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Wiskott-Aldrich syndrome protein (WASp) and relatives: A many-sided family

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LA PROTEÍNA DEL SÍNDROME DE WISKOTT-ALDRICH (WASP) Y SUS PARIENTES: UNA FAMILIA POLIFACÉTICA

RESUMEN

El síndrome de Wiskott-Aldrich (WAS) es una inmunodeficiencia primaria humana ligada al cromosoma X, caracterizada por inmunodeficiencia, microtrombocitopenia y eccema. El gen causante de WAS (WASP) codifica una proteína de 502 residuos llamada WASp. WASp da nombre a una familia de proteínas recientemente definida e involucrada en la transducción de señales desde la membrana celular al citoesqueleto de actina, así como en los procesos de desarrollo y activación celular. Además de su papel como organizador del citoesqueleto, WASp también es una proteína clave en la regulación de las señales procedentes de TCR/CD3 y que conducen a la activación transcripcional y proliferación de las células T. Además, WASp ha sido involucrada en la maduración de los timocitos. Así mismo, se ha mostrado que los miembros de la familia de WASp son importantes en el desarrollo de organismos multicelulares. Trabajos más recientes muestran que la familia WASp está involucrada también en diferentes enfermedades degenerativas. Esta revisión está centrada en la fisiología de la familia WASp, teniendo especial interés en la estructura de WASp, en la regulación intramolecular y en nuevos avances en terapia génica.

PALABRAS CLAVE: WASp/ Actina/ Citoesqueleto/ Linfocitos T/ IL-2/ Inmunodeficiencia.

ABSTRACT

The Wiskott-Aldrich syndrome (WAS) is a human X-linked primary immunodeficiency characterised by inmunodeficiency, micro-thrombocytopaenia and eczema. The gene that causes WAS (WASP) encodes a 502 residues protein designated as WASp. WASp gives name to a family of recently defined proteins involved in the signal transduction from the membrane to the actin cytoskeleton as well as in cellular development and activation processes. Besides its role as a cytoskeleton organiser, WASp is also a key protein involved in the regulation of signals coming from the TCR/CD3, which drive to T cell transcriptional activation and proliferation. Furthermore, WASp has been involved in thymocyte maturation. Likewise, WASp family members have been shown to be important in the development of multicellular organisms. Recent reports show that WASp family is also involved in different degenerative diseases. This review is focused on WASp family physiology, keeping special interest in WASp structure, intramolecular regulation, and new insights related to gene therapy.

KEY WORDS: WASp/ Actin/ Cytoskeleton/ T lymphocytes/ IL-2/ Immunodeficiency.

The Wiskott-Aldrich syndrome (WAS) is a human X-linked primary immunodeficiency characterised by immunodeficiency, micro-thrombocytopaenia and eczema[1].

The causing gene of WAS *(WASP)* was mapped in the Xp11.23 chromosomal region. WASP encodes a 502 residues protein, designated as WASp[2]. Wasp is essential in the development and activation of lymphocytes, as well as in the chemotactic and phagocytic processes that take place during the development of the immune response.

WASp is a highly conserved protein showing a 86% amino acid sequence homology between the human (hWASp) and mouse (mWASp) protein. Most studies have suggested that hWASp and mWASp have a pattern of RNA[2-4] and protein expression[5] restricted to cells of the haematopoietic lineage, which is consistent with the cell types affected in patients with WAS. Its reported subcellular location is predominantly cytoplasmic with a small amount found in the inner side of the plasma membrane (16%) and in the nucleus (3%)[6].

WASp gives name to a family of recently defined proteins involved in the signal transduction from the membrane to the actin cytoskeleton[7-9], as well as in cell development and activation processes. Neural WASp (N-WASp) is another member of the family whose expression is not limited to the haematopoietic lineage being rather expressed in brain, heart and lung[10]. A third member of the family is a suppressor of G-protein-coupled cyclic-AMP receptor (Scar) originally isolated from Dictyostelium[11]. There are three human Scar homologues SCAR1, SCAR2 and SCAR3, also designated as WASp-family verprolin-homologous proteins (WAVE1, WAVE2 and WAVE3). These proteins have a wider tissue distribution and show high homology with WASp at their carboxyl termini[12,13]. Finally, Las17/Bee1p and wasp are WASp related proteins found in *Saccharomyces cerevisiae* and *Drosophila melanogaster*, respectively[14,15], whose function still remains obscure.

STRUCTURE AND BIOCHEMICAL PROPERTIES OF WASP

The WASP gene includes 12 exons coding for the different domains found in the WASp protein. These domains play actin-regulatory functions as well as critical roles in the integration of cell signals delivered by a number of pathways[16].

