barbed ends of actin filaments continue to spontaneously extend until terminated by capping proteins such as gelsolin or capping protein muscle Z-line (CAPZ). Capping activity is modulated by anti-capping proteins, such as ENA (enabled) and VASP (vasodilator-stimulated phosphoprotein), which, by protecting the growing barbed ends from abundantly present capping proteins, promote continued filament extension (2,14).

Fine regulation of actin dynamics, necessary to control a diversity of cytoskeletal functions at different cellular locations, is orchestrated by several key cellular factors of which assembly is primarily mediated by the Rho family of small GTPases that include RhoA, Cdc42, and Rac (3). RhoA directly activates formins, which in resting cells exist in an autoinhibitory form. The Arp2/3 complex is also activated primarily by small GTPases, but indirectly through nucleation promoting factor (NPF) complexes (14–19). These complexes, which require a Wiskott–Aldrich syndrome protein (WASp) family (WASp or Neural-WASp) or a Wiskott–Aldrich syndrome family verprolin-homologous protein (WAVE 1, 2, or 3) member, plus several other proteins, such as Hem1, WIP, and Abi, activate Arp2/3 following the activation of WASp members by Cdc42-GTP (16,20) or WAVE members by Rac (21,22).

For optimal reorganization of the actin cytoskeleton, efficient disassembly of older actin filaments is necessary for promoting the forward movement of the actin cytoskeleton and for regenerating soluble G-actin substrate for new F-actin production. Disassembly is mediated by actin depolymerizing factor (ADF)/cofilin family of proteins that sever older, ADP-rich filaments (new F-actin is ATP-rich). Cofilin is activated by dephosphorylation of Ser-3 by Slingshot phosphatase (23) or chronophin (24) and inhibited by phosphorylation of Ser-3 by LIM kinases (LIMK) (25–28). For branched chains, disassembly is also mediated by Coronin, which both displaces the Arp2/3 complex from older branched points thereby destabilizing the branched chain and binds to Slingshot, which promotes the dephosphorylation (activation) of cofilin at same location (13).

CELLULAR FUNCTIONS DEPENDENT ON CORTICAL ACTIN CYTOSKELETAL DYNAMICS

The actin cytoskeleton participates in many different cellular processes because it provides the basic framework for a wide array of sub-cellular structures. As many of these processes such as cytokinesis and maintenance of cell morphology are common to most if not all cell types, this section will focus on cortical actin cytoskeletal structures more specifically associated with immune cell functions.

Actin cytoskeletal structures in membrane and cell motility

During migration and tissue invasion, cells utilize various subcellular structures for forward propulsion such as microvilli, filopodia, lamellipodia and lamella, membrane ruffles and podosomes that are regulated by the actin cytoskeleton. Filopodia and microvilli are slender actin cytoskeletal projections while lamellipodia/lamella, ruffles, and podosomes are flat sheet like structures.

Both filopodia and microvilli are composed primarily of parallel actin bundles, but differ somewhat in structure as well as in function. Filopodia, located at the leading edge of migrating cells, such as macrophages, are 1–5 μM -long projections originating from lamellipodia that act as cellular sensors able to adhere to the substratum via integrins (29). Filopodia formation requires both linear and branched-chained F-actin, and several actin cytoskeletal regulators have been implicated in this process, including the small GTPases Cdc42 and RIF, NPFs WASp and WAVE, Arp2/3 complex, the formin Dia2, anti-capping proteins ENA/VASP, motor protein myosin-X, and the F-actin crosslinking protein, Fascin, that promotes bundling (reviewed in (30)).

In contrast, microvilli, are shorter ($<0.5 \mu\text{M}$) actin cytoskeletal projections composed of linear actin filaments formed by formins, and are held together as bundles by several crosslinking proteins such as Villin, Fimbrin, and Epsin (reviewed in (31,32)). A high concentration of low affinity adhesion molecules, such as L-selectin and VLA-4, at the tips of microvilli allow rolling and tethering of circulating immune cells, until chemokine activation induces collapse of the microvilli and access to high affinity adhesion molecules, such as LFA-1 (32).

