WASP: a key immunological multitasker

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Abstract | The Wiskott–Aldrich syndrome protein (WASP) is an important regulator of the actin cytoskeleton that is required for many haematopoietic and immune cell functions, including effective migration, phagocytosis and immune synapse formation. Loss of WASP activity leads to Wiskott–Aldrich syndrome, an X-linked disease that is associated with defects in a broad range of cellular processes, resulting in complex immunodeficiency, autoimmunity and microthrombocytopenia. Intriguingly, gain of function mutations cause a separate disease that is mainly characterized by neutropenia. Here, we describe recent insights into the cellular mechanisms of these two related, but distinct, human diseases and discuss their wider implications for haematopoiesis, immune function and autoimmunity.

Scaffold protein
A protein that functions as a support to assemble a multiprotein complex.

ARP2–ARP3 complex
A complex composed of seven proteins, including ARP2, ARP3 and ARP complex protein 1 (ARPC1)–ARPC5. On its own, the complex has little activity but, when bound to an ARP2–ARP3-nucleation-promoting factor, it is activated to generate new actin filaments on pre-existing filaments.

Actin filaments
Formed from the nucleation of monomeric actin subunits during remodelling of the cytoskeleton.

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Wiskott–Aldrich syndrome protein (WASP) was the first identified member of a family of actin regulators, of which five are expressed in mammals (for detailed reviews see REFS 1,2). Although WASP expression is restricted to haematopoietic cell lineages, neural WASP (N-WASP) and WASP family verprolin homologous protein 1 (WAVE1; also known as WASF1), WAVE2 (also known as WASF2) and WAVE3 (also known as WASF3) are more widely expressed. WASP family members lack intrinsic catalytic activity; they function as scaffold proteins, transducing a wide range of signals from proteins or membranes to mediate dynamic changes in the actin cytoskeleton. They have a common modular structure that allows interaction with multiple distinct binding partners. The carboxyl terminus — consisting of the verprolin homology (V; also known as WH2) domain, the cofilin homology (C) domain and the acidic region (A) — is particularly conserved among WASP family members. The WASP family VCA regions bind a complex formed of actin-related proteins (ARPs), the ARP2–ARP3 complex, and recruits monomeric actin to stimulate and localize nucleation of branched actin filaments[3–7] (FIG. 1). Although WASP family proteins might also have important signalling activities that are independent of cytoskeletal rearrangements[8,9], stimulation of ARP2–ARP3-mediated actin polymerization is, to date, their most studied and best understood function. Based on the presence of a similar VCA domain, two new ubiquitously expressed WASP family-like proteins, WASP homologue (WASH) and WASP homologue-associated protein with actin, membranes and microtubules (WHAMM), have recently been shown to regulate actin dynamics in human cells, but these proteins require further characterization[10,11].

The importance of WASP-mediated cytoskeletal regulation in haematopoietic cells is shown by two human diseases that are caused by mutations in the *WAS* gene (which encodes WASP) (TABLE 1). Classical Wiskott–Aldrich syndrome (WAS) is an X-linked primary immunodeficiency that is characterized by low numbers of small platelets, easy bruising and prolonged bleeding, eczema and recurrent infections[12]. Autoimmunity and immunodeficiency-related haematopoietic cell malignancies are serious complications that occur in a substantial number of affected individuals[12,13,14]. Therefore, classical WAS has a severe clinical course (TABLE 1), resulting in premature death unless treated by bone marrow transplantation or gene therapy. There is, however, a range of clinical severity and milder variants of WAS, often known as X-linked thrombocytopenia (XLT) or attenuated WAS, have a more favourable outcome. Both classical and mild forms of WAS result from loss-of-function mutations of *WAS*, of which hundreds have been described (see WASPbase and the European Society for Immunodeficiencies). Although the correlation between genotype and phenotype is not exact, a complete absence of WASP expression generally results in classical WAS, whereas a low level of WASP expression as occurs with some missense mutations in the WASP homology 1 (WH1; also known as EVH1) domain is often associated with a milder clinical course in terms of dysregulated immunity, although thrombocytopenia is always present[14].

Recently, X-linked neutropenia (XLN) was identified as a clinically and biologically distinct WASP-associated disorder, resulting from the presence of a cluster of mutations that confer constitutive WASP activation[15,16]. As suggested by its name, the main clinical symptom of

XLN is neutropenia, which causes a predisposition to bacterial infection, and other clinical features, including myelodysplasia and other cytopenias[16,17] (TABLE 1). XLN seems to be much rarer than WAS; only four mutations (Leu270Pro, Ser270Pro, Ile276Ser and Ile294Thr) affecting a small number of patients have been reported so far[15,16] (H. D. Ochs, personal communication), giving a surprisingly variable phenotype.

In this article, we review recent advances in our understanding of the regulation of WASP expression and function and its role during normal adaptive and innate immune cell development and function. We discuss an evolving appreciation of how *WAS* mutations result in human disease through both protein deficiency and dysregulated activity. The pathogenic mechanisms underlying non-autoimmune microthrombocytopenias remain poorly understood and are not discussed in detail here.

Regulation of WASP expression and function

Auto-inhibition and activation of the ARP2–ARP3 complex. The modular structure of WASP facilitates regulation of the VCA domain activity through intramolecular and intermolecular interactions that alter its affinity for the ARP2–ARP3 complex, as well as its stability and subcellular localization. Several other factors have been reported to bind WASP, as single proteins or as part of a multimolecular complex, although the physiological relevance of these interactions is not completely known. These are summarized in TABLE 2 and have been reviewed in detail elsewhere[1]. The VCA domain of WASP is adjacent to a polyproline domain, which is also found in other family members. The rest of the amino-terminal region is more variable between WASP family members, but for WASP (and N-WASP) it consists of a WH1 domain, a basic region and a GTPase-binding domain (GBD).