The carboxi terminal region of WASp consists of two domains, a WASp homology region (WH2) and a terminal acidic region (A). WH2 comprises a verprolin homology (VH) domain and a cofilin homology (CH) domain. Thus, the carboxyl terminus of the protein is also designated as verprolin homology-cofilin homology-acidic region (VCA). The VCA region is essential for binding to actin and to a seven protein complex termed Arp2/3 (actin related protein) that initiates the nucleation of the actin filaments. Amino terminal to WH2, there is a proline-rich region that accounts for >15% of the entire protein sequence[2]. This polyproline region interacts, *in vitro*, with the Src homology 3 domain (SH3) of several signalling and adaptor proteins such as Nck, Fyn, cFgr, c-Src, p47phox, Grb2, Btk, Tec, PLcg1 e Itk[17-22]. However, *in vivo*, interaction of WASp has been only demonstrated for Nck[6], Grb2[17], Fyn[18] and Btk[23]. Nck is a ubiquitously expressed adaptor molecule that interacts with numerous proteins including signalling molecules downstream of the Rho family of GPT-ases. Nck is composed of one SH2 domain and three SH3 domains[24]. It has been shown that the first SH3 domain is both, necessary and sufficient for WAVE1 activation, another protein of the WASp family[25]. On the other hand, the Grb2 adaptor also possesses one SH2 domain but only two SH3 domains through which it interacts with WASp and N-WASp[17,21]. Similarly, Fyn interacts with WASp through its SH3 domain[18]. In the middle portion of the protein there is the GTP-ase binding domain (GBD) that regulates the activation of WASp. Amino terminal to GBD, there is a lysine-rich sequence that, at least for N-WASp, seems to be the binding site for the membrane phospholipid phosphatidylinositol-4,5-biphosphate (PIP2)[26,27]. The amino terminal region of WASp, N-WASp and Las17/Bee1p contains a WASp homology domain, called WH1, which is homologous to the EVH1 domain of ENA/VASP. The WH1 domain is involved in the regulation of the actin cytoskeleton[28] by binding to a proline-rich protein of 503 amino acids termed WASp interacting protein (WIP). WIP contains binding domains for actin monomers (WH2), profilin (a protein that binds actin and promotes its polymerisation), as well as for WASp at its carboxyl terminus. Since WIP lacks a GBD region, WASp serves as a bridge between Cdc42 and WIP, allowing Cdc42 regulation of WIP function[29]. WIP regulates WASp-mediated actin polymerisation and filopodium formation, and stabilises the actin filaments[29-31].

INTRAMOLECULAR REGULATION AND ACTIVATION OF WASP

In resting cells, WASp exists in an inactive conformation. WASp remains autoinhibited by means of the intramolecular interaction of the GBD hydrophobic core with the VC domain. This autoinhibited conformation is reinforced by the interaction of the acidic region at the carboxyl terminus with the basic region located amino terminal to GBD. This results in the occlusion of the Arp2/3 complex binding region[26,27,32-34].

This intramolecular interaction is thought to be disrupted by GTP-Cdc42 and PIP2 allowing WASp to be opened. This permits the interaction of the VCA region with the Arp2/3 complex and the globular actin and, consequently, enables the polymerisation of the actin cytoskeleton[16]. It has been proposed that not only GBD but also other regions of WASp are likely necessary for a tight *in vivo* interaction between Cdc42 and WASp[7-9,35,36]. However, it has been suggested that SCAR/WAVE proteins may act as effector molecules of the Rho GTP-ases by indirect mechanisms[3,37]. It has been demonstrated that IRSp53 forms a trimolecular complex binding to activated Rac through its amino terminus and to WAVE through the SH3 domain at its carboxyl terminus.

Small Rho GPTases are involved in the formation of actin-based structures (filopodia, lamellipodia, stress fibres) regulating multiple cellular functions such as cytoskeleton reorganisation, cell cycle progression, and vesicle trafficking and providing cells with a contractile mechanism[38].

WASp activation can also be regulated, at least in part, by the phosphorylation of specific residues. It has been described recently that phosphorylation of Y291 activates WASp[23,39]while dephosphorylation of WASp Y291 by PTP-PEST favours the adoption of an inactive conformation. PSTPIP is a substrate of PTP-PEST and serves as scaffold guiding PTP-PEST toward this specific dephosphorylation of WASp[40]. In resting cells, the constitutive interaction between WASp and WIP acts stabilising the inactive conformation of WASp. Cellular activation following TCR ligation results in the formation of a ZAP-70-Crkl-WIP-WASp complex, which is recruited to lipid rafts and to the immunological synapse. At the same time, TCR engagement results in PKCθ activation, which, in turn induces PKCθ-mediated phosphorylation of WIP. This disrupts the WASP/WIP complex and allows GTP-Cdc42 activation of WASp[41]. Furthermore, a recent report shows that regulation of tyrosine phosphorylation of WASp mediated by Fyn and PTP-PEST is required for coupling TCR engagement to WASp effector function and T cell activation. This shows key roles for Fyn and PTP-PEST in regulating WASp and implies that inducible WASp tyrosine phosphorylation can occur independently of Cdc42 binding, but unlike the Cdc42 interaction, it is absolutely required for WASp contributions to T cell activation[42]. Another recent study shows that N-WASP-dependent microspike formation is inhibited by forming binding protein 11 (FBP11), indicating that FBP11 regulates nuclear localisation of N-WASP and therefore negatively regulates N-WASP function in the cytoplasm[43]. It has been described recently that the C region of WASp, N-WASp and Scar have a conserved sequence motif, composed of several hydrophobic residues and one arginine residue,

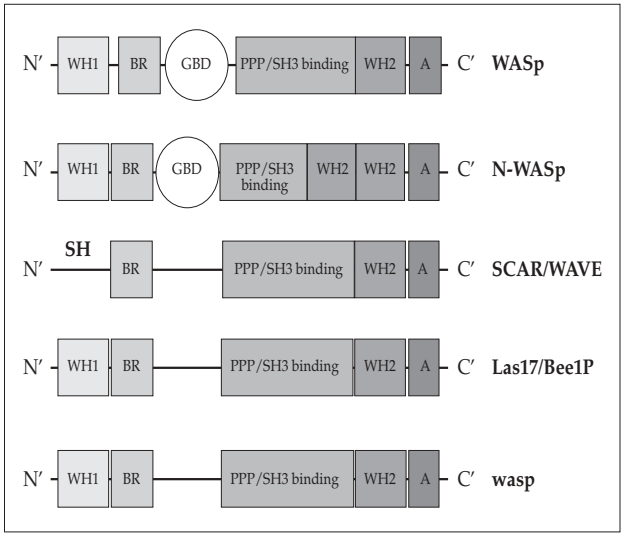

Figure 1. *Functional domains and structure of WASp family members. At the carboxyl terminal region of WASp exist a WASp homology region (WH2) and a terminal acidic region (A). WH2 comprises a verprolin homology (VH) domain and a cofilin homology (CH) domain. The polyproline region of WASp is a SH3 interacting domain. In the centre of WASp exist the GTP-ase binding domain (GBD) and a basic sequence of conserved lysine-rich basic residues (BR) that regulates the activation of WASp. At the amino terminus there is a WASp homology domain (WH1) homologous to the EVH1 domain of ENA/VASP. WASp family members present high homology with WASp at their carboxyl terminal region.*