Sheet-like actin cytoskeletal structures on cell surfaces, essential for cell migration and tissue invasion, include the lamellipodia and lamella, membrane ruffles, podosomes, and invadopodia, which, except for the lamella, consist predominantly of branched-chain actin filaments nucleated by the Arp2/3 complex. Lamellipodia are thin actin protrusions at the very leading edge of migrating cells while just under it sits the lamella, which consists of a less branched network of longer actin filaments formed by formins (33). In the lamellipodia, rapid actin treadmilling mediated by the Arp2/3 complex and ADF/cofilin is important for setting the direction of cell movement while forward propulsive force is generated by the

lamella (33). Membrane ruffles are sheet-like protrusions formed by a dendritic network of actin filaments unattached to a substratum that play a role in migration and receptor internalization (31). In addition to the Arp2/3 complex, formin mediated actin polymerization is also required for ruffle formation. Podosomes and invadopodia are protease-rich protrusions utilized during tissue invasion by macrophages, monocytes and/or metastatic cancer cells that facilitate degradation of the extracellular matrix allowing cells to advance.

The uropod, located at the opposite trailing end of migrating cells, is another actin-generated plasma membrane protrusion (reviewed in (34)). It is composed of parallel (to the axis of movement) actin filaments, a large diversity of cytoskeletal components, other signaling molecules, organelles, and adaptor proteins. Although the extent of its role in migration and other cellular functions is not known, the uropod can promote cell migration through RhoA-mediated activation of motor protein myosin II by facilitating detachment of the cell from the substratum and providing a force for forward movement. Furthermore, there is a higher concentration of microvilli on the surface of uropods that has been shown to enhance both sensing of the extracellular environment and intercellular interactions. The precise mechanism by which actin filaments are formed in the uropod is not yet known.

Cell-cell interactions

Regulation of the actin cytoskeleton is also critical for intercellular interactions, particularly the formation of the immune synapse (IS) and the cytotoxic T cell apparatus. For the former, the Arp2/3 complex and formin are both required for producing the dense actin network at the IS responsible for tight adhesion of T cells with antigen-presenting cells, while formins alone are needed for the reorientation of MTOC, a process necessary for generating and maintaining cell polarity (reviewed in (35)). In a similar manner, cytotoxic T cells form a fully functional F-actin-rich IS with target cells, but in addition require exocytosis of granules, a process mediated by centrosome polarization (36).

Endocytosis

Endocytic processes, including clathrin-mediated endocytosis, micropinocytosis, phagocytosis, and others, are virtually all dependent on actin cytoskeletal function (37). The various types of endocytosis are mediated by specific actin cytoskeletal regulatory proteins and actin structures. Thus, for example, phagocytosis induced by Fc γ Rs, which involves the generation of actin-propelled membrane protrusions that surround and engulf phagocytic particles, is mediated primarily by the Arp2/3 complex, while CR3-mediated phagocytosis, in which particles sink directly into the cell membrane, is mediated by both Arp2/3 and formin (38–40).

THE ROLE OF ACTIN CYTOSKELETAL REGULATORY PROTEINS IN NORMAL IMMUNITY AND AUTOIMMUNITY

Deficiencies in proteins that regulate the cortical actin cytoskeleton have increasingly been associated with immunodeficiency and/or autoimmune/autoinflammatory disease, indicating a critical role for these regulators in immune response and tolerance. In many instances, defects are primarily limited to the hematopoietic or immune systems because of restricted expression. In this section, the actin cytoskeletal regulatory proteins that play critical roles in the immune system will be reviewed, focusing on those whose primary function is regulation of the cortical cytoskeleton (Table 1). Other actin regulatory proteins, such as Zap-70, Lyn, Syk, and Abl, which affect diverse signaling and cellular processes in addition to the actin cytoskeleton, will not be discussed.