In the cytoplasm, WASP is mainly present in an auto-inhibited form in which the VCA domain interacts with a hydrophobic pocket in the GBD (FIG. 1), thereby concealing the binding sites for the ARP2–ARP3 complex[18]. The Rho family GTPase cell division cycle 42 (CDC42) was the first protein shown to bind WASP and is an important regulator of WASP function; interaction of GTP-bound CDC42 with the WASP GBD is thought to release the C terminus from auto-inhibition, allowing binding by the ARP2–ARP3 complex and actin nucleation[19]. Another Rho family GTPase, RAC1, might have a role in activation of N-WASP, but is probably not involved in WASP activation[20]. Although it is generally accepted that the binding of GTP-bound CDC42 to WASP is important for allosteric release from auto-inhibition, other signalling (for example, through the adaptor proteins non-catalytic region of tyrosine kinase 1 (NCK1) and NCK2) or post-translational events can also modulate WASP function both *in vitro* and *in vivo* and might, in some circumstances, provide an alternative mechanism for WASP activation that is independent of GTPase activity[20–23]. Phosphatidylinositol-4,5-bisphosphate ($PtdIns(4,5)P_2$) is an important regulator of actin organization mediated by both WASP and N-WASP and may be cooperatively dependent on NCK proteins[24]. WASP activation by CDC42 is mediated by binding of transducer of CDC42-dependent actin assembly 1 (TOCA1; also known as FNBP1L) to a complex between WASP (or N-WASP) and WASP-interacting protein (WIP; also known as WIPF1)[25]. Recently, it has been shown that an additional level of regulation is achieved through dimerization of WASP, which substantially increases the affinity of WASP for the ARP2–ARP3 complex[26].

The importance of tight regulation of WASP activity through auto-inhibition is shown by the clinical phenotype of XLN. The XLN-causing mutations are in the VCA binding region of the GBD and disrupt the hydrophobic pocket, either by change of charge or

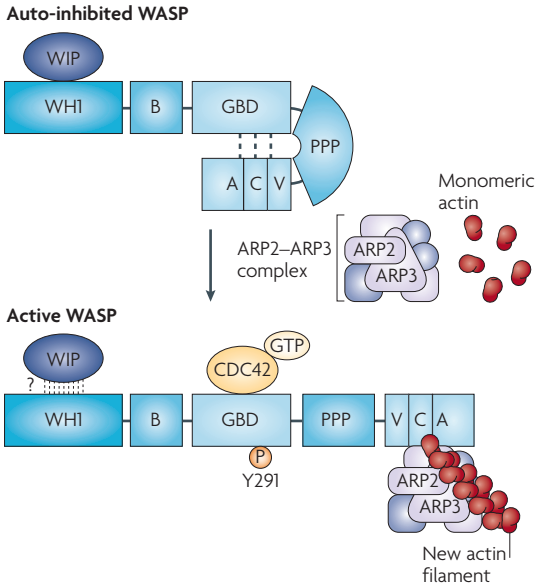

Figure 1 | **Domain structure of Wiskott–Aldrich syndrome protein.** Wiskott–Aldrich syndrome protein (WASP) family members are activated by a wide range of extracellular signals (including growth factors, cytokines and antigen), which are transduced through cell surface receptors. In its normal state, WASP has an auto-inhibited conformation in which an intramolecular interaction between the verprolin homology domain–cofilin homology domain–acidic region (VCA) domain and the GTPase-binding domain (GBD) is thought to prevent binding of the actin-related protein 2 (ARP2)–ARP3 complex and monomeric actin to the carboxyl terminus. The association of WASP-interacting protein (WIP) with the WASP homology 1 (WH1) domain is thought to stabilize the auto-inhibited configuration, although it is not clear whether WIP remains bound following WASP activation. The Rho family GTPase cell division cycle 42 (CDC42) is the main WASP activator, by binding to the GBD (possibly following docking by transducer of CDC42-dependent actin assembly 1 (TOCA1)), which causes allosteric release of the VCA from the GBD. Other factors, such as the adaptor protein non-catalytic region of tyrosine kinase 1 (NCK1), can also activate WASP independently of CDC42 (not shown). Phosphorylation of WASP tyrosine residue 291 (Y291) might either activate WASP alone or stabilize an active conformation initiated by other factors. Mutations in this region of WASP disrupt auto-inhibition and result in constitutive activation of WASP. B, basic domain; PPP, polyproline domain.

Thrombocytopenia
A lower number of circulating platelets than normal, owing to either the failure of production from bone marrow megakaryocytes or increased clearance from the circulation, predominantly in the spleen.

Rho family GTPases
A subfamily of small GTP-binding proteins that have key roles in rearrangement of the cytoskeleton. The nucleotide-bound state of these GTPases is generally regulated by guanine-nucleotide exchange factors (GEFs), which catalyse GDP–GTP exchange, and GTPase-activating proteins, which facilitate the hydrolysis of the bound GTP. Activation, by extracellular signals through various receptors, results in translocation to the plasma membrane thereby localizing their activity to discrete sites in the cell.

Table 1 | **A summary of WASP-related diseases**

Disease	Mutation type	Effect of mutation	WASP expression	Clinical features	Complications
Classical WAS	Nonsense, deletions, insertions, splice anomalies and missense mutations, especially outside exons 1–3	Loss of function	Usually absent	Microthrombocytopenia, moderate to severe eczema and recurrent or severe infections	Autoimmunity and haematopoietic cell malignancy
XLT	Most commonly missense mutations, especially in exons 1–3, or splice anomalies	Loss of function	Usually present at low levels	Microthrombocytopenia, mild to moderate eczema and no increased infections or recurrent minor infections	Autoimmunity
XLN	Missense mutations in the VCA binding domain	Disrupted autoinhibition	Present	Neutropenia, monocytopoenia, NK cytopoenia and myelodyplasia	Not determined

NK, natural killer; VCA, verprolin homology domain–cofilin homology domain–acidic region; WAS, Wiskott–Aldrich syndrome; WASP, WAS protein; XLN, X-linked neutropenia; XLT, X-linked thrombocytopenia.

perturbation of the secondary and tertiary structure, preventing normal intramolecular interaction with the C terminus. As a result, WASP auto-inhibition in patients with XLN is compromised and actin polymerization is dysregulated in terms of activity and localization in the cell[16].

