Current reviews of allergy and clinical immunology
(Supported by an unrestricted educational grant from Genentech, Inc. and Novartis Pharmaceuticals Corporation)

Series editor: Harold S. Nelson, MD

The Wiskott-Aldrich syndrome

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The Wiskott-Aldrich syndrome (WAS) is a rare X-linked disorder with variable clinical phenotypes that correlate with the type of mutations in the *WAS protein* (*WASP*) gene. WASP, a key regulator of actin polymerization in hematopoietic cells, has 5 well-defined domains that are involved in signaling, cell locomotion, and immune synapse formation. WASP facilitates the nuclear translocation of nuclear factor κB and was shown to play an important role in lymphoid development and in the maturation and function of myeloid monocytic cells. Mutations of *WASP* are located throughout the gene and either inhibit or dysregulate normal WASP function. Analysis of a large patient population demonstrates a phenotype-genotype correlation: classic WAS occurs when WASP is absent, X-linked thrombocytopenia when mutated WASP is expressed, and X-linked neutropenia when missense mutations occur in the Cdc42-binding site. The progress made in dissecting the function of WASP has provided new diagnostic possibilities and has propelled our therapeutic strategies from conservative symptomatic treatment to curative hematopoietic stem cell transplantation and toward gene therapy. (J Allergy Clin Immunol 2006;117:725-38.)

Key words: *Wiskott-Aldrich syndrome, X-linked thrombocytopenia, X-linked neutropenia, function of WASP, immune defects, scoring system, mutation analysis, mutational hotspots, genotype-phenotype correlation, hematopoietic stem cell transplantation, gene therapy*

Abbreviations used

Arp2/3:	Actin-related protein 2/3
CRIB:	Cdc42-and Rac-interactive binding
DC:	Dendritic cell
GTP:	Guanosine triphosphate
HSC:	Hematopoietic stem cell
IS:	Immune synapse
ITP:	Idiopathic thrombocytopenia
IVIG:	Intravenous immunoglobulin
NK:	Natural killer
N-WASP:	Neuronal Wiskott-Aldrich syndrome protein
PIP_2:	Phosphatidylinositol (4,5) biphosphate
WAS:	Wiskott-Aldrich syndrome
Toca-1:	Transducer of Cdc42-dependent actin assembly
WASP:	Wiskott-Aldrich syndrome protein
WIP:	Wiskott-Aldrich syndrome protein–interacting protein
XLT:	X-linked thrombocytopenia

The Wiskott-Aldrich syndrome (WAS) is an X-linked immunodeficiency disease with a characteristic clinical phenotype that includes thrombocytopenia with small platelets, eczema, recurrent infections caused by immunodeficiency, and an increased incidence of autoimmune manifestations and malignancies.[1-3] The identification of the molecular defect in 1994[4] has broadened the clinical spectrum of the syndrome to include chronic or intermittent X-linked thrombocytopenia (XLT), a relatively mild form of WAS,[5,6] and X-lined neutropenia caused by an arrest of myelopoiesis.[7] In this review we will describe the clinical presentations associated with mutations of the *WAS protein* (*WASP*) gene, the laboratory abnormalities, the known functions of WASP, and evidence for a strong genotype-phenotype correlation.

CLINICAL AND PATHOLOGIC MANIFESTATIONS

The incidence of the classic WAS phenotype has been estimated to be between 1 and 10 in 1 million individuals.[8,9] With broader awareness of the different clinical phenotypes, along with the availability of reliable diagnostic tools, the incidence might be much higher. Clinical manifestations suggesting WAS-XLT are often present at birth and consist of petechiae, bruising, and bloody diarrhea.[1] Excessive hemorrhage after circumcision is an early diagnostic sign. Eczema is a frequent

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Disclosure of potential conflict of interest: The authors have declared they have no conflict of interest.

Received for publication January 11, 2006; revised February 3, 2006; accepted for publication February 7, 2006.