Actin nucleators

Formins—Formins are the primary nucleators of linear F-actin polymerization, facilitate elongation, and promote actinomyosin-mediated contractions during lymphocyte chemotaxis (41,42). Mice deficient in diaphanous homolog 1 (*Diap1*, *Dial*, *Drf1*), a formin member expressed primarily in lymphocytes, have no developmental defects and are fertile (43). Immunological defects in these mice were limited primarily to T cells, with reduced numbers of CD4 and CD8 T cells in peripheral lymphoid organs and an impaired contact sensitivity response to dinitrofluorobenzene. *Diap1*^{-/-} T cells exhibited reduced trafficking to secondary lymphoid organs in vivo, as well as impaired chemokine mediated F-actin formation, cell polarization and chemotaxis in vitro (43). A more recent study showed that *Diap1*^{-/-} mice have expansion of the neutrophil population detectable as early as 8 weeks and that *Diap1*-deficient neutrophils had defective actin polymerization, polarization, and chemotaxis (44). Interestingly, both *Diap1*^{+/-} and *Diap1*^{-/-} mice develop late onset (100–450 days) myeloproliferative defects characterized by splenomegaly, loss of white pulp, expansion of granulocyte, monocyte/macrophage, and erythroid precursor populations, and myelodysplastic changes in the bone marrow that suggested similarities to human myeloproliferative and myelodysplastic syndromes (45).

Arp2/3 complex—The Arp2/3 complex is the only nucleator for branched chained F-actin and therefore deficiency of any component of this complex would be expected to have a major effect on cell function. Indeed, knockdown of either Arp2 or Arp3 in Jurkat T cells impairs formation of F-actin rich lamellipodia (7) and deficiency of the *Arp3* gene is early embryonic lethal at the blastocyst stage (embryonic day 3.5, E3.5) (46).

Nucleation promoting factors

WASp family—The WASp family of proteins, WASp and N-WASp, are the main Cdc42-activated NPFs for Arp2/3. WASp is expressed in all hematopoietic lineages except for erythrocytes while N-WASp is found in most cells. WASp deficiency is the most common Mendelian inherited human disease of an actin regulatory protein and, depending on the severity of the WASp defect, manifestations can range from the full-blown Wiskott-Aldrich syndrome (WAS) to X-linked thrombocytopenia (XLT) (reviewed in (47–49)). WAS patients typically develop thrombocytopenia, a high incidence (~80%) of eczema, and immunodeficiency, the latter of which is associated with an increased risk of infection and malignancy. Despite the immunodeficiency, there is paradoxically a high incidence (40–70%) of certain autoimmune diseases, most commonly autoimmune hemolytic anemia, followed by vasculitis, renal disease, and inflammatory bowel disease as well as isolated cases of a number of others (47–51). WASp deficiency is associated with impaired development, migration, activation, and/or adhesion of platelets, lymphocytes, neutrophils, mast cells, and dendritic cells, of which platelets and T cells are the most severely affected (reviewed in (47–49)).

Was-deficient mice exhibit similar defects in hematopoietic cells as WAS patients, but clinical manifestations differ somewhat, in that they consist primarily of immunodeficiency, thrombocytopenia and a late-onset inflammatory bowel disease (52,53). In contrast to WAS patients, however, in which there is only a single reported case of SLE (54), *Was*^{-/-} mice have a high incidence of antinuclear antibodies with high levels of anti-DNA (55). Studies in *Was*^{-/-} mice suggest that autoimmunity is caused in part by a progressive decline in Treg cell numbers with age (48,55,56), impaired clearance of apoptotic cells (57), and defective Fas ligand expression (49).