Tyrosine phosphorylation. Although several binding partners, including GTP-bound CDC42, have been shown to be responsible for activation of WASP (TABLE 2), little is known about the hierarchy of events and level of redundancy in the system. Furthermore, the mechanisms that control the localization of WASP activity to sites of new actin polymerization are poorly understood. Recently, phosphorylation of WASP and N-WASP at a conserved tyrosine residue (Tyr291 in human WASP) has been identified as an important physiological regulator of WASP activity. Crucially, this residue is located in the GBD, and its phosphorylation is thought to alter the charge and therefore stability of the auto-inhibited form of WASP. Mutation of a nearby amino acid (Ile294Thr), also located at an α-helical surface of the hydrophobic pocket, substitutes a non-polar amino acid for a polar amino acid and has been described in patients with XLN[16]. Phosphorylation of Tyr291 has been shown to positively regulate WASP activity in a CDC42-independent manner *in vitro*[27]. Accordingly, mutation of Tyr291 to a negatively charged glutamic acid (Tyr291Glu), which mimics phosphorylated Tyr291, enhanced actin polymerization *in vitro*. Several studies have examined the importance of WASP phosphorylation *in vivo* by studying the Tyr291Glu mutation and a Tyr291Phe mutation, in which tyrosine is substituted with a non-phosphorylatable non-polar residue[28–31]. Recently, knock-in mice with the equivalent mutations in mouse WASP (at the homologous residue Tyr293) have been generated[32]. Mice expressing a Tyr293Phe WASP mutant, which cannot be phosphorylated, developed many of the immune defects that occur in mice with a complete WASP deficiency, showing the importance of Tyr293 phosphorylation for normal WASP function. Surprisingly, however, the mutation Tyr293Glu, which was predicted to activate WASP function, resulted in a phenotype similar to that of WASP deficiency, but this mutation also markedly decreased the level of WASP expression. Expression levels were partly restored by treatment of cells with proteasome inhibitors, suggesting that Tyr293 phosphorylation might target WASP for proteasome-mediated degradation[32].

Phosphorylation of WASP seems to enhance its activity during many cell processes, including proliferation, phagocytosis, chemotaxis and the formation of adhesion structures and the immunological synapse. It might also mark WASP for degradation, thereby providing a mechanism for control of activity. The interplay between phosphorylation and regulation by other factors such as CDC42 is not known, although it has been proposed that efficient phosphorylation and dephosphorylation is dependent on binding of GTP-bound CDC42 to WASP[28,33]. However, it has also been proposed that tyrosine phosphorylation and normal T cell activation can occur independently of CDC42 binding[21]. WASP and N-WASP are also phosphorylated on adjacent serine residues (Ser483 and Ser484, respectively) in the VCA region at the junction of the C and A domains[34]. This is mediated by casein kinase 2 and is thought to be required for optimal WASP and N-WASP activity, although the importance of phosphorylation of these serine residues has not been studied in detail *in vivo*.

WASP degradation and the role of WIP. Degradation may be an important means by which WASP activity is regulated *in vivo* and the mechanisms involved have been the subject of several recent studies. As mentioned, phosphorylation might promote WASP degradation, whereas interaction with WIP might protect WASP from degradation[35–38]. WIP wraps around the WH1 domain of WASP, interacting with an extended surface[39,40]. Most of the WASP molecules in the cytoplasm are associated with WIP, which thereby regulates absolute cellular levels of WASP[37,41,42]. The WIP–WASP interaction is also important for localizing WASP to areas of active actin polymerization *in vivo*[35]. It is not clear whether the complex has to be disrupted during or before WASP activation, as has been suggested following T cell receptor (TCR) ligation[41]. Nevertheless, an absence of WIP in knockout mice leads to a marked decrease in cellular levels of WASP, which is partly reversible by treatment with calpain or proteasome inhibitors, indicating that WIP protects WASP from protease- and proteasome-mediated degradation[35,37]. This is clinically important

Calpain
One of a group of Ca^{2+}-activated cytoplasmic proteases that are found in many tissues and that hydrolyse various endogenous proteins, including neuropeptides and cytoskeletal proteins, as well as proteins from smooth muscle, cardiac muscle, liver, platelets and erythrocytes. Two subclasses are known: one with high Ca^{2+} sensitivity and one with low Ca^{2+} sensitivity.

Table 2 | **WASP binding partners**

WASP binding partner	WASP binding domain	Effect on WASP
WIP	WH1	Stabilizes WASP through formation of the WIP–WASP complex, which protects WASP from proteasomal degradation; may chaperone WASP to localize its activity; may have independent activity during cytoskeletal regulation
PtdIns(4,5)P_2	WH1 and/or basic domain (exact binding site unclear)	Potential WASP activator functioning synergistically with CDC42 and cooperatively with NCK1
ARP2 and ARP3	WH1 and VCA	Main effector complex for WASP activity through nucleation of branching actin polymerization; physiological relevance of binding to WH1 is not clear
GTP-bound CDC42	GBD	Activates WASP in GTP-bound state through disruption of autoinhibited conformation
TOCA1	Basic domain	May be required to dock CDC42 for WASP activation
Other GTPases: TC10 and RAC1	GBD	May contribute to WASP activation, although they bind with lower affinity than CDC42
SRC family tyrosine kinases: HCK, LCK, LYN, FYN and FGR	Polyproline	Activate WASP through tyrosine phosphorylation (Y291) and destabilization of autoinhibited conformation
TEC family kinases: BTK, ITK and TEC	Polyproline	May activate WASP
Adaptors: NCK, GRB, CRKL, syndapin, intersectin 2 and PSTPIP1	Polyproline	Activate WASP; NCK may be independent of CDC42 but interdependent with PtdIns(4,5)P_2; intersectin 2 and PSTPIP1 also localize WASP activity
PTPN12	Polyproline	Inactivates WASP through dephosphorylation of Tyr291
VASP	Polyproline	Activates WASP and localizes WASP activity
CK2	VCA	Activates WASP through serine phosphorylation
Monomeric actin	VCA	Activates ARP2–ARP3 complex

ARP, actin-related protein; BTK, Bruton's tyrosine kinase; CDC42, cell division cycle 42; CK2, casein kinase 2; GBD, GTPase-binding domain; GRB, growth factor receptor-bound protein; HCK, haematopoietic cell kinase; ITK, IL-2-inducible T cell kinase; NCK, non-catalytic region of tyrosine kinase; PSTPIP1, proline-serine-threonine phosphatase-interacting protein 1; PtdIns(4,5)P_2, phosphatidylinositol-4,5-bisphosphate; PTPN12, tyrosine-protein phosphatase non-receptor type 12 TEC, tyrosine kinase expressed in hepatocellular carcinoma; TOCA1, CDC42-dependent actin assembly 1; VASP, vasodilator-stimulated phosphoprotein; VCA, verprolin homology domain–cofilin homology domain–acidic region ; WASP, Wiskott–Aldrich syndrome protein; WH1, WASP homology 1; WIP, WASP-interacting protein.

Podosome
An adhesion structure that is found in various malignant cells and in some normal cells, including macrophages and osteoclasts. Podosomes are small (0.5 μm diameter) structures comprising an actin core surrounded by a ring containing typical focal-adhesion proteins, such as vinculin and paxillin.