that form an amphipatic helix necessary for the Arp2/3 complex activation[44]. Another study has recently identified two phosphorylation sites in the VCA domain of WASp at Ser483 and Ser484 that, when phosphorylated, increase the affinity of the VCA domain for the Arp2/3 complex seven-fold, what is required for an efficient *in vitro* actin polymerisation by the full-length WASp molecule[45].

PHYSIOLOGY OF WASP

The actin cytoskeleton is a dynamic network of filaments essential for the movement, polarisation, morphogenesis and division of cells[46-48]. The reorganisation of the actin cytoskeleton is a process regulated by WASp by means of the activation of the Arp2/3 complex. It has been demonstrated that recombinant WASp and N-WASp are weak activators of the Arp2/3 complex, while its association with GTP-Cdc42, PIP2, Nck and Grb2 drives to a marked enhancement of its activity. However, these factors have a weak effect individually, which suggests that they should act in a synergistic manner[26,27,33,49,50]. The reorganisation of the cytoskeleton is intimately related to T cell activation by the establishment of an immunological synapse between $CD4^+$ or $CD8^+$ T lymphocytes and the antigen-presenting cells

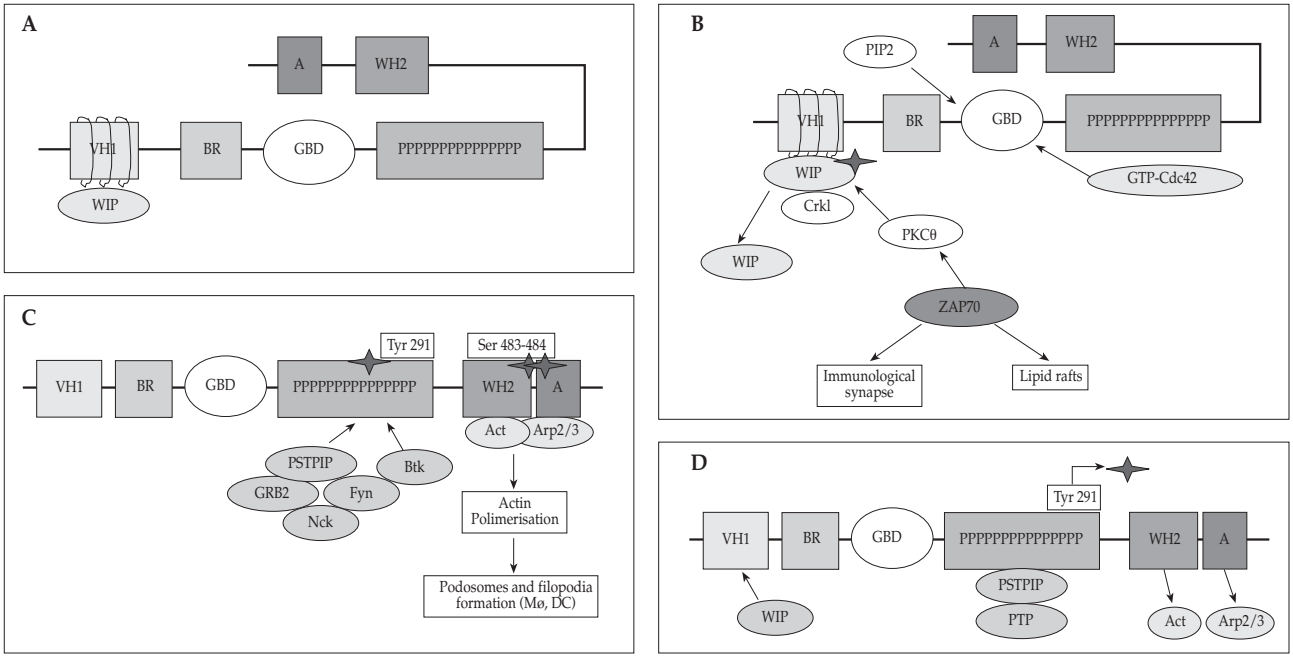

Figure 2. *Sequential process for WASp activation. A) In resting conditions, WASp presents an autoinhibited conformation due to an intramolecular interaction of the GTP-ase binding domain with the basic region and the VCA region at the carboxyl terminus, resulting in occlusion of the Arp2/3 complex binding domain. Furthermore, the constitutive interaction between WASp and WIP acts stabilising the inactive conformation. B) Cellular activation following TCR engagement results both in the formation of a ZAP-70-Crkl-WIP-WASp complex, which is recruited to lipid rafts and the immunological synapse, and in PKCθ activation, which, in turn, induces PKCθ-mediated phosphorylation of WIP that breaks the WASp/WIP complex. The break of WASp/WIP complex allows the disruption of WASp intramolecular interaction by means of the cooperative action of GTP-Cdc42 and PIP2 and thus, the release of the carboxyl terminus. C) Adaptor molecules work in a double way. First, recruiting WASp to the signalling site through the interaction of their SH3 domain and the polyprolyne rich region of WASp, allowing its colocalisation with Cdc42 and PIP2; and second, adaptor molecules phosphorylate WASp tyrosine residue 291 that contributes to WASp active conformation. Two more phosphorylation sites have been identified in the VCA domain at serines 483 and 484 that increase WASp activity. In its active conformation, WASp interacts with the globular actin (Act) and the Arp2/3 complex resulting in actin polymerisation, which is crucial to carry out a number of celullar processes necessary to develop a correct immune response. D) Dephosphorylation of WASp tyrosine residue 291 by PTP-PEST favours the adoption of the inactive conformation resulting in the disruption of the Arp2/3 complex and WASp interaction. Stars indicate sites of serine or tyrosine residue phosphorylation/ dephosphorylation.*

(APC)[51-53]. On the T cell side, this immunological synapse involves the formation of supramolecular activation clusters (SMACs) that comprise a central area (cSMAC) enriched in TCRs and co-stimulatory receptors (CD2 and CD28) and a peripheral area (pSMAC) with adhesion molecules (LFA-1). WASp is located at the site of contact of cell conjugates[54]. It has been demonstrated that WASp is recruited to lipid rafts immediately after the TCR/CD28 activation and it is required for the movement of these lipid rafts. It has also been demonstrated in cells from WASp-deficient patients, an impaired proliferation after TCR/CD28 engagement and a loss of the capacity to cluster and to increase the surface expression of the lipid rafts marker GM1[55].