WAVE family—The WASp-related WAVE proteins are the main Rac-activated NPFs for the Arp2/3 complex. Of the 3 members, WAVE 2 is expressed at high levels in

hematopoietic lineages, while WAVE1 and WAVE3 are mainly expressed in neuronal cells. Knockdown studies of WAVE2 in Jurkat T cells have documented its role in several pathways required for T cell activation including, lamellipodia and IS formation, Ca^{2+} flux, and downstream gene transcription following TCR engagement (58). WAVE2 is required for embryonic development and deficiency is associated with lethality at E10–12.5 (59,60).

WASp interacting protein (WIP)—WIP binds to the WH-1 domain of WASp and N-WASp and plays an important role in their regulation, resistance to calpain mediated degradation, and subcellular localization (61–63). In addition, WIP has WASp-independent actin cytoskeletal regulatory functions (61). WIP plays an essential role in T cell function with WIP deficiency associated with impaired actin reorganization, cell polarization, membrane protrusions, and IS formation resulting in reduced chemotaxis and TCR-induced activation, proliferation and IL2 production (61,64). In striking contrast, B cells exhibit enhanced activation and proliferation despite a similarly defective subcortical actin filament network (64). In macrophages and dendritic cells, WIP is required for optimal podosome formation (63,65,66) and, in mast cells and NK cells, for Fc ϵ R-induced degranulation and IL6 secretion (67,68).

Wipfl^{-/-} (WIP-deficient) mice are phenotypically normal at birth, but gradually develop worsening lymphopenia, granulocytosis and monocytosis, along with splenomegaly associated with increases in hematopoietic (mainly erythroid) tissue, but reductions in both B and T lymphocyte populations (64,69,70). Serum IgM and IgE levels are elevated and although humoral responses to T-independent antigens are normal, the T-dependent antibody response is virtually absent. The worsening immunological picture is associated with the development of inflammatory colitis, interstitial pneumonitis, glomerular nephropathy with predominantly IgA deposits, and inflammatory arthritis (69). Autoantibodies to nuclear and/or cytoplasmic antigens were found in all *Wipfl*^{-/-} mice tested. Overall, except for platelet defects, which are not found in *Wipfl*^{-/-} mice, the immunopathological consequences of WIP deficiency are similar to those observed in WAS patients and *Was*^{-/-} mice.

Hematopoietic protein 1 (Hem1)—Hem1 is a subunit of the WAVE complex and the essential Hem family member in hematopoietic cells. Hem proteins facilitate Rac-mediated activation and stabilization of the WAVE complex (71,72). Studies in mice with an ENU-induced loss-of-function mutation in Hem showed that Hem1 deficiency significantly impairs F-actin polymerization mediated by Rac-induced WAVE activation (72). Hem1-deficient T cells had reduced activation, proliferation, IS synapse capping, and adhesion following activation by anti-CD3, but proximal TCR-mediated signaling was intact and antigen-receptor engagement induced normal IL-2 and IFN- γ , and even increased IL-6 and IL-17 production. Phagocytosis and/or migration were defective in neutrophils and macrophages, but cytokine production was normal or increased in macrophages. Mice lacking Hem1 have defective development and expansion of $\alpha\beta$ -T cells predominantly at the DN to DP thymocyte stage, reduced numbers of mature B cells, a microcytic hypochromic hemolytic anemia similar to Rac-deficiency due to RBC fragility, neutrophilia, and increased glomerular mesangial matrix and cellularity of undetermined etiology.