Aorta–gonad–mesonephros region
An embryonic site in which the development of definitive haematopoietic stem cells (HSCs) occurs. It comprises the aorta and developing reproductive and excretory (mesonephros) systems. In this haematogenic site, HSCs are concentrated in the aortic region.

X-chromosome inactivation
In females, a single, randomly selected X chromosome is inactivated during early embryogenesis to avoid an imbalance of X-linked genes. This process is controlled by the *XIST* gene, which produces a large non-protein-encoding RNA that triggers widespread gene silencing on the same X chromosome. If one X chromosome encodes a gene that impairs cell growth or survival, development of cells with the non-silenced normal chromosome is favoured. This is known as apparent non-random X-chromosome inactivation.

as missense mutations in the WH1 domain of WASP are some of the most common mutations occurring in patients with attenuated forms of WAS. Because of the extended nature of the binding interface between WASP and WIP, different missense mutations disrupt the intermolecular interaction in a similar way to each other, resulting in accelerated degradation and decreased levels of WASP in patients with these genotypes[43]. However, these missense mutations do not fundamentally affect WASP function, as the mutant proteins retain the ability to support actin polymerization to levels at least equivalent to wild-type WASP when expressed at similar levels *in vitro*[35]. In addition, despite lower levels of expression of missense mutant proteins *in vivo*, WASP-dependent actin structures, including macrophage or dendritic cell (DC) podosomes, are formed, albeit at lower levels than normal[44]. It remains to be clarified whether these mutations disrupt normal regulation and localization of WASP activity, although this seems probable as WIP binding, which facilitates recruitment to the plasma membrane and might attenuate WASP activity, is decreased or abolished[45]. It also seems probable that, in addition to the role of WIP as a chaperone for WASP, it can regulate the cytoskeleton independently of WASP, although the details of this pathway remain to be clarified. Recently, a female patient with genetic deficiency of WIP has been reported to have a WAS-like phenotype, which is consistent with findings in an established mouse knockout model[46] (S. Giliani, L. Notarangelo and R. Geha, unpublished observations).

WASP function in haematopoiesis

WASP is expressed by all haematopoietic cell lineages and precursor cells, including haematopoietic cells from the earliest site of definitive human haematopoiesis in the aorta–gonad–mesonephros region of the developing embryo[47]. However, the functional importance of WASP is likely to vary at different stages of haematopoietic cell development. Human female carriers of *WAS* mutations are known to exhibit apparent non-random X-chromosome inactivation of mutant *WAS* in peripheral haematopoietic cell lineages and earlier progenitors (rarely, inadvertently skewed X-chromosome inactivation results in a WAS-like phenotype in females)[48]. One possibility is that the migration of WASP-deficient haematopoietic stem cells from the fetal liver to the bone marrow is impaired such that the development of cells expressing wild-type WASP is favoured[49]. However, WASP seems to have a redundant

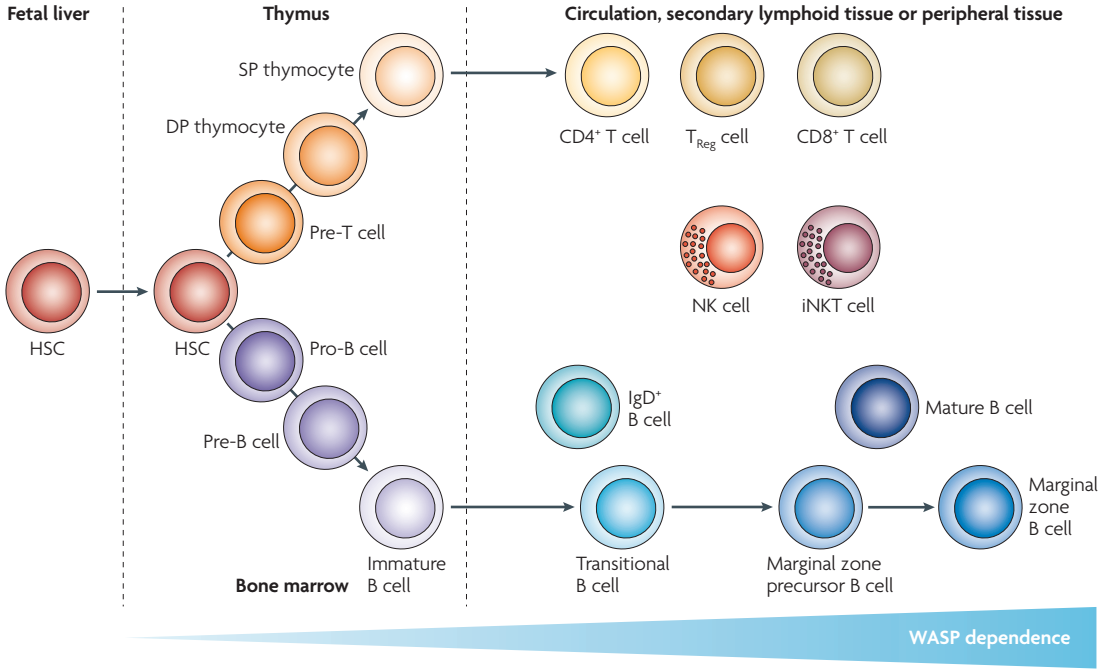

Figure 2 | **Wiskott–Aldrich syndrome protein and lymphocyte homeostasis.** Wiskott–Aldrich syndrome protein (WASP) is expressed by all leukocytes, but dependence on WASP for homeostatic regulation varies with cell lineage and stage of maturity. The mechanisms of WASP-mediated regulation of individual subpopulations remain to be clarified, but WASP may affect cell differentiation, survival and migration. Although the migration of haematopoietic stem cells (HSCs) that lack WASP expression is decreased compared with wild-type cells, there is no widespread defect of haematopoiesis in humans or mice. In WASP-deficient mice, early stages of B cell development proceed normally in the bone marrow but homeostatic defects of peripheral subpopulations are apparent. Homeostasis of mature B cells from patients with Wiskott–Aldrich syndrome is abnormal, but further studies of specific subsets of B cells are needed. Early T cell development also proceeds normally in WASP-deficient mice, but defects in homeostasis are apparent from the double positive (DP) thymocyte stage onwards. Humans with WASP mutations have fewer circulating $CD8^+$ T cells but normal numbers of $CD4^+$ T cells, regulatory T (T_{Reg}) cells and natural killer (NK) cells, although WASP expression confers a survival advantage on these subpopulations after WASP reversion mutations. The effect of WASP mutations on invariant NKT (iNKT) cells has only been studied in mice, in which numbers are reduced. WASP does not seem to be required for the homeostasis of myeloid lineage cells. SP, single positive.

role during haematopoiesis, as early progenitors of several cell lineages develop normally in WASP-deficient mice[50–54]. In humans with WAS there is also no consistent evidence for a generalized defect in haematopoiesis, and the cytopenias affecting several lineages seem to be related to autoimmune destruction or late cell differentiation and survival defects, which is consistent with a role for WASP as a key regulator of haematopoietic homeostasis. The surprising lack of (or subtle) effect on haematopoiesis might result from the partially redundant function between WASP family proteins, as has been described during thymic development[53].