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0091-6749/$32.00

doi:10.1016/j.jaci.2006.02.005

TABLE I. Clinical phenotypes associated with mutations of the *WASP* gene

	WAS	XLT	IXLT	XLN
Phenotype				
Thrombocytopenia	+	+	(+)	−
Small platelets	+	+	+	−
Eczema	+/++/+++	−/+	−	−
Immune deficiency	+/++	−/(+)	−	−
Infections	+/++	−/(+)	−	+*
Autoimmunity and/or malignancies	Frequent	Possible	−	−
Congenital neutropenia	−	−	−	+
Disease scores	3, 4, or 5	1, 2, or (5)†	<1	0
WASP mutations	Nonsense; frame shift caused by deletions, insertions; splicing defects	Missense (exons 1-3); inframe deletions or insertions	Missense	Missense in Cdc42-binding site
WASP expression	Absent or truncated	Present, reduced quantity	Present, normal quantitiy	Present
Treatment				
IVIG	Yes	No (with exceptions)	No	No
HSCT	Yes at an early age	Might be considered if there is a sibling donor	No	?
Splenectomy	No	Might be considered‡	No	No

IXLT, Intermittent XLT; *HSCT*, hematopoietic stem cell transplantation; *XLN*, X-linked neutropenia.
*Infections typical for neutropenia.
†Patients with XLT with a score of 1 or 2 might progress to a score of 5. Incidence of autoimmunity and malignancies are less in XLT than in WAS.
‡Splenectomy results in increased platelet numbers and reduced bleeding but causes a marked increase in sepsis, requiring continuous antibiotic prophylaxis.

manifestation of classic WAS during infancy and childhood. The most consistent finding at diagnosis of both classic WAS and XLT is thrombocytopenia and small platelets. Infections, including otitis media with drainage of mucoid purulent material, pneumonia most often caused by bacteria and rarely by *Pneumocystis carinii*, and skin infections, are frequent complaints during the first 6 months of life. Patients with XLT have less problems with eczema and infections and often receive misdiagnoses of idiopathic thrombocytopenia (ITP), considerably increasing the actual age of diagnosis. X-linked neutropenia caused by missense mutations in the Cdc42-binding domain does not resemble classic WAS or XLT. To delineate these strikingly different clinical phenotypes (Table I), we have generated a simple scoring system.[10]

Defects of the immune system

The severity of the immune deficiency can vary from family to family, depending largely on the mutation and its effect on protein expression.[11,12] Both T- and B-lymphocyte functions are affected. During infancy, the number of circulating lymphocytes might be normal or moderately decreased.[13,14] By 6 years of age, lymphopenia caused by reduced T-lymphocyte numbers is a common finding in patients with classic WAS and might be due to accelerated cell death observed in patients with classic WAS, although not in those with XLT.[15,16]

The number of B cells might be normal or moderately decreased.[17] Serum IgG levels are generally within normal range, IgM levels are moderately decreased but can be normal or increased, and IgA and IgE levels are frequently increased. Antibody responses are adequate to some antigens and insufficient to others.[3,13,18] Consistent findings are low isohemagglutinin titers, markedly decreased responses to polysaccharide antigens, and low antibody titers associated with defective class-switch recombination after immunization with the T cell–dependent neoantigen bacteriophage ΦX174.[13] In a multicenter retrospective review, antibody responses to a variety of protein antigens, including diphtheria and tetanus toxoid, and conjugated *Haemophilus influenzae* B vaccine were reported to be abnormal in the majority of patients with WAS; in contrast, antibody responses to live virus vaccines were mostly normal.[3] As expected, patients with XLT were found to have a more robust response to bacteriophage ΦX174, with amplification and isotope switching that is often comparable with that seen in healthy control subjects.[19] Abnormal T-cell function is suggested by diminished but not absent lymphocyte responses to mitogens,[18] decreased proliferative responses to allogeneic cells,[13] and immobilized anti-CD3 mAb.[20] Skin test results for delayed-type hypersensitivity were abnormal in 90% of patients studied.[3] An increased incidence of *Pneumocystis carinii* pneumonia also points to a significant T-cell defect in classic WAS. Recent studies suggest that B-cell function is equally affected. EBV-transformed B lymphoblasts derived from patients with WAS have reduced levels of F-actin and defective actin polymerization,[21] and B cells from patients lacking WASP have defective cell motility (as discussed later).

