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Review

The myosin family: unconventional roles of actin-dependent molecular motors in immune cells

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ABSTRACT

Myosins comprise a family of ATP-dependent motor proteins that are best known for their role in muscle contraction and their involvement in a wide range of other eukaryotic motility processes. Recent phylogenetic analysis places myosins into 35 highly diverse classes. Although these actin-based molecular motors have been characterized extensively, and much is known about their function in different cellular compartments, there is little information available about these molecules in hematopoietic cells. The available data establish that myosins expressed by immune cells are able to support general tasks, such as maintaining plasma membrane tension, moving and secreting vesicles, aiding in endo- and exocytotic processes, and promoting the adhesion and motility of cells. Additionally, however, myosins are involved in highly specialized functions, such as regulating cell activation, IS-induced signaling, and the severing of microfilaments via the control of GTPases. In this review, we summarize the current understanding of myosins in leukocytes, with emphasis on the emerging roles of these molecular motors in immune functions. *J. Leukoc. Biol.* 91: 35–46; 2012.

Introduction

Myosins constitute one of the largest and most divergent protein families in eukaryotes. These molecules are heteromeric complexes formed by one or two heavy chains and a variable number of light chains.

Heavy chains are characterized by the presence of three well-defined regions: the motor, the neck, and the tail domains [1]. The motor, or head, domain has the main function of binding to actin in an ATP-dependent manner [2, 3]. The neck domain, consisting of varying numbers of IQ motifs, allows the binding of light chains and serves as a lever arm that permits motor domain movements [2]. Finally, the tail domain represents the most variable region of myosins and has various lengths and functions, which depend on the motifs included in its sequence, such as coiled-coil dimerization regions, FERM, MyTH4, or SH3 domains for protein–protein interactions and PH domains for lipid interactions, among others [2].

Light chains are constituted by calmodulin or calmodulin-related proteins, which serve as "cervical collars" to maintain the rigidity of the heavy chain's neck. Changes in light-chain binding and resultant changes in the flexural rigidity of the neck in the heavy chain, play critical roles in regulating mechanochemical properties of the different myosins [2].

In terms of biological functions, myosins are involved in many cellular tasks, such as organelle trafficking [3], cytokinesis [4], the maintenance of cell shape [5], and muscle contraction [6].

Recent phylogenetic analysis of myosins [7–9] from different organisms grouped these molecular motors into 35 classes, which include the 40 different proteins expressed in mouse and human (**Table 1**). For nomenclature purposes, the accepted abbreviation of these proteins is Myo. In the case of the class II myosins, the abbreviation Mhc is used in this review, according to recent myosin sequence analyses reported in CyMoBase, a database for information about cytoskeletal and motor proteins (www.cymobase.org) [8].

Among the known myosins, only a subset has been predicted to be present in hematopoietic cells by transcriptome

Abbreviations: C1=zinc (Zn^{2+}) binding domain, CONACyT=Consejo Nacional de Ciencia y Tecnología, cSMAC=central supramolecular activation cluster, FERM=ezrin/radixin/moesin/band 4.1 domain, GTD=globular tail domain, IQ=isoleucine-glutamine, IS=immune synapse, KE=lysine/glutamine-rich domain, L2=loop 2 insertion, LCK=lymphocyte-specific protein tyrosine kinase, MLCK=myosin light chain kinase, MLL=mixed lineage leukemia, MTOC=microtubule organizing center, MyTH4=myosin tail homology domain 4, NHT=nonhelical tail region, NMMHC=nonmuscle myosin heavy chain, PDZ=postsynaptic density protein 95, disc large, zonula occludens-1, PGR=putative globular domain, PH=pleckstrin homology domain, PYK-2=proline-rich tyrosine kinase-2, RA=Ras association-like domain, RhoGAP=Rho GTPase-activating protein domain, ROCK=rho kinase, *sh-1=shaker-1*, SH3=src homology domain 3, siRNA=small interfering ribonucleic acid, TH=tail homology region, WASP=Wiskott-Aldrich syndrome protein

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TABLE 1. Abundance of Myosins Expressed by Immune Cell Populations

Gene	Probe	B	DC CD4	DC CD8	MF	MO	Neu	NK	T CD4	T CD8	Citations
MYO1A	10366994	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO1B	10354432	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO1C	10378697	*3*	*2*	*2*	*2*	*2*	*2*	*2*	*1*	*1*	10, 11
MYO1D	10389022	*1*	*1*	*1*	*1*	*1*	*2*	*1*	*1*	*1*	
MYO1E	10586781	*3*	*2*	*1*	*3*	*1*	*1*	*2*	*1*	*1*	12, 13
MYO1F	10443980	*1*	*3*	*2*	*3*	*4*	*5*	*3*	*1*	*2*	14
MYO1G	10384154	*2*	*2*	*2*	*2*	*3*	*2*	*2*	*2*	*2*	15, 16
MYO1H	10524515	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH1	10562856	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH2	10377055	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH3	10377018	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH4	10377117	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH6	10419900	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH7	10415140	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH7B	10477673	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH8	10377148	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH9	10430201	*4*	*3*	*3*	*2*	*3*	*5*	*4*	*4*	*4*	17–20
MYH10	10377319	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH11	10437885	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH13	10377181	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH14	10562856	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH15	10436128	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYH16						ND					
MYO3A	10469637	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO3B	10472649	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO5A	10587107	*2*	*2*	*2*	*2*	*2*	*2*	*1*	*1*	*1*	21
MYO5B	10456653	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO5C	10587150	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO6	10587446	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO7A	10565634	*1*	*1*	*1*	*2*	*1*	*1*	*1*	*1*	*1*	22
MYO7B	10457967	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO9A	10585956	*1*	*2*	*2*	*2*	*1*	*1*	*1*	*1*	*1*	
MYO9B	10572533	*2*	*3*	*2*	*2*	*2*	*2*	*2*	*2*	*2*	23
MYO10	10423293	*1*	*1*	*1*	*2*	*1*	*1*	*1*	*1*	*1*	24
MYO15	10376615	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO16	10570029	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO18A	10378914	*2*	*1*	*2*	*2*	*2*	*2*	*2*	*2*	*1*	25
MYO18B	10532563	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO19	10379840	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	*1*	
MYO35						ND					