Actin stabilizing protein

Hematopoietic lineage cell specific protein (HS1)—HS1 is the leukocyte-specific homologue of cortactin. It increases the half-life of branched chain actin networks by stabilizing the binding of Arp2/3 to F-actin (73,74) and provides binding sites that can localize Lck, PLC γ , and particularly Vav1, to the cortical actin cytoskeleton enhancing Arp2/3 activation (75). HS1 is required for optimal antigen-receptor-mediated activation of

B cells, and to a lesser extent T cells, and in T cells this was related to HS1 promoting sustained F-actin accumulation at the IS (75,76). In other in vitro knockdown studies of NK cells, reduced expression of HS1 was associated with defective lysis of target cells, cell adhesion, and chemotaxis (77). Despite these cellular defects, HS1-deficient mice, exhibit normal lymphoid development with normal cellularity of thymus, spleen, lymph node, and peritoneal cavity (76). In these mice, the T-independent humoral response to TNP-Ficoll (type II response) was partially impaired while the T-dependent response was unaffected. A slight effect of HS1 deficiency on platelet function was observed in one, but not another study (78,79).

De-polymerizing proteins

Coronin—Coronins, which localize to regions of actively forming cortical branched-chain actin filaments, play an important role in promoting cytoskeleton remodeling through their coordinated actions on the Arp2/3 complex and cofilin (80–82). These activities include the direct regulation of Arp2/3, displacement of Arp2/3 from branched actin filaments, and enhancement of cofilin-dependent disassembly of actin filaments. Recent studies indicated that these functions may be controlled by the predominant ATP or ADP state (indicative of the age) of the F-actin (83,84).

In mammals, there are seven Coronin members, of which the best characterized, Coronin-1A, is expressed primarily in hematopoietic cells. Coronin-1A is required for the function and survival of T cells with Coronin-1A deficiency specifically associated with defective chemotaxis, reduced antigen-receptor-mediated activation and Ca^{2+} flux, loss of mitochondrial membrane potential, and spontaneous apoptosis, primarily in the single positive thymocyte and naïve T cell subsets (85–89). Mice deficient for Coronin-1A have markedly reduced numbers of peripheral T cells, particularly the naïve CD4 T cell subset, due to defective thymic egress, impaired migration to peripheral lymphoid organs, reduced activation, and increased spontaneous apoptosis. Consequently, T-dependent humoral response and germinal center formation are impaired (86). In macrophages, Coronin-1A is also required for calcineurin activation and blocking of mycobacteria delivery to lysosomes (a process critical for intracellular survival of mycobacteria), but otherwise is not essential for other actin cytoskeletal-associated functions (90).

Coronin-1A deficiency, caused by a 2 bp deletion in exon 3 on one allele plus a complete deletion of *CORO1A* on the other, was recently identified in a patient with $\text{T-B}^+\text{NK}^+$ phenotype severe combined immunodeficiency (SCID) (89). From infancy, there was markedly reduced T cell numbers despite a normal sized thymus, recurrent severe infections, and reduced antibody responses to immunization, consistent with impaired T helper function and the findings in Coronin-1A-deficient mice.

A function-impairing mutation in *Coro1a* was also recently identified as the genetic alteration responsible for the lupus-associated *Lmb3* locus in mice (86). This *Coro1a* allele was unexpectedly derived from the nonautoimmune strain and therefore it was a lupus-suppressing variant. This finding raised the possibility that allelic variants of other actin regulatory genes that alter normal immune function could also modify susceptibility to lupus and/or other autoimmune diseases and, importantly, suggested the possibility of targeting such genes for therapy.

Actin severing proteins

Cofilin—The actin-severing activity of the ADF/cofilin family of proteins, which generates barbed ends for continuous actin polymerization and also, more importantly, depolymerizes older ADP-rich actin filaments, is essential for actin reorganization (reviewed in (91)). In

mammals, there are three members: CFL1 (n-cofilin), expressed in most cells; CFL2 (m-cofilin), expressed in muscle cells, and ADF (or destrin, Dstn), expressed primarily in epithelial and endothelial cells. In T cells, cofilin (CFL1) was shown to be required for optimal IS formation, proliferation and cytokine production (92,93). However, partly because of its ubiquitous expression, *Cfl1*^{-/-} mice are embryonic lethal at E10 because of defective neuronal development (94).