In contrast to the minor role of WASP in early haematopoietic cell development, differentiated or mature haematopoietic cells in WASP-deficient hosts have a significant growth and/or survival disadvantage in humans and mice (FIG. 2). Furthermore, naturally occurring reversion mutations, which restore WASP expression to normal levels, are present in up to 11% of patients with WAS. This remarkably high frequency and the presence of multiple distinct reversion mutants within a single patient[55] support a strong selective advantage for WASP-expressing cells in humans, although this advantage is restricted to mature lymphocyte lineages (T cells, natural killer (NK) cells and mature B cells) but not myeloid cells[36,56–60]. Similarly, in mice, competition assays *in vivo* and *in vitro* indicate that WASP is required for the development and survival of mature lymphoid cell lineages (including single-positive thymocytes and, to a greater extent, peripheral $CD4^+$ and $CD8^+$ T cells, natural regulatory T (T_{Reg}) cells, invariant natural killer T (iNKT) cells and mature B cells) whereas myeloid cell lineages that express normal WASP levels show little or no selective advantage[52,54]. In bone marrow transplantation and more recently in human gene therapy clinical trials similar patterns of selective accumulation of mature $WASP^+$ lymphocytes have been observed. Further work is required to clarify the lineages and stages of development in which WASP function has most importance for homeostatic regulation *in vivo*, and how disturbed WASP function contributes to disease-related abnormalities in these systems.

Patients with XLN have been reported to have partially arrested myeloid cell development with variable myelodysplasia. Although this does not imply a physiological role for WASP in haematopoiesis, it indicates

that dysregulated WASP activity can severely compromise cell growth and development. Interestingly, cells expressing constitutively active WASP mutants showed defective localization of actin polymerization (which occurred throughout the cell instead of at the plasma membrane) that seems to result in mitosis defects that promote apoptosis, aneuploidy and failure to undergo cytokinesis[61]. It is not clear why neutrophil differentiation is particularly affected whereas monocytes and NK cells are affected to a lesser extent in patients with XLN; it is possible that it relates to the higher steady-state level of production and turnover of the neutrophils than of other lineages.

WASP function in adaptive immune cells

Compromised humoral and cellular adaptive immunity is a hallmark of classical WAS. There are often lower total T cell numbers in the peripheral blood of patients with WAS early in life than in normal individuals, possibly owing to abnormal thymopoiesis[62]. Attrition of the immune system is also accelerated, and over time patients with classical WAS can become profoundly lymphopenic owing to decreased thymic output and lowered peripheral T cell survival rates. T cell proliferation in response to TCR stimulation (with CD3-specific antibody) is almost universally defective *in vitro*, and the clonal distribution of TCR β-chain families is usually disturbed[12,63,64]. Defects in immunoglobulin production result in low levels of IgM and high levels of IgA and IgE. Antigen-specific T and B cell responses, particularly to polysaccharides, are also impaired in patients with WAS. The range of infections that occur in these patients are, unsurprisingly, those typically associated with humoral and T cell impairments[12].

T cells. WASP-deficient T cells have marked intrinsic abnormalities, indicating that cytoskeletal regulation is crucial for their normal function. Their morphology is disturbed with fewer microvillus-like surface projections[64], although the functional relevance of this decrease for cell rolling and adhesion has not been reported[65]. However, one study has reported that mouse and human WASP-deficient T cells have normal filopodial protrusions[66], which may relate to variable cell activation states in these studies. More importantly, WASP is normally recruited to the immunological synapse, possibly mediated by binding of CD2-associated protein (CD2AP) to proline-serine-threonine phosphatase-interacting protein 1 (PSTPIP1), which interacts with the SRC homology 3 (SH3) domain of WASP. In the absence of WASP, the localized assembly of filamentous actin and recruitment of other immunological synapse proteins is impaired in response to TCR stimulation[41,67,68]. However, this defect can be overcome depending on the stimulation used for synapse formation, which probably relates to the antigen dose and strength of co-stimulation[69]. TCR-mediated signalling and proliferation are similarly compromised, either as a direct consequence of immune synapse disruption or as a result of the role of WASP as a scaffold protein for assembly of effective signalling complexes. In different experimental systems, WASP-deficient T cells fail to polarize cytokines normally in the cell and show a variable block in their secretion[70–72]. This may result from an indirect effect of WASP on gene transcription, or it could indicate an important post-transcriptional role for WASP in T cell effector function.

Interestingly, mice in which WASP and N-WASP are both deleted exhibit a severe defect in thymopoiesis, indicating that although there may be some redundancy between these proteins, both have important and unique roles in T cell development[53]. Recently, a new human combined immunodeficiency has been described that is associated with mutations in dedicator of cytokinesis 8 (DOCK8)[73,74]. This is notable because DOCK8 is reported to be a GTP-exchange factor (GEF) for CDC42, and deficiency in DOCK8 would, therefore, be predicted to result in some phenotypic features in common with WASP deficiency. In fact, there are striking clinical and immunological similarities, both in humans and in a recently reported mouse model of DOCK8 deficiency[75]. The severity of the phenotype may be greater than that of WASP deficiency alone because the GEF activity of DOCK8 may not be restricted to CDC42, or some CDC42-mediated functions that are WASP independent may be affected. In addition, DOCK8 deficiency may compromise the functions of both N-WASP and WASP.

B cells. For years, little was known about B cell function in patients with WAS despite their clear defects in humoral immunity. Several recent studies have addressed this issue in mouse models and in human cells. It is clear that WASP deficiency perturbs B cell homeostasis with a greater effect on some B cell subpopulations than others, resulting in the selective depletion of circulating mature B cells, splenic marginal zone precursors and marginal zone B cells in particular[52,54]. Similarly, in humans, a WASP reversion mutation in common lymphoid progenitor cells conferred a selective advantage on mature and memory B cells but not their immature counterparts[52].