CD2 cross-linking induces the formation of a macromolecular complex consisting of CD2-CD2AP-PSTPIP1-WASp. The interaction of the SH3 domain of PSTPIP1 with the proline-rich region of WASp allows WASp recruitment to the area of contact between T cells and APC, allowing the initial actin polymerisation required for immunologic synapse formation during T cell activation. On the contrary, it has been shown that CD2-mediated actin polymerisation is abrogated in $WASp^{-/-}$ T cells[56].

The recruitment of WASp to the T cell:APC contact zone occurs independently of its activation by Cdc42, indicating that the activation of WASp requires the integration of multiple signals. Cdc42 localisation occurs in an antigen-dependent way, through its interaction with GEF Vav, in a process in which the Lck and ZAP-70 kinases are required[54]. This is a way to ensure WASp activation only when WASp is located with GTP-Cdc42 in the signalling site and not when it is in another part of the cell.

Focusing firstly on WASp as a *cytoskeleton organiser*, cells of the haematopoietic lineage present a series of defects as a consequence of a faulty actin regulation. T lymphocytes from WAS patients show abnormal morphology with a reduced number of surface microvilli and of the mucin

CD43. Likewise, they present a defect in the establishment of the immunological synapse and in TCR/CD3 mediated transduction of activation signals, what results in impaired actin polymerisation and receptor clustering and internalisation, as well as in failure to produce IL-2. All these defects finally result in defective cell activation and proliferative responses[55,57-60]. It has also been shown that TCR-mediated actin polymerisation is markedly reduced in WASp-deficient mouse thymocytes and T cells[60,61]. A cell model expressing a WASp form devoid of its carboxyl terminus (the Arp2/3 complex interaction domain) shows that this domain is essential to carry out the actin polymerisation[62]. Likewise, another model proposes that after TCR stimulation a multimolecular complex consisting of Fyb/SLAP (Fyn-binding protein/SLP-76-associated protein), SLP-76, Nck, Vav, WASp, proteins of the family Ena/VASP and Arp2/3 is formed, linking TCR-mediated signalling and actin cytoskeleton remodelling[63]. WASp associates with the endocytic adaptor intersectin-2 and localises it to sites of TCR endocytic activity, suggesting its implication in this process[64]. It has been also shown that WASp and Cdc42 are involved in stromal-derived factor 1 (SDF-1) mediated chemotaxis of T cells[65].

The role of WASp in B lymphocytes has not been clearly established. One study suggests a normal B cell receptor (BCR)-mediated signalling in WASp-deficient human B lymphocytes[66], while another study suggests a defective signalling[67]. Later on, a study in mouse B cells null for WASp, has shown a normal signalling and BCR clustering indicating that, at least in mice, WASp is not required for the proliferation induced by the BCR. This suggests that TCR and BCR mediated signalling have different WASp requirements. A possible functional redundancy carried out by proteins of the WASp family such as the N-WASp could exist in B cells[61]. Defective antigen presentation could also be an underlying factor in WAS immunodeficiency. A recent study using WASp-deficient B lymphocytes and dendritic cells (DC) has shown that WASp is dispensable for processing and presentation of soluble antigens, but not for efficient presentation of particulate antigens[68]. B lymphocytes from WAS patients present, like T lymphocytes, an abnormal morphology with shortened microvilli, which could be related with the humoral, aggregation and search of targets defects[69].

NK cells and $CD8^+$ T lymphocytes also form an immunological synapse with their targets[70]. Thus, $CD8^+$ T lymphocyte and NK cells deficient in WASp show a failure in cytotoxicity as a consequence of a defective immunological synapse caused, in last term, by the defect in actin polymerisation and lipid rafts polarisation[71]. A recent study shows that the mature activating NK cell immunological synapse is formed in distinct stages in a WASp-dependent manner, being the CD2, CD11a, CD11b and F-actin accumulation in the pSMAC and the perforin accumulation in the cSMAC, sequential processes with distinct cytosqueletal requeriments[72].