WD repeat domain 1 (Wdr1)—Wdr1 is the mammalian homologue of actin interacting protein 1 (Aip1) that promotes actin depolymerization by primarily enhancing the F-actin severing activity of cofilin (95–97). Deletion of Wdr1 is embryonic lethal at E9.5 consistent with its essential role in actin cytoskeletal function (97). Mice with an ENU-generated hypomorphic variant, called redeal (*rd*), however, were found to be healthy and fertile and survived to at least 14 months, but interestingly these mice developed a progressive autoinflammatory disease characterized by neutrophilic infiltrates in the skin and extremities, and a megakaryocytic thrombocytopenia with platelet counts of ~20% of normal levels due to defective platelet production and function. The autoinflammatory manifestations were not dependent on lymphocytes and autoantibodies were not detected. These findings indicate that Wdr1 is particularly important for neutrophil and platelet development and function.

Other actin cytoskeletal regulatory proteins

Dedicator of cytokinesis 2 (DOCK2)—DOCK2 is a hematopoietic cell-specific member of the CDM (*Caenorhabditis elegans* Ced-5, mammalian DOCK180 and *Drosophila melanogaster* myoblast city) family that functions upstream of Rac in regulating the actin cytoskeleton. It is required for chemotaxis of lymphocytes, neutrophils, and plasmacytoid DC, for IS formation and activation in T cells, and for integrin-activation of B cells (98–102). DOCK2 deficient mice, although otherwise healthy and fertile, have reduced numbers of T cells, B cells, and plasmacytoid DC (but not macrophages or myeloid DC) in peripheral lymphoid organs because of reduced homing capacity (98,99). Of clinical relevance, DOCK2 deficiency was shown to enable long-term survival of complete MHC-mismatched cardiac allografts in mice (103). This documents the potential utility of DOCK2 in transplantation and further supports the possibility of targeting actin cytoskeletal regulators to treat pathological or undesired immune responses.

Actin binding protein 1 (Abp1, SH3P7, HIP-55)—The mammalian ortholog of yeast actin-binding protein 1, Abp1, is ubiquitously expressed and appears to function primarily as an accessory protein in clathrin-mediated endocytosis through interactions with Cdc42 and dynamin (a GTPase that drives the scission of newly formed vesicles from cell membrane) (104,105). Deficiency of Abp1 slightly, but significantly, reduces TCR-induced activation, proliferation, and IL-2 production (106), as well as BCR-mediated antigen internalization and presentation (107). *Abp1*^{-/-} mice have increased fetal deaths, are small at birth, and have multiple organ defects that include significant neurological deficits, heart dilatation, and severe interstitial lung fibrosis (104,106). Their lymphoid development is normal, but primary and secondary T-dependent antibody responses are slightly reduced and with age moderate splenomegaly develops.

CONCLUDING REMARKS

Proper functioning of the immune system requires cells that are highly motile and able to migrate into tissues and blood vessels, interact with other cells, and endocytose membrane-bound and soluble material, all processes that depend on the dynamic activities of the actin cytoskeleton. Recent advances in this area, that have begun to define components and basic

mechanisms, have notably revealed critical and specific roles for cortical actin regulatory proteins in normal and pathological immune responses. From these studies several conclusions can be derived. First, deficiency of actin regulators can result in defects limited to the immune system or sometimes to a single immune cell type. Thus, although the cellular processes mediated by the actin cytoskeleton are common to many cell types, the specific actin regulatory protein in a particular cell population can have more limited distribution. Second, lack of an actin cytoskeletal regulator can result in immunodeficiency, autoimmunity, autoinflammatory disease, or a combination of these manifestations. Third, allelic variants of actin cytoskeletal regulatory genes can alter susceptibility to lupus and likely other autoimmune diseases. Fourth, studies suggest that therapeutic targeting of actin regulatory proteins might be effective in autoimmunity and transplantation rejection. Further studies in this relatively nascent area should continue to yield new and important discoveries relevant to human immunological disorders.