Impaired B cell homeostasis results from an intrinsic B cell defect that persists after transplantation of WASP-deficient bone marrow to wild-type recipient mice; the extent of the defect, which differs between B cell populations, directly correlates with the level of WASP expression[52]. The reason for deficiency of marginal zone B cell populations does not seem to relate to inadequate survival signals propagated from the B cell receptor (BCR) or B cell-activating factor (BAFF) receptor, but may result from abnormal homing to or retention of the cells in the marginal zone. Two lines of evidence support this hypothesis: first, WASP-deficient marginal zone B cells fail to migrate in response to sphingosine 1 phosphate, which is implicated in marginal zone homing[54], and second, WASP is required for normal integrin clustering and function downstream of BCR engagement, which is thought to mediate the retention of B cells in the marginal zone[52]. As an additional consequence of impaired BCR and integrin signalling, the immunological synapse is poorly formed by WASP-deficient B cells, which may compromise

Aneuploidy
The occurrence of one or more extra or missing chromosomes, which leads to an unbalanced chromosome complement.

Immune synapse
A discrete contact site classically formed at the point of contact between an antigen-presenting cell (APC) and a T cell. Similar synapses have been described in other immune cells such as natural killer or cytotoxic T cells, where the synapse is formed with a target cell. It is important in establishing cell adhesion and polarity, is influenced by the cytoskeleton and transduces highly controlled secretory signals, thereby allowing the directed release of cytokines or lytic granules towards the APC or target cell.

Marginal zone
A region at the border of the white pulp of the spleen.

Marginal zone B cell
A mature B cell that is enriched in the marginal zone of the spleen. They recognize antigen through semi-invariant receptors, which stimulates their rapid differentiation into antibody-secreting cells. They are thought to be important for host defence against circulating blood-borne pathogens.

Integrin
A member of a large family of transmembrane proteins that traverse the plasma membrane as heterodimers of α- and β-subunits. They have an important role in mediating the interaction of cells with extracellular matrix components, such as fibronectin, and in mediating intracellular cytoskeleton arrangement.

Invariant NKT (iNKT) cell
A lymphocyte that expresses a particular variable gene segment, Vα14 (in mice) and Vα24 (in humans), precisely rearranged to a particular Jα (joining) gene segment to yield T cell receptor α-chains with an invariant sequence. Typically, these cells co-express cell surface markers that are encoded by the natural killer (NK) locus, and they are activated by recognition of CD1d molecules presenting glycolipid antigens.

cognate B cell activation; however, this is not known to directly compromise class switching[76]. WASP-deficient mice also fail to generate normal humoral responses *in vivo* as a consequence of intrinsic B cell defects, which are not rescued by provision of help from wild-type T cells[76]. Therefore, several questions remain with regard to the importance of WASP during B cell homeostasis and the generation of physiological humoral responses. However, it seems increasingly likely that WASP deficiency in B cells is a prime contributor to both immunodeficiency and autoimmunity (particularly haematological) in patients with WAS.

Invariant NKT cells. The invariant NKT (iNKT) cell population has attracted recent interest in the context of WAS because of the suggested role of these cells in the clearance of microorganisms, tumour surveillance and autoimmunity[77,78]. This subset bridges innate and adaptive immune compartments, expressing both NK cell receptors and a semi-invariant αβ TCR. iNKT cells are activated by glycolipid peptide antigens presented by CD1d molecules; in response, iNKT cells secrete cytokines, including interleukin-4 (IL-4) and interferon-γ (IFNγ), and mediate antimicrobial and antitumour immunity.

It is now clear that WASP has an important, non-redundant role in iNKT cell homeostasis and function[79,80]. Like other T cell lineages, WASP seems to be more important for iNKT cell peripheral homeostasis than thymic production, as decreased numbers of iNKT cells have been observed in the spleen and liver, but not the thymus, of WASP-deficient mice[79]. iNKT cell maturation in the thymus is abnormal, showing decreased progression to mature phenotypes. Competitive cell transfer assays indicate that WASP confers a selective advantage to peripheral iNKT cells, which is consistent with the lower numbers of circulating iNKT cells in humans with WAS or XLT[80]. Homeostatic defects do not seem to relate to impaired proliferative capacity of peripheral iNKT cells but may, in part, be explained by lower levels of CD11a cell surface expression, which is normally required for iNKT cell tissue retention[79]. In addition, mouse WASP-deficient iNKT cells respond poorly to glycolipid peptide antigens, probably as a result of defective TCR signalling, with a consequent decrease in release of IL-4 and IFNγ. Interestingly, patients with X-linked lymphoproliferative disease (XLP), which is caused by mutations in SH2 domain protein 1A (*SH2D1A*), are particularly vulnerable to Epstein–Barr virus infection and malignancy, which may in part also relate to a deficiency of iNKT cells.

WASP function in innate immune cells

Innate immune cell types have been studied in the context of WASP deficiency, and unsurprisingly various functional defects have been described (FIG. 3). Human WASP-deficient myeloid lineage cells exhibit impaired phagocytosis[81,82], which is consistent with an important role for WASP in the formation of the actin-rich