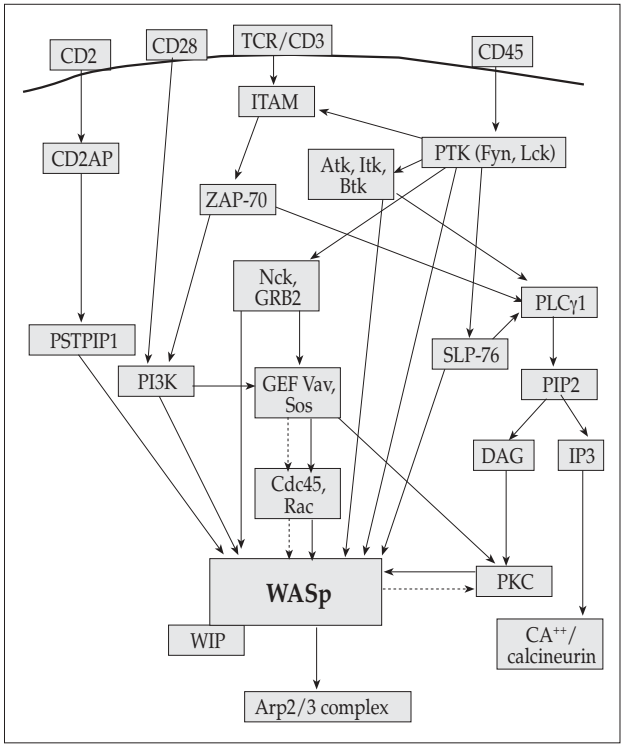

Figure 3. *Intracellular signalling pathways following TCR/CD3 cross-linking. WASp integrates different signals from diverse activation pathways. WASp plays a crucial role in TCR/CD3 signalling pathway leading to T cell actin cytoskeletal rearrangement and IL-2 transcriptional activation.*

To carry out a correct multieffector immune response it is necessary that the immune cells have the capacity to respond to activator signals and directional and migratory stimuli[16]. Macrophages and DC from WAS patients present defects in the polarisation and extension of filopodia which result in a defective chemotaxis in response to colony-stimulating factor 1 (CSF1)[73]. Monocytes null for WASp present, likewise, an alteration in motility in response to monocyte chemoattractant protein (MCP1) and to macrophage inflammatory protein (MIP1)[74]. A lack of podosomes has been observed in WASp-deficient macrophages and mature DC, resulting in a reduced ability to adhere to intercellular cell-adhesion molecule 1 (ICAM1)-coated surfaces[75-77]. A recent report proposes that podosomes provide an essential link between directional cell protrusion and achievement of DC translocation by establishing new dynamic anchor points at the leading edge of the cell in a process in which WASp is involved. Furthermore, the temporal regulation

of podosome assembly during DC maturation also suggests that they may be most critical for early movement, perhaps during transmigration across the lymphatic endothelium[78]. Besides these cell types, podosomes are also found in osteoclasts and in some transformed cells. Future studies focused on regulation of WASp-like proteins of podosomes could open new therapies for the control of the osteoporosis and tumour cell metastasis. In WASp null cells, Fcγ receptor (Fcγ-R)-mediated phagocytosis is delayed and the actin-rich phagocytic cup is poorly formed[79,80]. A molecular complex has been described consisting of Fyb/SLAP, SLP-76, Nck, VASP and WASp that links the actin cytoskeleton to the Fcγ receptor signalling during the phagocytosis in human macrophages[81]. Recently it has been reported in mast cells from WASP-deficient mice that IgE-dependent degranulation, cytokine secretion, tyrosine phosphorylation of phospholipase C-gamma (PLC-γ), Ca^{2+} mobilisation, cell spreading and redistribution of cellular F-actin were diminished, suggesting that WASP regulates FceRI-mediated granule exocytosis, cytokine production and cytoskeletal changes in mast cells[82].

Wasp has been involved not only in actin regulation but also in the transmission of signals coming from the TCR/CD3 complex which drive to T cell transcriptional activation and proliferation.

One of the most outstanding alterations present in T cells from WAS patients is the co-existence of a defect in CD3-mediated intracellular signalling with a normal response to other mitogens (the WAS paradox). This fact suggests that WASp plays a critical role in CD3-mediated signalling while it seems not to be required in the allogeneic response. This alteration has been broadly described in cells from WAS patients[57,58] and from mice knockout for WASp[60,62], and is owed partly to a defect in the IL-2 production in response to CD3 stimulation[57,60].

Tyrosine phosphorylation of proteins after TCR engagement is not altered in T cells from WAS patients and from WASp knock-out mouse models, which present the same phosphorylation pattern than T cells from healthy controls[57,60]. Likewise, it has been described that activation of the MAP kinase pathway after TCR cross-linking is normal in WASp-deficient mouse T cells[60].

T cells transduce signals across the membrane through the TCR/CD3 complex by means of the cytoplasmic domains of the subunits of CD3[83] which contain immunoglobulin receptor family tyrosine-based activation motifs (ITAM) that are crucial to couple TCR to intracellular tyrosine kinases[84,85]. Two protein tyrosine kinases (PTK) of the Src family, p59Fyn and p56Lck, are associated with the TCR/CD3 complex[83]. Fyn and Lck are dephosphorylated by CD45,