We analyzed microarray data previously reported in the NCBI Gene expression Omnibus (GEO, www.ncbi.nlm.nih.gov/geo/) under accession number GSE15907 [26]. This information is also available from the assembled data of the ImmGen consortium (www.immgen.org). The platform employed was Affymetrix 1.0 ST MuGene GeneChip. The table shows data for only one representative probe for each gene (probe ID shown). We extracted expression levels of the different myosins present in mouse leukocytes. Within each data set, myosin genes are ordered by the level of expression. Taking the most abundant myosin gene as 100%, we calculated the percentage of expression of subsequent myosin genes. Values below 5% were considered with very low expression (denoted as *1*). Values above 5.1% were distributed among four equal populations, the first one representing the low-expressing myosins (denoted as *2*), the next including the medium-expressing myosins (denoted as *3*), the next including high-expressing myosins (denoted as *4*) and the last for the very high-expressing myosins (denoted as *5*). The populations shown are as follows: B, B lymphocytes; DC CD4, $CD4^+$ dendritic cells; DC CD8, $CD8^+$ dendritic cells; MF, peritoneal macrophages; MO, monocytes; Neu, neutrophils; NK, NK cells; T CD4, $CD4^+$ T lymphocytes; T CD8, $CD8^+$ T lymphocytes; ND, not determined. When available, citations column includes representative references where corresponding myosin proteins have been confirmed to be expressed by leukocytes.

analyses (highlighted in Table 1) [26]. Interestingly, these molecular motors present differential expression patterns across typical immune cell populations; this may be related to specific cell functions. Here, we enumerate the properties and known roles of myosins in the different cells of the immune system, basing our review on recent studies, which have begun to reveal more specialized functions of the myosin family.

MYOSIN I: CONTROLLING CELL MEMBRANE TENSIONS AND DYNAMICS

Myosin I refers to a class of single-headed molecular motors, which regulates a number of cellular functions, including intracellular transport, the formation of cell-surface projections, the regulation of the cytoskeleton, and the regulation of mem-

brane-related events, which includes exocytosis, endocytosis, and phagocytosis [27].

Myosins of the myosin I class contain a single heavy chain of 110–140 kDa [28, 29], are monomeric, and unlike conventional myosin II, do not form filaments. The single heavy chain is divided into the head (or motor), neck, and tail domains. The motor domain contains the ATP binding site and the actin binding site. This domain is followed by the neck domain, which contains one or more regions of 29 aa with the consensus sequence IQXXXRGXXXR, referred to as IQ motifs [30]. The sequence and number of IQ motifs change among myosin I isoforms. The neck region is implicated in the binding of myosin light chains, which in vertebrate and yeast class I myosins, correspond to calmodulin molecules [2]. Following the neck is the C-terminal tail region. The tail domain is divided into three regions referred to as TH regions 1, 2, and 3 (TH1, TH2, and TH3), respectively [31]. TH1 is enriched in basic residues and is involved in the binding of membrane phosphoinositides [32, 33]. The TH2 domain, which is rich in glycine, proline, and alanine/glutamine, contains an ATP-insensitive actin binding site [34]. The TH3 domain is a SH3 domain [35], which is a protein–protein interaction region (**Fig. 1**).

Class I myosins containing all three TH domains have been called "long-tailed", whereas those containing only the TH1 region are known as "short-tailed". In the human and the mouse, eight different genes encode the eight heavy chains of class I myosins. Short-tailed myosins include Myo1a, Myo1b, Myo1c, Myo1d, Myo1g, and Myo1h, whereas Myo1e and Myo1f are long-tailed [36]. These proteins have been implicated in nuclear transcription, lamellipodia generation in motile cells, brush-border dynamics of proximal-tubule cells of the kidney, and the adaptation of mechanoelectrical transduction in hair cells, as well as in exocytosis, adhesion, and vesicular trafficking in hematopoietic cells [27, 36, 37].

Short-tailed Myo1c and Myo1g

Myo1c has three IQ sites that bind to two to three calmodulins in the absence of Ca^{2+} [38]. Myo1c expression varies widely in tissues, with enrichment in actin-rich structures near the plasma membrane [39–41] and in phagocytic cups [42] in some cells. It is a highly versatile motor that regulates numerous functions, including mechanotransduction at sensory hair cells of the inner ear [43], insulin-induced movement of GLUT4-containing vesicles [44], and in its nuclear isoform, interaction with RNA polymerase I and RNA polymerase II, with putative roles in transcription and directed, long-range chromosome movement [45–48].

Myo1c has also been identified in association with the plasma membrane of B lymphocytes, with enrichment of Myo1c at actin-based structures, such as the dendrite-like extensions of these cells [10]. Recently, Myo1c was detected in