Abbreviations

Abi	Abl interacting adaptor protein
Abl	Abelson non receptor tyrosine kinase
ADF	actin depolymerizing factor
Arp2/3 complex	actin related protein 2/3
BCR	B cell receptor
Cobl	Cordon-Bleu
CR3	complement receptor 3
DC	dendritic cell
ENU	<i>N-ethyl-N</i> -nitrosourea
F-actin	filamentous-actin
FcγR	Fc fragment of IgG
FH	formin homology
G-actin	globular-actin
Ig	immunoglobulin
IL2	interleukin-2
Lck	leukocyte-specific protein tyrosine kinase
LFA-1	leukocyte function-associated antigen 1
LIMK	LIM kinase
Lmod	Leiomodin
MHC	Major histocompatibility complex
MTOC	microtubule organizing center
NK cells	natural killer cells
NPF	nucleation promoting factor
N-WASp	neural Wiskott-Aldrich syndrome protein
PLCγ	Phospholipase Cγ

RBC	red blood cell
RIF	Rho in filopodia
SLE	Systemic lupus erythematosus
Syk	spleen tyrosine kinase
TCR	T cell receptor
Treg	regulatory T cell
VLA-4	$\alpha 4\beta 7$ integrin very late antigen-4
WAS	Wiskott-Aldrich syndrome
WAVE	Wiskott-Aldrich syndrome family verprolin-homologous protein
WH-1	WASp homology-1
Wipf1	WAS/WASL interacting protein family, member 1; gene for WIP
Zap70	zeta chain associated protein kinase

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Figure 1. F-actin assembly and disassembly

Branched-chain F-actin dynamics: 1) Small GTPases, such as Cdc42, Rac1, and RhoA, are activated by extracellular stimuli. 2) In resting cells, WASp family of NPFs, WASp and WAVE/SCAR, remain in an inactive conformation. Binding of Cdc42 or Rac1 activates WASp or WAVE/SCAR, respectively. 3) These in turn bind actin and the Arp2/3 complex stabilizing the latter in its active conformation, which induces nucleation of new filaments at a 70° angle on existing mother filaments. 4) Barbed ends extend until they are terminated by capping proteins such as capping protein muscle Z-line (CAPZ) and gelsolin. 5) Binding of anti-capping proteins such as Ena and VASP, promotes filament extension by protecting barbed ends and by promoting the addition of actin monomer units bound to profilin. 6) HS1 (the leukocyte specific homologue of cortactin) binds to and stabilizes Arp2/3 complexes at branch points, which displaces WASp or WAVE/SCAR. 7) At the aging end of the F-actin network, Coronin and Slingshot replace the Arp2/3 complex at branched points leading to variable angles and instability of F-actin branches. 8) Cofilin and Wdr1 induce F-actin depolymerization by severing and enhancing the off-rate of actin monomers. 9) Thymosin β 4 (Thy β 4) sequesters actin monomers (G-actin) and inhibits actin polymerization. 10) Cofilin is inhibited by LIM kinase (LIMK). LIMK it self is activated by two different pathways, activation of ROCK (Rho-associated, coiled-coiled containing protein kinase) by RhoA and activation of PAK (p-21-associated kinase) by Rac1 and Cdc42. 11) Cofilin is activated by Slingshot mediated dephosphorylation.

Unbranched F-actin assembly: 12) Actin filaments are polymerized by formins such as mDia1 following activation of the specific formin by RhoA. Profilin catalyzes this process.