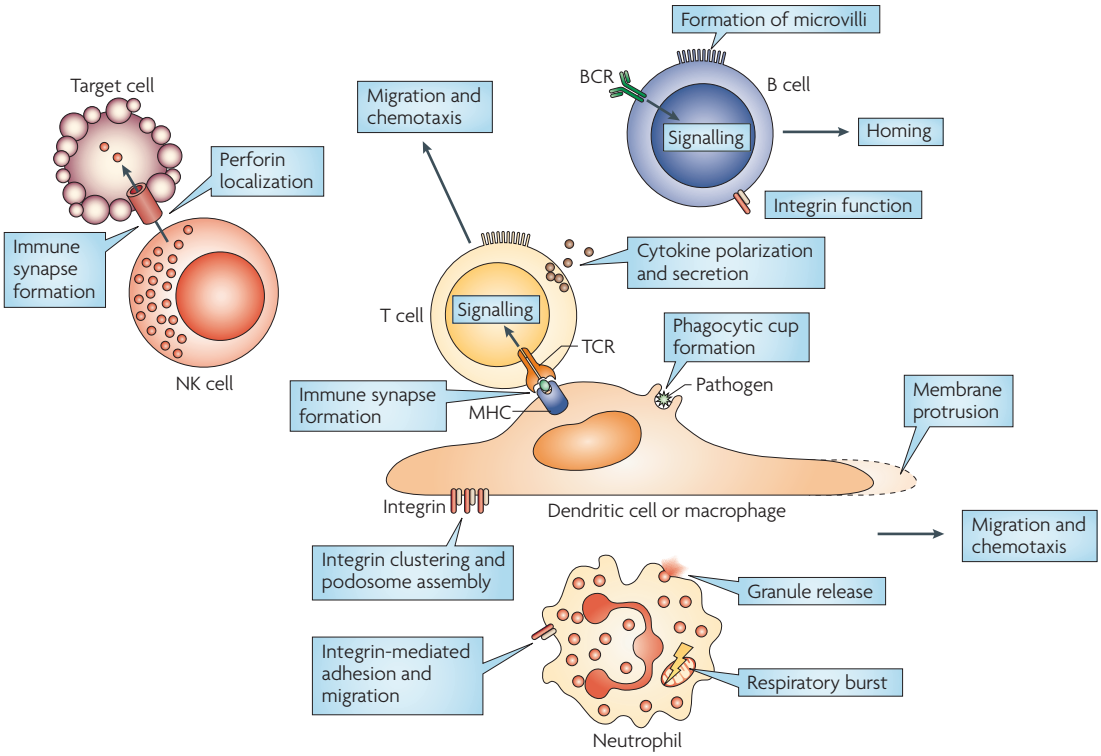

Figure 3 | **Wiskott–Aldrich syndrome protein function in immune cells.** Wiskott–Aldrich syndrome protein (WASP) is required for many functions in lymphoid and myeloid immune cells. Many of these, such as phagocytosis and podosome assembly, relate to the role of WASP in regulating the polymerization of new branched actin filaments. Other functions of WASP depend on its activity as a scaffold protein for assembly of effective signalling complexes downstream of antigen receptor or integrin engagement. BCR, B cell receptor; NK, natural killer; TCR, T cell receptor.

phagocytic cup[83]. In addition, monocytes, macrophages, DCs and osteoclasts from WASP-deficient humans and mice show almost completely abrogated assembly of podosomes[84,85]. Podosome assembly is also impaired to a lesser extent in cells derived from patients that express low levels of functional WASP[44], although the relevance of this level of expression for cell function has not been tested. Similarly, the formation of filopodia and sustained lamellipodia at the migrating edge of macrophages and DCs is defective, and net migration, including chemotaxis to specific chemoattractants, is impaired in these cell types[84,86,87]. Motility defects have been confirmed *in vivo*, as DC homing from peripheral tissues to the T cell zones of secondary lymphoid tissues is kinetically and quantitatively impaired in WASP-deficient mice[88,89]. Indeed, impaired migration and homing of several cell lineages may be a significant pathogenic mechanism in WAS. DC priming of T cells is also impeded by defects in cell-to-cell interactions, which are likely to be exacerbated by abnormal DC-mediated induction of immune synapse formation in T cells[89,90]. Mutations that result in constitutively active WASP also impair myeloid cell function, as podosome assembly and cell polarity are dysregulated, and phagocytosis of particulate antigen is abrogated in affected human macrophages[16].

In addition to actin remodelling, efficient cell migration generally requires interactions involving integrins. These are linked processes, as actin filaments and integrin molecules are physically connected through adaptor proteins such as vinculin and talin[91]. Although it is clear that WASP deficiency results in the disruption of actin assembly, integrin function is likely to be indirectly impaired. In support of this, the β2 family of integrins, which are found in myeloid cell podosomes, fail to cluster in the absence of WASP[92]. Similarly, β2 integrin clustering is defective in WASP-deficient human and mouse neutrophils, resulting in impaired integrin-dependent functions that range from adhesion and migration to degranulation and activation of the respiratory burst[93]. Migratory defects affect both immune cell homing to sites of inflammation and initiation of adaptive immune responses in secondary lymphoid tissues. In addition, it is possible that delayed cell migration kinetics could lead to ectopic maturation of innate immune cells, such as DCs, causing tissue inflammation through cytokine or chemokine release and recruitment of other leukocytes, for example in WAS-associated eczematous skin disease.

As well as providing immune surveillance against pathogens, innate immune cells have an important role in antitumour immunity. The high rate of haematopoietic malignancy associated with WAS relates, in part, to inadequate immune responsiveness to oncogenic viruses such as Epstein–Barr virus, and possibly also to defective immune surveillance. NK cells in particular are required to kill both virally infected and tumorous cells. In the absence of WASP, NK cell function is abnormal[94,95]: cytotoxic activity is defective as a result of impaired immune synapse formation between an NK cell and its target cell, impaired perforin localization at the immune synapse and defective signalling downstream of CD16 engagement. *In vivo* NK cell activation may be further compromised in WAS by poor intercellular interactions, particularly with DCs, which are required for optimal NK cell activation through a DC–NK cell immune synapse[96]. There is also some evidence that WASP may be important for NK cell signalling, particularly for activation of nuclear factor of activated T cells 2 (NFAT2) and nuclear factor-κB (NF-κB) after ligation of the activating receptor NKp46 (but independently of actin polymerization and cytoskeletal rearrangement)[8]. In contrast with other key leukocyte populations, which are present in normal or decreased numbers in patients with WAS and XLT, peripheral blood NK cell percentages are often increased, which may in part compensate for impaired NK cell function[94,95]. Examining the function of WASP-deficient NK cells *in vivo* and specifically in tumour control are important areas for future work. WASP, therefore, has many roles during normal adaptive and innate immune cell function, which underlie the broad immunodeficiencies that are characteristic of patients with classical WAS, and may provide important clues for determining the pathogenesis of WASP-associated autoimmunity.