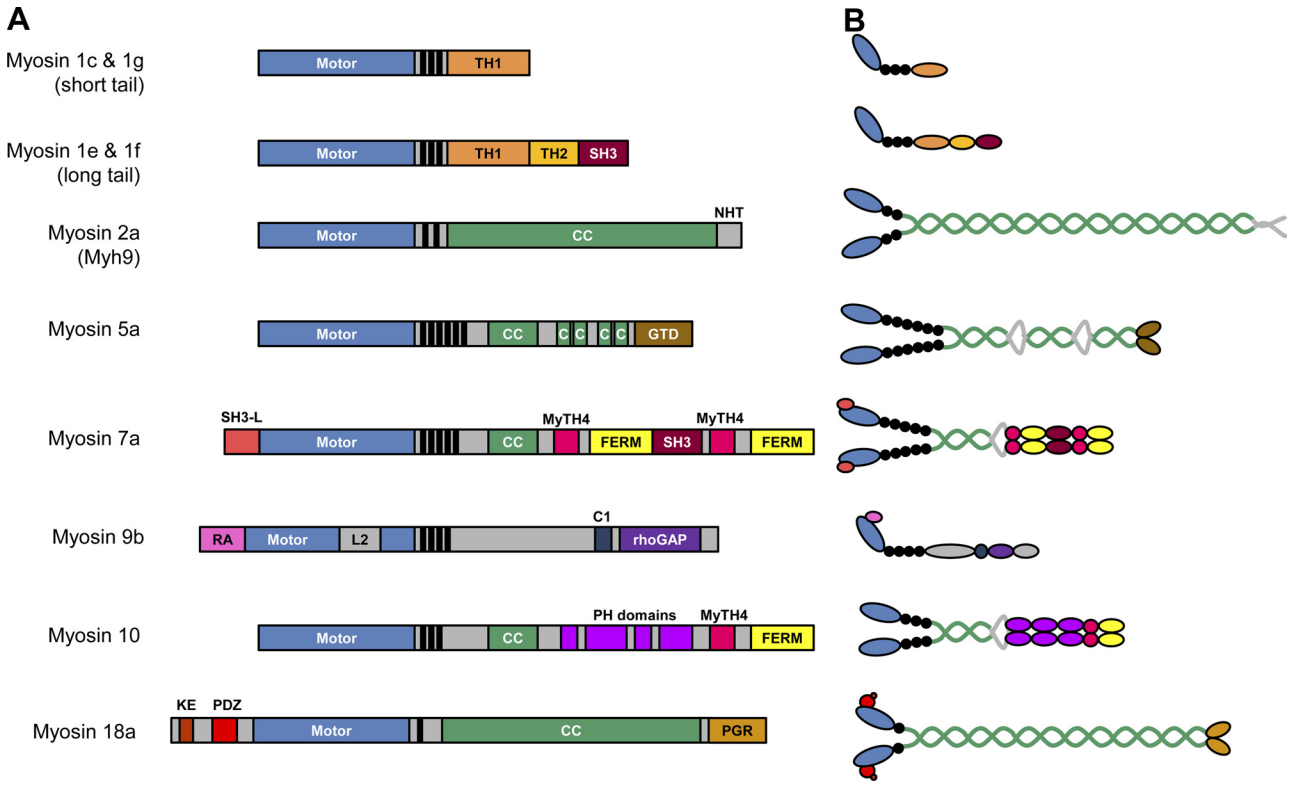

Figure 1. Structures of myosins expressed by leukocytes. (A) Structure of the heavy chains of myosin classes discussed in this review. (B) Schematic representations of myosin structures. Figure is based on figures of Mermall et al. [25]. Colored boxes represent different regions predicted by sequence homology: CC, Coiled-coil domains; SH3-L, SH3-like domain.

lipid rafts of B cells and showed strong colocalization with MHC-II. B lymphocyte, transfected with a dominant-negative form of Myo1c or specific siRNA for knock-down purposes, produced alterations in their spreading and their antigen-presenting capabilities, as denoted by the reduced numbers of activated T cells obtained after cocultures [11].

Myo1g is another short-tailed class I myosin, highly represented in leukocytes, according to microarray databases [26, 49]. By immunoblot analyses of different tissues and cell lines, this protein was identified recently as being an exclusively hematopoietic motor protein [15, 16].

Mass spectrometric proteomic profiling of lymphocyte proteins indicates that Myo1g is actually the most-abundant class I myosin expressed by T lymphocytes [16], where as defined by immunofluorescence assays, it localizes to the plasma membrane, particularly enriched at cell-surface microvilli, and associates in an ATP-releasable manner to the actin cytoskeleton (see Fig. 2) [15, 16]. Besides its localization, it has been proposed that this molecular motor may have a role in regulating cell membrane deformation, according to experiments that demonstrate a decreased elasticity of siRNA Myo1c-depleted T cells [15].

Long-tailed Myo1e and Myo1f

Myo1e and Myo1f have differential expression in mouse and human tissues [50]. Both myosins are highly expressed in immune cells, but expression can be biased toward particular cell populations such as Myo1e in B cells and Myo1f in neutrophils (Table 1).

Some evidence of long-tailed, class I myosin function in immune cells has been described, particularly for Myo1f. *MYO1F−/−* mice showed deficient neutrophil mobilization and were susceptible to infection by *Listeria monocytogenes* [14]. Additionally, migration of Myo1f-deficient neutrophils was impaired on integrin ligand-coated surfaces [14]. β2-Integrins are important for adhesion and motility of granulocytes in ECMs. These adhesion molecules are stored in neutrophil granules, which allow for a rapid increase in the surface expression of β2-integrins when required [51]. When Myo1f is absent, neutrophils show excessive adherence to integrin ligands and increased exocytosis of β2-integrin-containing granules [14]. It has been proposed that the cortical actin web of the cell acts as a barrier that prevents exocytosis of these granules [52]. Thus, it is possible that Myo1f regulates the integrity of the cortical actin web (see Fig. 2) and that the network becomes loose when these motors are absent, allowing more and larger granules to be secreted [53].

Interestingly, the Myo1f gene is fused to the MLL gene in acute monocytic leukemia [54]. It is not known how MLL-Myo1f fusion proteins or misregulation of Myo1f plays a role in the generation of leukemia. Further analysis of Myo1f-deficient mice will be helpful to solve this question.

Myo1e was initially found associated with the F-actin-rich region at cell–cell contact areas, suggesting a relationship with the formation of cortical actin structures [55, 56]. The presence of Myo1e around the phagosomes in macrophages implied a role for this motor in the closure of phagocytic cups (see Fig. 2) [12, 13]. Additionally, it has been demonstrated that human Myo1E interacts with dynamin and synaptojanin-1 in epithelial cells and that the expression of a dominant-negative form of the protein inhibited transferrin endocytosis. This suggests a role for Myo1e in clathrin-mediated endocytosis [57].