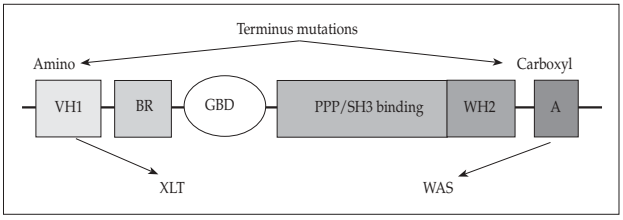

Figure 4. *Genotype/phenotype correlation in WAS. Mutations at the carboxyl terminus region result in lack of protein expression or in the expression of a truncated protein and therefore in classic WAS phenotype. However, mutations at the amino terminus region result in protein expression leading to XLT phenotype.*

which activates them and allows that, in turn, PTKs phosphorylate ITAMs. This allows the recruitment and activation of ZAP-70, a PTK belonging to a group of PTKs different from that of the Src family[86,87]. PTKs are involved in multiple signalling cascades that lead to T cell activation. On one hand, ZAP-70 activates PLC-γ, which is recruited to the plasma membrane by tyrosine-phosphorylated p36. The latter is associated to the plasma membrane and facilitates the interaction of PLC-γ with its substrates, the inositol phospholipids[85,88]. PLC-γ catalyses the hydrolysis of phosphoinositol biphosphate (PIP2) and thereby generates inositol triphosphate (IP3) and diacylgylcerol (DAG). IP3 increases the intracellular calcium concentration that activates the calcineurin phosphatase, which, in turn, activates members of the NFAT transcription factor family. DAG, together with calcium, activates the protein kinase C (PKC) which, in turn, activates the transcription factor NFκB. On the other hand, ZAP-70 activates the phosphatidylinositol-3 kinase (PI3K), allowing the PI3K recruitment to the plasma membrane where its catalytic subunit (p110) can phosphorylate its main substrate, PIP2, generating PIP3[89]. PIP3 interacts with the pleckstrin homology domain (PH) of multiple molecules such as the members of the TEC family (Btk, TEC, Itk), associating them to the membrane where they are activated by the Src family kinases[90,91]. Once activated, TEC family members regulate the activity of the PLC-γ. This puts, at least in part, the calcium/calcineurin and the PKC pathways under the control of PI3K[91]. It has been shown the *in vitro* interaction of the regulatory subunit of PI3K (p85) with WASp[22]. Since PIP2 is needed together with Cdc42 for WASp activation, the regulation of the phosphatidylinositols metabolism carried out by PI3K and PLC-γ should participate in WASp activity.

Likewise, WASp possesses at its amino terminus a PH domain, which allows its recruiment to the cellular membrane by PIP3. Recently it has been described that residues 83-93 of WASp can bind to the catalytic domain of Src kinases inhibiting their activity, what represents a new way of

regulating PTK activity[92]. The proline-rich region of WASp and WIP can interact, in turn, with the PH domain of the TEC family members. The relationship of WASp with these molecules suggests a communication between WASp and PLC-γ. Available evidence suggest that TEC kinases can be related with WASp-mediated actin regulation. A recent work has demonstrated that Itk$^{-/-}$ and Itk$^{-/-}$Rlk$^{-/-}$ T cells present a defect in actin polymerisation and in the conjugate formation in response to antigens presented by APCs[93]. They show that although a normal recruitment of WASp and the Arp2/3 complex to the immunological synapse exists, there is a defective local activation of Cdc42 and WASp, what indicates a requirement of Itk in Vav recruitment to the immunological synapse. On the other hand, PI3K is also involved in GEFs (Sos, Vav) regulation. Their activity is also regulated by the PTKs through adaptor proteins (Grb2, Nck, SLP-76) that, in turn, activate the Ras pathway and molecules of the family of the Rho-GTPases (Rho, Rac, Cdc42). TCR engagement induces the formation of p36/Grb2/Sos complexes related with Ras activation[94]. The Ras pathway is also regulated by GTPase activating proteins (GAP) which are activated by PTKs. Following Ras and Rho GTPase activation, different signalling cascades are triggered, of which the mitogen-activated protein kinases pathway is the best known. Ras activates Raf triggering a cascade that drives to ERK-1 and ERK-2 activation that finally activate Fos, a component of the transcription factor AP-1[85]. The Rho-GPTases activate p38 and JNK that in turn activate c-Jun, another component of AP-1. Therefore, in TCR/CD3 signalling, WASp appears to be a protein that plays an important role in T lymphocyte activation, thanks to its interaction with key signalling molecules. In fact, it has been indicated that WASp could be a member of the LAT complex (linker for the activation of cells T) composed by adaptor proteins associated to the plasma membrane (PLC-γ, Cbl, Vav, SLP-76 and Grb2) that play an important part in the activation of the T cell coupling TCR cross-linking in the plasma membrane to distal signalling cascades[95].