WASP is also involved in innate immunity. In normal natural killer (NK) cells, WASP can easily be detected in the immunologic synapse, together with F-actin. In contrast, NK cells derived from patients with WAS lack WASP and show a markedly reduced accumulation of

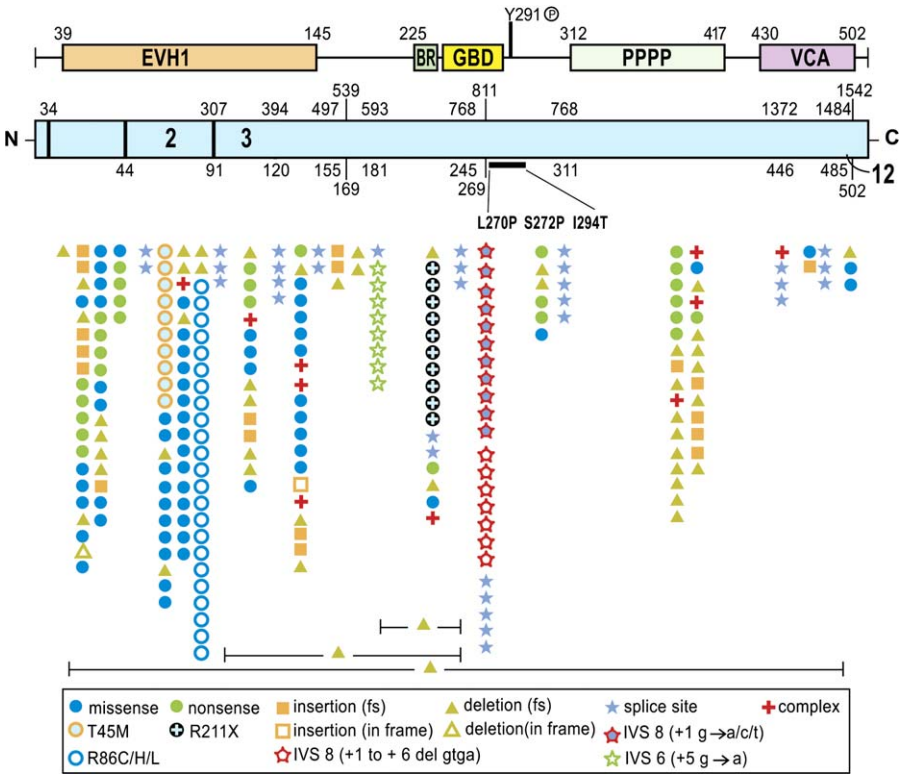

FIG 1. Schematic representation of the *WASP* gene, which encodes a protein with 12 exons and 5 major functional domains. The mutations identified in 270 unrelated WAS families are visualized according to their location in the exons and the exon-intron junctions. Each *symbol* represents a single family with WASP mutation. Missense mutations are located mostly in exons 1 through 4. Deletions, insertions, and nonsense mutations are distributed throughout the *WASP* gene. Splice-site mutations are found predominantly in introns 6, 8, 9, and 10. The symbols for specific WASP mutations shown in the *box* represent the 6 hot spots described in the text. *EVH1*, Ena/VASP homology 1 domain; *BR*, basic region; *GBD*, GTPase binding domain; *PPPP*, proline-rich region; *VCA*, verpolin cofilin homology domains/acidic region.

F-actin in the immunologic synapse. As a direct result, patients with WAS have defective cytolytic NK cell function.[22,23] This inhibition of NK cell–mediated cytotoxicity is associated with a reduced ability of these cells to form conjugates with susceptible target cells and to accumulate F-actin at the site of contact. The involvement of the actin cytoskeleton in cell migration and cell trafficking of myeloid cells, macrophages, dendritic cells (DCs), and Langerhans cells makes these cells particularly vulnerable to mutations of the *WASP* gene. Similarly, phagocytosis of particulate antigens and apoptotic cells is highly dependent on the formation of a specialized phagocytic actin cup, which is unsurprisingly compromised in cells deficient in WASP.[24,25] A striking deficiency observed in patients with WAS is the complete failure to assemble podosomes in monocytes, macrophages, and DCs, resulting in a severe defect of adhesion and motility.[26] Transfection of full-length *WASP* cDNA into WASP-deficient macrophages restores chemotaxis in response to colony-stimulating factor 1.[27]