NONMUSCLE MYOSIN II: BEYOND CELL BODY CONTRACTION

Nonmuscle myosin II is a class II myosin, which is ubiquitous in many organisms and cell types. It plays a role in several processes, including migration [58], adhesion [58], cytokinesis [59], and the maintenance of cell-shape integrity during development and in adult organisms [60, 61]. In mammals, three different NMMHCs exist: II-A (Mhc9), II-B (Mhc10), and II-C (Mhc14). Each is encoded by the following genes, respectively: *MYH9*, *MYH10*, and *MYH14* [36, 62, 63].

Each NMMHC presents a head domain at the amino-terminal region and a coiled-coil domain terminating with a NHT section at the carboxyl-terminus. Two NMMHCs (230 kDa) form a homodimer and bind two pairs of light chains, one commonly referred to as regulatory light chain and the other, as essential light chain, both regulating motor function [58]. The myosin molecule is bifunctional, in that it contains two amino-terminal motor domains, which can bind to actin and hydrolyze ATP, and a tail domain, which is responsible for filament formation (Fig. 1). The two heads are separated from the coiled-coil α-helical region by a neck domain, which binds light chains. The bipolar filaments that these proteins form are considerably smaller than those formed by skeletal and cardiac myosin [64, 65].

As shown in Table 1, the dominant NMMHC in hematopoietic cells is Mhc9 (NMMHC-IIA), which has been studied extensively in different immune responses involving lymphocytes.

In T cells, Mhc9 has been associated with a variety of functions, and it has been found to participate in processes, such as cell migration, receptor segregation, or even the establishment of the IS with APCs [17, 66]. In relation to cell motility, the structure of Mhc9 could suggest its function: the coiled-coil nature of the tail allows homodimers of myosin II family members to form large oligomers, which through combined action, are able to bind adjacent actin filaments and contract one toward the other. This enhances tension in certain parts of the cell, aiding in control of, for example, movement or cytokinesis [67, 68]. In other motile cells, such as *Dictyostelium*, retrograde flow during crawling is partially controlled by myosin II, and its participation is also necessary for efficient migration. In this way, accumulation of Con A-labeled receptors into the rear of migrating *Dictyostelium* requires myosin II [69, 70]. Similarly, motile T cells present myosin II clusters that originate from membrane protrusions at one pole of the cell; as migration progresses, these clusters move backward toward the opposite pole of the lymphocyte [71]. This movement has been related with retrograde flow of TCR toward the rear edge of moving T cells [72], suggesting a role of Mhc9 in surface molecules positioning during leukocyte migration.

To generate a functional adaptive immune response, leukocytes, especially T cells, must be able to migrate between blood

and secondary lymphoid organs and even sites of injury or infection elsewhere in the body [73, 74]. The motility of T lymphocytes is in part regulated by the differential localization of certain proteins, such as integrins, at the front and rear edges of the cell [75]; interestingly, Mhc9 has been found to interact with such molecules in crawling T cells.

Integrins are a large family of proteins, which act as adhesion molecules and as receptors that are able to transmit information about the chemical and mechanical properties of the external environment into the cell (a process termed outside-in signaling), facilitating leukocyte trafficking as one of their main functions [76].

All integrins are transmembrane heterodimers, composed by α- and β-subunits. Integrins exist on the cell surface mainly in an inactive form, until they receive stimulating signals from other receptors (via inside-out signaling), which cause a shift in their conformation from a bent, compact shape to an extended, open one [76]. The binding of cytoplasmic proteins to carboxy-terminal ends of both subunits is an essential part of the integrin activation process, as these interactions provide, at the same time, stabilization of the extended integrin conformation and connections to the cytoskeleton [76, 77].

The four leukocyte-specific β2-integrins (αLβ2, αMβ2, αXβ2, αDβ2) are found on T cells, with αLβ2 (LFA-1) being is the most abundant [78] and allowing cell arrest and migration on surfaces expressing the LFA-1 ligand, ICAM-1 [79].

Signaling events that follow direct LFA-1 engagement include tyrosine phosphorylation of PLCγ1 [80] and activation of tyrosine kinases, such ZAP-70 [81], PYK-2, FAK [82], and a high rate of actin polymerization [83].

In vitro experiments have shown that binding of primary human T cells to ICAM-1-coated surfaces (via their membrane LFA-1) causes, within 1–2 min, their polarization, leading to random cell motility 2–3 min later [84].

As well as migrating randomly, T cells also migrate under the direction of chemotactic stimulants such as chemokines [77, 85].

Chemokines are small, soluble proteins of ~70 aa residues with a MW of 8–10 kDa [86]. They act as potent chemoattractants of a large variety of mononuclear cell types to sites of inflammation or secondary lymphoid organs. Based on the positions of two conserved cysteine residues in their amino-terminal end, chemokines can be divided into four subfamilies: CC, CXC, CX3C, and C [87, 88]. CXC chemokines are primarily involved in the activation of neutrophils, whereas CC chemokines stimulate other leukocytes such as monocytes, lymphocytes, and basophils [89].

Chemokine receptors are a group of transmembrane proteins that belong to the superfamily of GPCRs [90, 91]. They possess seven-transmembrane helices and transmit signals from extracellular ligands to intracellular biological pathways via heterotrimeric G-proteins [89].

T cells responding to chemokines rapidly polarize, forming a leading lamellipodium and filopodia-containing structure protruding toward the source of stimulus (leading edge) and a trailing uropod projection, along with a central region, which serves as subcellular compartments for the polar distribution of various organelles, lipid raft components, receptors, and signaling molecules during migration [75].