Table 1
Immune alterations associated with deficiency in actin cytoskeletal regulator proteins

Protein *	Gene *	Species	Actin-related function	Defects in cellular function	Major Immune cells affected	Phenotype **
Arp2/3 complex	<i>Arp3</i>	Mouse	Nucleation	migration (lamellipodia), activation (IS)	T cells	embryonic lethal
mDia1	<i>Diap1</i>	Mouse	Nucleation	activation (MTOC organization), Chemotaxis (cell polarization)	T cells, Neutrophils	splénomegaly, immunodeficiency
WASP	<i>WAS</i>	Human	NPF	migration and adhesion (lamellipodia, podosomes), activation (IS), antigen presentation	platelets, T cells, B cells, dendritic cells	immunodeficiency, macrothrombocytopenia, autoimmunity, cancer
	<i>Was</i>	Mouse		chemotaxis and adhesion (lamellipodia, podosomes), activation (IS), antigen presentation, phagocytosis	platelets, T cells, B cells, macrophages, neutrophils	immunodeficiency, thrombocytopenia
WAVE2	<i>Wasf2</i>	Mouse	NPF	Rac mediated migration, lamellipodia formation, dorsal ruffling	MEF (mouse embryonic fibroblast) ***	embryonic lethal
WIP	<i>Wipf1</i>	Mouse	Stabilizing and recruitment of WASp	chemotaxis (cell polarization, lamellipodia, podosomes), activation (IS), de-granulation, B cell hyperactivity	T cells, B cells, Dendritic cells, Macrophages, Mast cells	immunodeficiency, autoimmunity
Hem1	<i>Hem1</i>	Mouse	WAVE complex subunit	development, activation (IS), Migration (lamellipodia, podosomes), adhesion, phagocytosis	T cells, B cells, neutrophils, macrophages	immunodeficiency, neutrophilia, splénomegaly, nonautoimmune hemolytic anemia
HS1	<i>Hcls1</i>	Mouse	Stabilizing protein	Activation (IS)	T cells, B cells	normal
Coronin-1A	<i>CORO1A</i>	Human	De-polymerizing factor		T cells	immunodeficiency
	<i>Coro1a</i>	Mouse		chemotaxis (lamellipodia), activation (Ca ²⁺ flux), spontaneous apoptosis,	T cells	immunodeficiency reduced T-D Ab responses suppression of lupus
Cofilin	<i>Cfl1</i>	Mouse	Severing			embryonic lethal
Wdr1	<i>Wdr1</i>	Mouse	Cofactor for cofilin	Hypomorph-chemotaxis (cell polarization)	neutrophils platelets	KO-embryonic lethal Hypomorph-macrothrombocytopenia and autoinflammatory disease
DOCK2	<i>Dock2</i>	Mouse	Activation of Rac	Chemotaxis (lamellipodia, maintenance of cell polarity)	T cells, B cells, Neutrophils, pDCs	immunocompromised, suppression of allotransplant rejection
Abp1	<i>Dbn1</i>	Mouse	Adaptor protein	Activation, BCR internalization (endocytosis), antigen presentation	T cells, B cells	reduced survival, progressive neurological defects, splénomegaly, dilated heart, interstitial lung fibrosis

* Arp2/3 complex (actin related protein 2/3); mDia1 (mammalian diaphanous related formin -1), *Diap1* (diaphanous homolog 1); WASp (Wiskott-Aldrich syndrome protein), WAVE2 (Wiskott-Aldrich verprolin homologous protein 2), *Wasf2* (WASP family, member 2); WIP (WASP interacting protein), *Wipf1* (WASP/WASL interacting protein family, member 1); Hem1 (Hematopoietic protein 1); HS1 (Hematopoietic lineage cell specific protein), *Hcls1* (hematopoietic cell specific Lyn substrate 1); *Coro1a* (coronin, actin binding protein 1A); *Cfl1* (cofilin 1, non-muscle); *Wdr1* (WD repeat domain 1); DOCK2 (Dedicator of cytokinesis 2); Abp1 (mammalian actin binding protein 1), *Dbn1* (debrin-like)

** Gene knockout unless otherwise indicated.
 *** Cellular function was only assessed in MEFs.