Platelet abnormalities

Thrombocytopenia associated with small platelet volume is a consistent finding in patients with mutations of the *WASP* gene; exceptions are those with missense mutations within the Cdc42-binding site (Fig 1).[7,28,29] Platelet counts can vary from patient to patient within a family, from family to family, or within individual patients, with the numbers of platelets being as low as 5000/mm^3 or as high as 50,000/mm^3. Intermittent thrombocytopenia associated with unique amino acid substitutions in the *WASP* gene have been described in 2 families.[6] In most patients with WAS-XLT, the mean platelet volume is half (mean platelet volume = 3.8-5.0 fl) that of healthy control subjects.[13] After splenectomy, platelet counts and platelet volume increase but are still less than those of healthy control subjects.[30,31] This partial recovery of platelet counts after splenectomy suggests that thrombocytopenia in patients with WAS-XLT is at least in part due to platelet destruction in the spleen or other reticular endothelial organs.[32,33] Increased expression of phosphatidylserine on the surface of circulating platelets from patients with WAS has been interpreted as an indication for increased phagocytosis and destruction of phosphatidylserine-positive platelets in the spleens of patients with WAS-XLT.[34] An alternative explanation is a decrease in platelet production. This possibility is suggested by the observations that autologous platelet survival in patients with WAS-XLT is only

moderately reduced (5 ± 1.3 days [SD]).[13] A persistent finding has been a decrease in platelet turnover, which was found to be approximately 30% of the value observed in healthy subjects, indicating a significant platelet production defect. Because the marrow megakaryocyte mass is normal or increased in patients with WAS-XLT,[13,31] it has been suggested that ineffective thrombocytopoiesis is at least in part responsible for the low platelet count.

As a consequence of the platelet defect, the majority (84%) of patients with WAS have a history of bleeding, including epistaxis, oral bleeding, hematemisis and melena, and petechiae, purpura, or both. Life-threatening bleeding, including oral, gastrointestinal, and intracranial hemorrhage, occurred in 30% of patients with WAS, with intracranial hemorrhage observed in only 2%.[3]

Eczema

Eczema is one of the characteristic findings that originally differentiated WAS from ITP.[1] The typical skin lesions resemble acute or chronic eczema in appearance and distribution. A history of eczema, mild or severe, transient or consistent, was reported by 81% of a large cohort of patients with WAS.[3] In its most severe form, eczema is resistant to therapy and persists into adulthood. Molluscum contagiosum, herpes simplex, or bacterial infections can develop in areas of the skin affected with eczema. Patients with XLT have either mild and transient eczema or none at all.[11,12] It has been hypothesized that defective chemotaxis of DCs and Langerhans cells plays a role in the local generation of antigen-specific (most likely bacterial) T cells that are responsible for the development of eczema.[35] The eczema tends to be worse in families with a history of atopic diathesis, a finding suggesting that genes responsible for allergies might have a modifying affect.

Autoimmune manifestations

Autoimmune diseases are frequent in patients with WAS, being present in 40% of a large cohort of patients.[3] Most commonly reported is autoimmune hemolytic anemia, followed by vasculitis, renal disease, Henoch-Schönlein–like purpura, and inflammatory bowel disease. Other less frequent autoimmune diseases include neutropenia, dermatomyositis, recurrent angioedema, uveitis, and cerebral vasculitis. The incidence of autoimmune disease in patients with XLT is generally less frequent than in patients with classic WAS. However, a recent report form Japan suggests that autoimmune diseases are equally frequent in Japanese patients with a low clinical score, representing XLT, as in those with a high clinical score, representing WAS. IgA nephropathy, often causing chronic renal failure and requiring dialysis or renal transplantation, was a frequent complication in Japanese patients with the XLT phenotype.[11] A retrospective review of risk factors, clinical features, and outcomes of autoimmune complications in patients with mutations of WASP from a single center further underlines the importance of this problem.[36] Of 55 patients with WAS, 40 had at least one autoimmune or inflammatory complication. Autoimmune hemolytic anemia was detected in 20 patients with onset before the age of 5 years; after 5 years, hemolytic anemia was present in 29%, neutropenia in 25%, vasculitis (including cerebral involvement) in 29%, inflammatory bowl disease in 9%, and renal disease in 3%. A high serum IgM concentration was a significant risk factor for the development of autoimmune disease or early death.