Chemokine receptors and integrins, which localize to the leading edge of migrating T cells, sense chemoattractant gradients and allow adherence to the substratum, respectively [75]. Together, these receptors stimulate the activity of various kinases (including PYK-2 and FAK) [75, 92], adaptor proteins (such as vinculin and talin) [93, 94], and guanine nucleotide exchange factors (such as proto-oncogene vav 1) [95], which ultimately regulate the activation of Rho GTPases and their F-actin regulating effectors, including WASP, WASP family verprolin-homologous protein 2, and diaphanous-related formin 1 [96–98]. This results in F-actin polymerization at the leading edge to drive forward movement and adhesion and causes the uropod to undergo cycles of substrate attachment, release, and retraction. Thus, this coordinated adhesion and detachment, along with actin-mediated propulsion, allow T cells to "crawl" or "chemotax" along blood vessel walls and to transmigrate through vascular endothelium into and through surrounding tissues [75].

Interestingly, the activity of Mhc9 in T lymphocyte migration is also controlled by integrin/chemokine receptor-induced signals. Active forms of MLCK and ROCK are also detected during lymphocyte migration [58, 99]. Both molecules are capable of phosphorylating regulatory light chains, thereby activating Mhc9. These kinases are segregated spatially in the T cell: MLCK operates at the leading edge to control T cell adhesion and lamellipodial extension, whereas ROCK is involved in the retraction of the trailing edge [84].

Mhc9 has also been shown to interact with LFA-1 at the rear of migrating T lymphocytes, and the inhibition of the association between the molecules resulted in excessive uropod elongation, defective tail detachment, and decreased lymphocyte migration over ICAM-1-coated surfaces [66]. Interestingly, inactive LFA-1 is selectively localized to the posterior of polarized T lymphocytes, whereas active LFA-1 is localized to their anterior [66]. Thus, during T lymphocyte migration, uropodal adhesion depends on the LFA-1 activation state, and Mhc9 serves as one of the mechanical links between LFA-1 and the cytoskeleton, critical for LFA-1 detachment.

On the rear side of the cell, an association between Mhc9 and the C-terminal end of chemokine receptors CXCR4 and CCR5 was detected (see Fig. 2). Analysis by confocal microscopy showed that CXCR4 and this motor protein colocalize at the leading edge of migrating T lymphocytes [100]. Consequently, Mhc9 could not only have a role at the rear side of the cell but also may be participating in the regulation of directed migration on a chemoattractant gradient.

Although it may not be surprising that Mhc9 functions as a regulator of T cell motility, this highly versatile molecule has also been implicated in other relevant immune processes of these cells. One such process is the establishment of the site of interaction between a T cell and an APC: the IS. This is a crucial event for the adaptive immune response.

The initial step in IS formation is interaction between the TCR and the specific MHC-peptide on the APC. This engagement leads to actin-dependent microcluster formation and to the recruitment of signaling proteins to form a "microsignalo-

some" [101, 102]. The TCR microsignalosome recruits tyrosine-phosphorylated forms of LCK, ZAP70, and linker of activated T cells and excludes certain molecules such as inhibitory phosphatase CD45 [101–105]. Through adhesion exerted by integrins, the contact area then expands in a phenomena usually referred to as spreading, during which, TCR microclusters continue forming at the outer edge of the interface [104, 105]. These microclusters move to the center of the IS, where they coalesce into larger aggregates to form the nonmobile cSMAC [104]. As there is low tyrosine phosphorylation in the cSMAC, it has been suggested that the cSMAC is the site of inactivation of old clusters by internalization, whereas new microclusters continue forming at the periphery [102, 104, 106]. The formation and movement of new TCR microcluster-based signalosomes toward the cSMAC sustain signaling [104].

Although there is a great deal of controversy related to cSMAC formation, the force necessary for protein rearrangement in the IS has recently been partially attributed to microfilament/myosin-driven contraction. When blebbistatin, an inhibitor of Mhc9 function, is used to treat T cells, TCR microclusters at IS do not experience the centripetal movement described previously, and conjugates of inhibitor-treated T cells and APCs are less frequent and show diminished stability when compared with conjugates of untreated T cells and APCs [17]. In addition, blebbistatin treatment reduces the levels of phosphorylated ZAP-70 and phosphorylated LCK at the T cell side of the synapse and inhibits the maintenance of calcium fluxes that are induced in the lymphocytes immediately after IS establishment [17]. These data lead to the hypothesis that Mhc9 has a direct impact on T cell signaling, which increases the activation of these cells after interaction with APCs (see Fig. 2).

It is important to note that APCs also have an important role in IS formation and that B lymphocytes can act as efficient APCs. Antigen capture and presentation on MHC-II molecules by B lymphocytes are mediated by their surface BCR; the transport of vesicles containing BCR-antigen complexes and compartments containing MHC-II must fuse, allowing for efficient processing of the antigen for presentation [107]. It was observed that Mhc9 is activated upon BCR engagement and that it associates with MHC-II-invariant chain complexes [108]. Mhc9 inhibition or depletion affects the convergence and concentration of MHC-II and BCR-antigen complexes into antigen-processing compartments. In addition, specific T cell activation, mediated by cognate peptide-presenting B cells, is impaired in cells lacking this myosin activity [108]. Consequently, Mhc9 has been proposed as a key motor protein in BCR-driven antigen processing and presentation.

NK cells are lymphocytes of the innate immune system, which defend against cancerous and virally infected cells. NK cells are activated by cross-linking of their germline-encoded receptors. Upon the recognition of target cells via these receptors, NK cells promote lysis of the target cells by directed secretion of lytic granules. These granules are a form of secretory lysosomes and contain molecules such as perforin and granzymes [18].

Mhc9 function has been related to NK cell cytotoxicity [109, 110]. In primary human NK cells and human NK cell lines treated with blebbistatin or siRNA against Mhc9, cytotoxicity was decreased significantly. These NK cells were capable of conjugating with target cells and polymerizing actin at the IS, and they were capable of polarizing activation receptors, the MTOC, and lytic granules to the IS; however, they were unable to degranulate [109].