WAS and autoimmune processes

Autoimmunity is a common and clinically important complication of WAS that is often present in early childhood and can occur in both severe and attenuated cases of the disease[43]. Antibody-mediated cytopenias are the most frequent manifestation of autoimmune reactions but various vascular and organ-based autoimmune processes have also been reported[12,13] (TABLE 3). Mice with a deficiency of WASP or an inability to activate WASP through phosphorylation have increased titres of antinuclear antibodies but do not develop overt autoimmunity. Although considerable research is still required to understand the aetiology and cellular mediators of WAS-associated autoimmunity, several recent reports have helped to clarify the issue. It has been shown by several groups that T_{Reg} cells fail to function normally in WASP-deficient humans and mice[97–100]. WASP-deficient T_{Reg} cells fail to proliferate normally in response to TCR stimulation and their suppressive activity is impaired *in vitro*. This defect in T_{Reg} cell function was shown to be functionally important *in vivo*, as WASP T_{Reg} cells fail to control autoimmunity in several mouse models, including multiorgan autoimmune inflammation, radiation-induced autoimmune colitis and a T cell transfer model of colitis[97,98]. WASP-deficient T_{Reg} cells express decreased levels of activation markers, including CD25 (the IL-2 receptor α-chain), and their suppressive activity can be partially rescued *in vitro* by administration of exogenous IL-2. This is relevant because WASP-deficient effector T cells are poor secretors of IL-2, which might augment intrinsic T_{Reg} cell dysfunction. However, the provision of normal levels of IL-2 by wild-type effector T cells was not sufficient to restore disease control *in vivo*[98]. Interestingly, WASP-deficient T_{Reg} cells also produce less of the anti-inflammatory cytokine IL-10 and lack tissue homing markers such as CD103 (also known as

Lamellipodia
Thin sheet-like processes, which extend at the leading edge of moving cells in an actin-dependent manner, promoted by the Rho family GTPase RAC1.

Respiratory burst
A large increase in oxygen consumption and reactive oxygen species generation that accompanies the exposure of neutrophils to microorganisms and/or inflammatory mediators.

Eczematous skin disease
A clinical process that is associated with severe pathological changes to the skin, which is characterized by redness, oozing, crusting and loss of pigmentation. Histologically, it is characterized by epidermal changes of intracellular oedema, spongiosis or vesiculation.

Antinuclear antibodies
Heterogeneous autoantibodies against one or more antigens present in the nucleus, including chromatin, nucleosomes and ribonuclear proteins. Antinuclear antibodies are found in association with many different autoimmune diseases.

Table 3 | **WASP-related autoimmunity**

Disease feature	Humans	Mice	Refs
Clinical occurrence			
Antibody-mediated cytopenias	Present in over a third of WAS patients	Present in WASP-deficient mice (mild)	12,13,14,102
Vasculitis, especially skin and cerebral	Present in up to a third of classical WAS patients	Not determined	12,13,14
Arthritis	Present in up to a third of WAS patients	Not determined	12,13,14
Colitis	Present in some WAS patients (<10%)	Spontaneous in WAS-deficient SV129 strain and induced in mice with WAS-deficient T_{Reg} cells	12,13,14,97,98
Nephritis	Present in some WAS patients (<5%)	Not determined	12,13,14
DNA-specific antibodies	Detected in some WAS patients (prevalence not known)*	Detected in WASP-deficient mice	32,98
Mechanistic evidence			
Reduced T_{Reg} cell numbers	No	Yes	97,98,99,100
T_{Reg} cell homeostasis defect	Yes	Yes	98,99
T_{Reg} cell functional defect	*In vitro*	*In vitro* and *in vivo*	97,98,99,100
T_{Reg} cell homing defect	Not known	Probable	97,98
B cell dysfunction	Autoantibodies, particularly blood cell-specific	DNA-specific autoantibodies	12,13,14,32,98
Reduced apoptotic cell clearance	Decreased IgG-mediated macrophage phagocytosis *in vitro*	Decreased phagocytosis of apoptotic cells *in vitro*	81,82
Defective DC maintenance of peripheral tolerance	Impaired DC antigen uptake, antigen presentation and migration *in vitro*	Impaired DC antigen uptake, antigen presentation, T cell priming and migration *in vitro* and *in vivo*	84,88,89,90
Invariant NKT cells	Decreased numbers, function not yet reported	Decreased numbers and function	79,80

DC, dendritic cell; NKT cell, natural killer T cell; T_{Reg} cell, regulatory T cell; WAS, Wiskott–Aldrich syndrome; WASP, WAS protein. *A.J.T. and S.O.B. unpublished observations

αE integrin), P-selectin, E-selectin and CC-chemokine receptor 4, which may in part explain why WASP-deficient T_{Reg} cells are almost entirely absent in inflamed peripheral tissues and found in decreased numbers in secondary lymphoid tissues[97,98]. Although WASP does not seem to be required for the thymic generation of natural T_{Reg} cells, it has a crucial role in their peripheral homeostasis. Mouse WASP-deficient T_{Reg} cells have decreased survival and are outcompeted by $WASP^+$ counterparts in competitive-repopulation assays. The role of T_{Reg} cells in the autoimmunity of human WAS is less clear. Although $WASP^+$ T_{Reg} cells have a selective advantage *in vivo* after spontaneous reversion mutation, peripheral T_{Reg} cell numbers are normal[98–100], suggesting that any contribution to human disease is likely to result from intrinsic cellular dysfunction.

Given the broad effects of WASP deficiency on immune cells, other alterations of immune function probably also contribute to the development of autoimmunity, and might vary depending on the type of autoimmune process and the target tissue. These include defective phagocytosis and clearing of apoptotic cells and the production of autoantibodies owing to intrinsic B cell dysfunction[12–14,82,98]. Curiously, autoimmune disease can persist, or arise *de novo*, after allogeneic bone marrow transplantation and this might indicate partial reconstitution in which some recipient (that is, WASP-deficient) cells remain[101]. More detailed analysis of this patient group may clarify the relative importance of myeloid and lymphoid lineages and of T_{Reg} cells in the aetiology of WAS-associated autoimmunity.

Concluding remarks

WASP deficiency or dysregulation in humans and corresponding mouse models have greatly enhanced our understanding of the role of the actin cytoskeleton in haematopoietic and immune cell function. It is clear that WASP is required not only for specific cellular activities but also for overall immune cell homeostasis. At present, the cell lineages in which WASP has the most important biological function in terms of physiological immunity and protection against immunological disease are not clearly defined. The underlying mechanisms of several key features of WASP-associated disease remain to be determined, most importantly the development of autoimmunity. For curative treatment of severely affected patients with WAS, haematopoietic stem cell transplantation remains a highly effective option, and gene therapy (through addition of a functional *WAS* transgene) has proved encouraging in early clinical trials. In the future, targeted therapies that enhance the stability of mutant WASP may also emerge from a clearer understanding of the biochemical processes involved in the disease and will offer a rational treatment option to a wider range of patients in whom disease may not necessarily be immediately life-threatening but nevertheless is associated with considerable morbidity. Finally, given the multiple immunological tasks coordinated by WASP through regulation of the actin cytoskeleton, pharmacological intervention in WASP-mediated signalling pathways could eventually evolve as a rational strategy for the treatment of other diseases, including autoimmunity and cancer.

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