The IL-2 gene transcriptional machinery integrates multiple types of biochemical information using diverse transcription factors that, when optimally activated by different signalling pathways, determine whether the gene is expressed or not[96]. Some of these factors, such as Oct-1 and Spl-like factor, are constitutive. Other factors such as NFAT, NFkB and AP-1 need to be activated by different pathways, as indicated above. If one of these factors is not activated the expression of IL-2 is totally inhibited. The inability of the other unaffected factors to work is due to the fact that no factor can interact stably with its target site in the IL-2 enhancer unless all the factors are present[96]. This suggests that the failure in CD3-mediated IL-2 production seen in WASp-deficient cells is due to a failure in the activation of one or a number of these transcription factors. It has been described that the WH1 region of WASp is required for NFAT-dependent IL-2 transcription. Likewise, transgenic mice overexpressing the N-terminus of WASp do not produce IL-2 upon TCR stimulation, while actin cytoskeleton reorganisation remains intact[95]. This work together with the one of Silvin et al.[62] support a role for WASp in CD3/TCR-mediated transcriptional activation independent of its role in actin polymerisation. It has been described that SLP-76 overexpression in Jurkat cells increases the activity of NFAT and AP-1, while Vav overexpression increases only that of NFAT. Both molecules act synergistically regulating IL-2 gene expression and reflect that a cooperation exists between different activation pathways[97]. Likewise, Vav and PKC are functionally related in spite of the fact that a physical interaction has not been shown between both molecules[97,98]. Recently it has been demonstrated that SLP-76 coordinates Nck-dependent WASp recruitment with Vav-1/Cdc42-dependent WASp activation at the T cell:APC contact site[99]. New studies are necessary to determine whether the relationship between Vav and PKC is mediated by WASp and to shed light on the cross-talk between WASp and the molecules of the signalling pathways described previously. This cross-talk may occur in a two-way direction, not only by regulating WASp activity but rather by WASp regulating the activity of these molecules and therefore, of the pathways that they integrate. WASp would act increasing the activation of these molecules to achieve an optimal activation of these pathways and therefore, of the transcription factors that lead to the production of IL-2. A recent study shows that the absence of WASp does not block completely the signalling pathways coming from the TCR, but rather it avoids the amplification mechanism required for an optimal activation, that is to say, WASp plays a crucial role diminishing the activation threshold. This way, WASp could be regulating the calcium flux by regulating these pathways. Some recent results demonstrate that the calcium flux is diminished after CD3 stimulation in cells from WAS patients[55]. Nevertheless, other authors have described that the calcium flux upon CD3-mediated stimulation is not altered in cells from WAS patients[97]. It has been proposed that these differences can be due to the fact that the intensity of the defect is variable from patient to patient depending on the mutation that presents.

Zhang et al described in mice null for WASp, a defect in *thymocyte maturation* due to an impaired progression of CD4–CD8– (double-negative) precursors from the CD44-CD25^{+} stage to the CD44-CD25- stage[60]. Later on they demonstrated, in mice that express WASp devoid of its VCA

domain (WASpΔVCA), a severe early block in T lymphopoiesis associated with impaired TCRαβ expression and a consequent failure to generate single-positive $CD4^+$ and $CD8^+$ T cells. These later defects, which have not been observed in $WASp^{-/-}$ mice, are associated with a defect in actin polymerisation and a failure in the terminal differentiation of double negative thymocytes. These observations suggest that WASp functions in T cells can be mediated, at least in part by other proteins whose effector activity is impeded by WASpΔVCA expression[100]. On the contrary, a recent study suggests that differentiation and survival of B lymphocytes is minimally WASp-dependent[101].

The WASp family of proteins has not only been involved in development of haematopoietic lineage cells. In $N\text{-}WASp^{-/-}$ mice a lethal embryonic mutation was observed, which is reasonable, given the wide tissue distribution of N-WAS[102,103]. Recently, $WAVE1^{-/-}$ mice have been generated and showed sensorymotor retardation and defects in learning and memory, which reflect the restricted expression of WAVE1 in brain[104]. Studies in Drosophila mutant for the WASp family genes reflect the importance of these molecules in the multicellular organisms. Mutant $WASp^{-/-}$ flies were viable but showed abnormal differentiation of neurons, which was caused by a defect in the cellular division that resulted in the generation of an excessive number of neurons[15]. Likewise, in this study the WASP gene was related to the components of the Notch-signalling pathway, which has a connection with neural differentiation, indicating that WASp is a key signal transducer of the Notch pathway. The $WAVE/Scar^{-/-}$ flies presented a more severe phenotype than that of $WASp^{-/-}$. It consisted on a generalised defect in actin cytoskeleton organisation during early development, suggesting that the main activator of the Arp2/3 complex during early development is WAVE/Scar and not WASp[105,106]. A recent work shows that WAVE2-regulated actin reorganisation might be required for proper cell movement and that a lack of functional WAVE2 impairs angiogenesis *in vivo*[107]. Another recent study shows a non-redundant role for WAVE2 in mouse embryogenesis and a critical role for WAVE2 in actin-based processes downstream of Rac that are essential for cell movement[108]. Two recent studies have related the WASp family of proteins to the Alzheimer´s disease (AD). One of the studies demonstrates that the protein levels of N-WASp, WISH and WAVE are significantly increased in the brain of AD patients. Additionally, colocalisation of these proteins with actin filaments is observed in abnormal dendritic processes, suggesting that they could participate in the neurodegenerative aberrant sprouting in AD neurons[109]. AD is characterised by the accumulation of extracellular amiloid-beta fibrils, and microglia cells are considered to participate in the pathways that lead to clearance of amiloid-beta . The second study shows that WAVE and Rac co-localise with F-actin in the lamellipodia of phagocytic microglia, suggesting that WAVE and Rac could participate in the phagocytosis of the amiloid-beta carried by microglia[110]. Elevated expression of S100A4 protein is associated with metastatic tumour progression. A recent work shows that S100A4 co-localise with Arp3 and N-WASp at the leading edge of lamellipodia formation and suggests that the identification of the responsible molecules for locating S100A4 to the lamellipodial structures could help to know the mechanism by which S100A4 regulates metastasis[111].