Some groups found Mhc9 directly attached to the surface of lytic granules by ultrastructural and biochemical studies. As Mhc9-coated lytic granules are able to bind F-actin, there is likely a role for this myosin in directing granule transport (see Fig. 2). New techniques also support that idea, such as data obtained by total internal reflection microscopy, which suggest that myosin II motor function physically guides the motion of granules into the actin network at the IS [110].

One of the most significant examples of Mhc9 function is in regulating genesis and contractile activity of platelets. In vitro studies on cultured megakaryocytes showed that myosin IIA inhibits proplatelet formation. The loss of myosin IIA function as a result of *MHC9* mutations promotes proplatelet formation and may trigger premature platelet release, resulting in rare disorders that are characterized by abnormal giant platelets, low circulating platelet numbers, and bleeding tendency with variable severity, known as macrothrombocytopenia [19]. Giant platelets only residually express mutant dysfunctional Mhc9, which cannot participate in the reorganization of cytoskeletal contractile structures [111].

Through its interaction with actin, Mhc9 participates in the agonist-induced cytoskeletal reorganization of platelets [112]. Previous studies have provided in vitro and in vivo evidence implicating Mhc9 in platelet deformation, granule motility, platelet spreading on ECM, as well as thrombus growth and stabilization [20, 113–115].

Although *MHC9*−/− mice showed embryonic lethality [116], conditional knockout mice present a marked thrombocytopenia, combined with a strong increase in bleeding time and the absence of clot retraction [20]. Interestingly, some human genetic disorders are generated by defects in Mhc9, including four autosomal dominant disorders: May-Hegglin, Fechtner, Sebastian, and Epstein syndromes. The common feature of all four diseases is macrothrombocytopenia. Some patients show later onset of deafness, cataracts, and glomerulonephritis [117].

MYOSIN V: A VERSATILE MOTOR THAT LINKS ACTIN AND THE MICROTUBULE CYTOSKELETON

Myosin V class members are dimeric proteins. Each myosin V heavy chain has a molecular weight of ~210 KDa [118, 119]. After the motor domain, which is not significantly different from other myosins, is the neck domain, with IQ motifs capable of binding six light chains. The light chains are always calmodulin or calmodulin family members. Mouse myosin 5a contains only calmodulin light chains [120]. After the neck domain is the tail region, which consists of long stretches of a predicted coiled-coil-forming sequence, interrupted by small regions of low-probability coiled-coil formation. Myosin 5a dimerizes near this stalk region to form a two-headed mole-

cule [118, 119]. At the end of each heavy chain is a GTDs, which has been implicated in cargo transport (Fig. 1). Interestingly, the activity of mammalian myosin 5a is regulated by molecular folding, in which the GTD fold back and interact with the motor domains to form a compact, triangular-shaped molecule [121, 122].

Myosin V class members are recognized as cargo-carrying, processive motors. It has been established that this motor moves processively along actin tracks via a "hand-over-hand" lever-arm mechanism that gives 36-nm steps [123].

The function of this protein has been well studied in different cell types and involves the movement of many types of cargo, including melanosomes [124], secretory vesicles [125], ER [126], and even certain mRNAs [127]. Interestingly, this protein has interactions, not only with microfilaments but also with numerous cytoskeleton molecules, such as microtubules, kinesin motors, intermediate filaments, and organelle-docking proteins, i.e., the melanophilin/Rab 27a complex in melanosomes [128].

In terms of the function of myosin 5a in hematopoietic cells, analysis of macrophages and DCs derived from the *dilute* mouse, a mouse with functionally null *MYO5A* mutations, revealed that microtubule-dependent, intracellular movement of phagosomes is significantly faster in these mutant mice than in control cells [129], and it is possible to observe an accumulation of these vesicles at the perinuclear region of cells. This same increase in microtubule-dependent organelle movement has also been observed in *dilute* melanocytes [21] and neurons [130]. This finding has given rise to a model in which organelles contain microtubule motors and Myo5a, thus allowing continued, actin-based movement in regions of the cell devoid of microtubules (see Fig. 2). Additionally, in regions where organelles are moving along microtubules, interactions of Myo5a with microfilaments in the cytoplasm could allow retardation of organelle movement [21, 130].

MYOSIN VII: REGULATION OF VESICULAR TRAFFIC

Vertebrates synthesize two class VII myosins: Myo7a and Myo7b, from two different genes [131, 132]. Both class VII myosins have similar structures. Their motor domains share high homology with other myosins, and their neck regions are composed of five IQ motifs that mediate the association with calmodulin light chains. Their long-tail region has different domains, including a predicted coiled-coil domain (which is only present in Myo7a), followed by two repeats, each containing a MyTH4 and a FERM, separated by a SH3 [7, 9, 133]. Additionally, an N-terminal SH3-like subdomain is present in both proteins adjacent to the motor domain. These domains are shown in Fig. 1 [133].

Some observations have led to the hypothesis that myosin 7a may influence the tension at subcellular emplacements and move cargo along microfilaments [134–136]. Defects in Myo7a cause phenotypic anomalies in *Drosophila*, zebrafish, and mice. In humans, loss-of-function mutations in the *MYO7A* gene cause Usher syndrome type I, a dual-sensory defect that combines sensorineural deafness and retinitis pigmentosa, leading to blindness [137].

Class VII myosins have also been implicated in endocytosis. Myosin 7a is highly expressed in cochlear hair cells [138], and mice with mutations in *MYO7A* (*sh-1*) are deaf as a result of defects in their neurosensory epithelium [139]. Uptake of cytotoxic aminoglycoside antibiotics by hair cells is blocked in certain *sh-1* mutant alleles [140]. Phagocytosis of photoreceptor outer-segment disks by the retinal pigmented epithelium is also impaired in *sh-1* mice [141]. Inhibition of the removal of these ingested phagosomes led to a delay in phagosomal/lysosomal fusion, indicating a possible role for this motor in vesicle trafficking [141].

According to data mentioned previously [140, 141], it is possible that Myo7a could also have a role in vesicular trafficking in hematopoietic cells. In uptake assays with fluorescently tagged *Escherichia coli*, *sh-1*-treated DCs exhibited deficiencies in phagocytosis [142]. It has been proposed that the loss of Myo7a could have adverse effects on the coupling of the cortical actin array to the plasma membrane, which is underlying the incipient phagocytic cup (see Fig. 2).

MYOSIN IX: SIGNALING REGULATORS OF RHO GTPASES

Vertebrates have two class IX myosin genes: *MYO9A* and *MYO9B*. Myo9b is the only characterized motor of this group, and although it is a single-headed protein, it has processivity and high velocity, taking multiple steps along microfilaments before dissociating [22].

The motor domain of Myo9b differs from the head domains of other myosins. The Myo9b head domain has two insertions: the first is a RA (see Fig. 1), which is unable to bind Ras but may mediate association with other proteins [23, 143]. The other insertion is the L2 (see Fig. 1), which consists of basic amino acids and has no sequence similarity to other known domains [144]. The neck domain mediates the binding of four light chains of calmodulin or calmodulin-related proteins [145]. The tail region has two different domains: the C1, which contains a motif that is able to bind two zinc ions, and a RhoGAP domain, which interacts with the small GTPase Rho (Fig. 1). This domain accelerates the GTP-hydrolysis of Rho, switching it from its active GTP-bound state to its inactive GDP-bound state. It therefore confers to Myo9b the ability to act as a negative regulator of Rho signaling (see Fig. 2) [146–148].

According to studies in *MYO9B−/−* mice, Myo9b is a key signaling molecule in the control of membrane protrusions of hematopoietic cells. *MYO9B−/−* macrophages have a "contracted" morphology, characterized by a paucity of lamellipodia, actin cytoskeletal polarization, and their low velocity in a chemoattractant gradient. Rho signaling is overactive in these cells as a result of the lack of RhoGAP activity of this protein, so inhibitors of Rho were able to restore the phenotype [22].

In vivo assays have also shown that chemoattractant-induced recruitment of monocytes to the peritoneum is impaired in *MYO9B−/−* mice. This implies a direct role for this motor in cell motility and directed migration [22].

MYOSIN X: FORMATION OF ACTIN-DEPENDENT MEMBRANE STRUCTURES

Myosin X has unique structural tail domains that confer class-specific functions. Human myosin 10 is a 2058-aa protein with head and tail domains separated by a region that is predicted to form a coiled-coil domain. This suggests that myosin 10 heavy chains exist as dimers [149]. The most unusual feature of these motors is the presence of three PH domains (Fig. 1), which are probably in direct association with the membrane [150]. The C-terminal end contains a MyTH4 and a FERM domain, which allow this myosin to bind to and potentially transport multiple proteins [36]. It has been shown that Myo10 binds actin, microtubules, vasodilator-stimulated phosphoprotein, products of PI3K, and β-integrins [151–156]. Myosin 10 is enriched in actin-rich protrusions, such as the edges of lamellipodia, membrane ruffles, and the tips of filopodia [149]. It was reported that myosin 10 accumulates at the tips of filopodia, in agreement with the recent demonstration that this protein is a motor, which moves in a typical manner toward the barbed ends of actin filaments [157]. These facts indicate that this motor could be important in processes that induce the protrusion of the plasma membrane (**Fig. 2**).

An example of Myo10 function in hematopoietic cells is given by osteoclasts derived from macrophages: on glass, osteoclasts generate podosomes, which are foot-like processes containing a core of F-actin. To facilitate bone resorption, osteoclasts generate an actin-rich sealing zone composed of densely packed, podosome-like units [158]. Microscopy studies showed that Myo10 was associated with the outer edges of immature podosomes and sealing zones, suggesting a possible role in the generation and/or positioning of these structures. This is supported by the fact that siRNA-mediated suppression of Myo10 led to a decreased cell/sealing-zone perimeter, along with decreased motility and resorptive capacity; in contrast, when osteoclasts overexpress Myo10, there is an increase in podosome-like formation and resorptive capacities [158].

The presence of MyTH4 domains in actin-based motor proteins, which have been related directly with microtubule binding [153], is particularly interesting, as it suggests that these myosins act as direct linkers between the microtubule and microfilament cytoskeletal networks without a requirement for