Actin-based-motility (ABM) has been studied in several intracellular pathogenic organisms. Thus, ABM in *Listeria monocytogenes* is a process regulated by the bacterial protein ActA and the Arp2/3 complex, although N-WASp is not required[102,112]. ABM of Vaccinia virus is carried out by the interaction of the viral protein A36R with Nck, which facilitates the recruitment of N-WASp by WIP[102,113-115]. In a similar way, *Escherichia coli* uses its protein Tir to recruit Nck, N-WASp and the Arp2/3 complex[116,117]. *Shigella flexneri* ABM depends on the direct interaction of its protein IcsA with N-WASp, which results in N-WASp activation and subsequent recruitment of WIP[113,118,119]. A recent study has shown that *Mycobacterium marinum* is capable of actively inducing actin polimerisation within macrophages using host proteins such as the Arp2/3 complex and VASP, which localise throughout the actin tails, and WASp that localises exclusively at the actin-polymerising pole of *M. marinum*[120]. Two other reports suggest that rickettsial ABM is independent of N-WASp and the Arp2/3 complex[121,122]. Similarly, another study shows that ABM of *Burkholderia pseudomallei* involves the Arp 2/3 complex but not N-WASp and Ena/VASP proteins[123]. ABM enables these pathogens to invade and to spread in the host cells, which causes the disease. Thus, the understanding of the mechanisms involved in ABM of pathogens will increase the knowledge of the pathogeny of these intracellular organisms and will therefore allow the development of new therapies.

GENOTYPE AND PHENOTYPE IN WAS

Recently, new mutations have been identified in the WASP gene that, together with those already well-known and the advances in the laboratory techniques, are allowing studies focused on establishing a correlation between *WASP* mutations and the clinical phenotype of WAS patients.

Patients with classic WAS present a broad spectrum of mutations (deletions, insertions and splice-site mutations) that usually result in lack of protein expression or in the expression of a truncated protein at the carboxyl terminus region[124]. As mentioned above, this region is involved both in actin cytoskeleton reorganisation and in the development

of cellular processes, therefore resulting in the most severe form of the disease. A study carried out in 50 patients with mutations in the *WASP* gene shows that all the patients with misssense mutations were WASp positive while patients with non sense mutations and deletions were WASp negative[125]. Each patient's clinical phenotype was correlated with the presence or absence of the protein what indicates that WASp expression can be a useful tool in predicting the long-term prognosis in WAS/XLT. A recent study suggests that the termination codon mutation causes reduced mRNA stability, resulting in the absence of WASp expression[126].

The clinical phenotype of WAS is represented by microthrombocytopaenia presenting from small haemorrhages to life threatening gastric or intracraneal haemorrhages. WAS T lymphocytes present a defective CD3-mediated response, being the clinical consequence a high susceptibility to viral, pyogenic and opportunistic infections. Likewise, B lymphocytes are affected, presenting deficient antibody responses particularly against polysaccharide antigens, as well as low levels or absence of isohemagglutinins[16,124].

Patiens with X-linked thrombocytopaenia present less severe immunological and platelet alterations. Most of the patients present misssense mutations at the amino terminus region resulting in a reduced expression of partially functional protein[1,16]. Most of the mutations found affect exons 1-5, and therefore, the WH1 domain of WASp, leading to impaired WIP-WASp interactions[30]. Arg86 and the proximal acidic residues Asp77, Glu98 and Gln100 have been identified as hot spot point mutations that disrupt the WH1 hydrophobic region and thus are critical for WIP-WASp interaction[127].

X-linked neutropaenia or mielodisplasia has been described recently and is caused by mutations in GPT-ase binding domain (GBD) of WASp[128]. It is thought that these mutations (Leu270Pro and Ile294Thr) disrupt the hydrophobic core of the protein producing a failure in autoinhibition. The patiens presented neutropaenia and monocytopaenia in the first mutation and panleucopaenia with displasia in the three cellular lineages of the bone marrow, as well as high levels of spontaneous apoptosis in the progenitor's cellular populations in the second mutation. However, patients did not present microthrombocytopaenia[16]. The clinical phenotype is presented by the male patients meanwhile female carriers have no clinical sings. This is explained because in obligate female heterozygotes only the wild type X-chromosome is active, while X-chromosome bearing the mutation is non-randomly inactivated. However a recent paper reports about a girl presenting WAS phenotype due to a skewed X-inactivation that favours the WASP-mutated allele[129].

Currently, treatment strategies are variable and individualised depending on the centre and on the patient. However, there are some trends that consist of intravenous gammaglobulin and prophylactic antibiotics in the majority of patients while splenectomy is less used[130]. At present, the only effective curative treatment is stem-cell transplantation. However, in many cases this treatment is unsuccessful, due to the capacity of patients T cells to develop an allogenic response. The frequent lack of suitable donors and the potential of severe complications associated with bone marrow transplantation, make the development of gene therapies for WAS a desirable target. Thus, new gene therapies are under study to develop a safe and effective cure. Functional correction of T cells from WAS patients by transduction with an oncoretroviral vector encoding WASp has been shown[131]. Recently, another report shows the correction of the defects in T-cell-mediated immunity to influenza virus in a mouse model knock out for WASP, by oncoretroviral vector-mediated gene transfer into repopulating haematopoietic cells[132]. It has been shown that retrovirus-mediated WASP gene transfer, both in primary T lymphocytes and in transformed T cell lines derived from WAS patients, corrects WAS T cell disfunction[133]. Another group has documented a selective advantage of wild type over knock-out cells in mouse lymphoid tissue. They show the rescue of T-cell signalling and amelioration of colitis upon transplantation of retrovirally transduced haematopoietic stem cells in mice, providing proof of principle that the WAS-associated T-cell signalling defects can be improved using this treatment without overt toxicity, what may encourage clinical gene therapy trials[134].

CONCLUDING REMARKS

WASp family members are emerging as a group of proteins involved in multiple and important cellular processes not only restricted to haematopoietic cells. Therefore, new studies that keep deepening in the physiopathology of WASp members could open new therapeutic ways not only for WAS but also for other autoimmune and degenerative diseases such as cancer. Furthermore, new insights on gene therapy would not only help to provide WAS patients with a definitive cure but also could serve as a starting point for the application of gene therapy in other diseases.

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