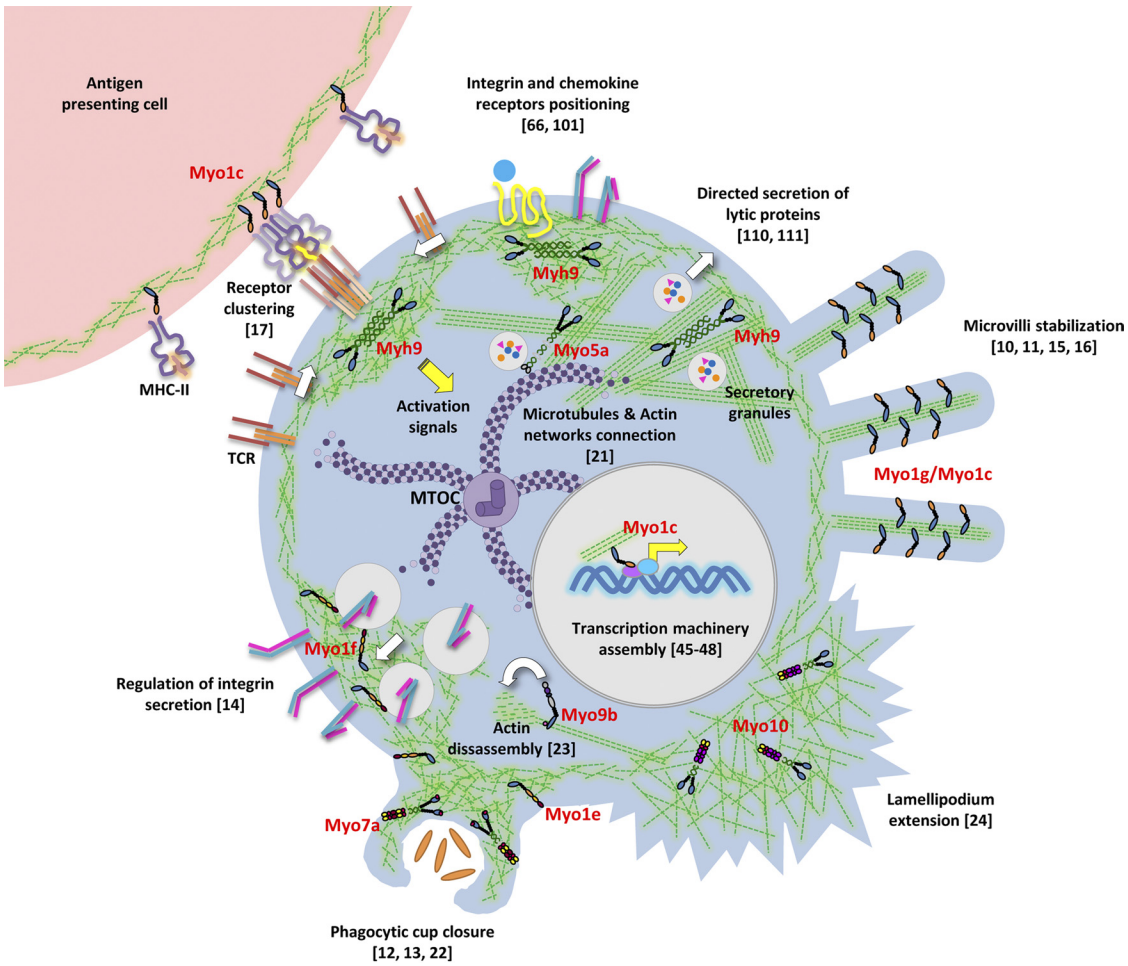

Figure 2 . Myosin family functions in leukocytes. Several roles of myosins in immune cells are depicted in this imaginary leukocyte, which shares the properties and structures of the different hematopoietic cell populations presented in Table 1.

other protein intermediates. Evidence of this hypothesis is given by the observation that Myo10 siRNA or dominant-negative transfected cells could not properly position podosomes following cold-induced microtubule disruption [158], as it has been demonstrated that microtubule integrity is required for podosome patterning in osteoclasts [24].

Although transcriptomic analysis [26] found *MYO10* expression in macrophages (as shown in Table 1), further investigation is needed to clarify whether membrane protrusions of highly motile cells, such as leukocytes, can contain and depend on the functions of Myo10 for their regulation.

MYOSIN XVIII: UNCONVENTIONAL TAIL DOMAINS

Among the less-studied myosins are the proteins grouped in the XVIII family. There are two subclasses: a and b [159]. Myosin 18a was first isolated from bone marrow stromal cells of mice and was designated as MysPDZ [160]. Myosin 18a has also been found in humans and *Drosophila* [160, 161]. It has two splice variants, designated as α and β, in humans and mice [162].

Like other myosins, Myo18a-α has a motor domain with an ATP binding motif in its N terminus and only one IQ motif in its neck region. In its C-terminal end, Myo18a-α has a coiled-coil domain, followed by a PGR (see Fig. 1) [160], which is expected to mediate the formation of a two-headed molecule (Fig. 1). Myo18a-α has three distinct features that distinguish it from other classes of myosins. First, the motor domain of Myo18a-α lacks any canonical actin binding sequence, although this protein, at least partially, colocalizes with microfilaments [161]. At its N terminus, Myo18a-α has a KE, which is conserved among some nuclear proteins and centromere/microtubule binding proteins and is thought to participate in nuclear targeting and microtubule binding. Finally, downstream from the KE-rich domain, this myosin has a PDZ domain, which is known to be involved in protein–protein interactions [163]. In mice, this myosin is expressed abundantly in stromal cells of the bone marrow and thus, likely plays a role in hematopoietic cell development [160].

Myosin 18a-β is expressed in several hematopoietic cell lines; myosin 18a-α is expressed in only a subset of these cell lines, but it is widely distributed in the epithelial cell lines, such as stromal cells. Myosin 18a-β is expressed exclusively in macrophages before terminal differentiation, but after terminal differentiation, the expression of Myo18a-α is highly induced [163]. Recently, an isoform of Myo18, p110 myosin, was identified, which may come about through post-translational processing of Myo18a-α and/or Myo18a-β via the phosphorylation of tyrosine residue(s) after the induction of macrophage differentiation by CSF-1 treatment [163]. This potentially implies that the regulation of expression of the three isoforms of Myo18a is important for macrophage differentiation.

CONCLUDING REMARKS

The conventional view that myosin polymerizes into filaments in muscle cells and has a role in muscle contraction has been expanded greatly by the study of nonmuscle myosins in nonmuscle cells. Myosins perform many different cellular functions, including organelle trafficking, cytokinesis, maintenance of cell shape, and cell motility. Many functions have been described in sessile and motile cells. Myosins aid in defining microdomains and in limiting the migration of molecules through associations with integrins, cell–cell adhesion molecules, and growth factor receptors. Myosins may also have a role in signaling functions. The use of microarrays has revealed the expression of different myosins with remarkable specificity, elucidating not only the type of myosin expressed but also differences among different cell populations. Among the nearly 40 different myosins expressed in mouse and human, only a subset of these has been found in hematopoietic cells. These molecular motors show differing patterns of expression across immune cell populations, a fact that may indicate specific cell functions. The information available is scarce and limited to analysis of only a fraction of the myosins that have been examined in a limited number of laboratories. Further analysis of the expression and functions of myosins in lymphocytes and other immune cells will certainly reveal more specialized functions. The ultimate goal is to identify the importance of myosins in controlling the quantity and quality of an immune response and their possible association with disease.

AUTHORSHIP

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