Malignancies

Malignant tumors can occur during childhood but are more frequent in adolescents and young adults with the classic WAS phenotype. In the North American cohort studied by Sullivan et al,[3] malignancies were present in 13%, with an average age at onset of 9.5 years. Considering the increasing life expectancy, it is reasonable to assume that the incidence of malignancies will further increase as patients with WAS get older. The most frequent malignancy reported is B-cell lymphoma, often EBV positive, which suggests a direct relationship with a defective immune system. WAS-associated malignancies have a poor prognosis, as illustrated by the fact that only 1 of the 21 patients who had a malignancy was alive more than 2 years after establishing the diagnosis.[3] The incidence of malignancies in patients with the XLT phenotype is unknown but is less than in classic WAS.

Laboratory findings

All hematopoietic stem cell (HSC)–derived lineages are functionally abnormal in patients with WAS, including lymphocytes, platelets, neutrophils, DCs, and Langerhans cells. This is not surprising because WASP is expressed in $CD34^+$ stem cells and their progeny.[37] Although thrombocytopenia is a consistent finding in patients with WAS-XLT, the differentiation from ITP is often difficult because immunodeficiency and eczema might be absent at the initial evaluation. Laboratory evidence to establish the diagnosis, especially in patients with XLT, is therefore of prime necessity. The presence of small platelets is considered pathognomonic of WAS-XLT. Abnormal T- and B-lymphocyte functions are a consistent finding in classic WAS. The absence or reduced quantity of WASP in lymphocytes is the best confirmatory test short of mutation analysis.[38] Lymphopenia is a consistent finding in classic WAS and might be present at an early age.[14]

Iron deficiency anemia is common in infants and children with WAS-XLT because of the constant loss of red blood cells. This can be partially corrected with increased dietary intake of iron. Chronic infection might further impair the production of red blood cells, and autoimmune Coombs-positive hemolytic anemia is a frequent complication and needs to be recognized early for proper therapy.

Patients with WAS respond to infections or to autoimmune disease with increased sedimentation rates and increased C-reactive protein levels. Vasculitis is a common complication affecting small and large arteries; deposits of IgA-containing immune complexes have been found in purpuric skin lesions and in the blood vessel walls of the small bowel of patients with WAS with a Henoch-Schönlein purpura–like vasculitis.

Histopathology

A gradual loss of cellular elements occurs in the thymus and lymphoid organs.[18] Lymph nodes and spleens from patients with WAS consistently show depletion of small lymphocytes from T-cell areas, the prominence of the reticulum cell stroma, and the presence of atypical plasma cells often associated with plasmacytosis and extramedullary hematopoesis.[39] Progressive depletion of the marginal zone involving B cells was also observed in a study of spleens obtained from patients with WAS undergoing splenectomy.[40] These histologic abnormalities might explain the defective *in vivo* antibody responses to polysaccharides and selected protein antigens.

MOLECULAR BASIS

By studying DNA from large WAS families with multiple affected members, the *WASP* gene was mapped to the region Xp11.22-Xp11.3.[41] On the basis of these mapping data, Derry et al[4] isolated the *WASP* gene by means of positional cloning and demonstrated mutations in lymphoblastoid cell lines derived from patients with WAS-XLT. The *WASP* gene (Fig 1) encodes a 502-amino-acid intracellular protein (WASP) expressed exclusively in hematopoietic cells.

WASP is an activator of actin polymerization

WASP is a member of a distinct family of proteins that participate in the transduction of signals from the cell surface to the actin cytoskeleton. These proteins are organized into evolutionarily conserved modular domains that regulate activity and subcellular localization.[42,43] Other members of this family include the more widely expressed neuronal WASP (N-WASP) and WASP family verprolin–homologous protein proteins (also known as suppressor of G protein–coupled cyclic AMP receptor), of which 3 mammalian isoforms have been identified. They are all characterized by a C-terminal tripartite domain containing a common actin monomer-binding motif, WASP-homology domain 2 (WH2) or verprolin homology domain (V), and a central acidic region that is capable of activating the actin-related protein (Arp) 2/3 complex, a potent nucleator of actin polymerization.[44] Because the structure and biochemical interaction between WASP and N-WASP are very similar, functional data obtained from experiments studying either of these 2 proteins are considered relevant for both proteins, although their tissue distribution is different.

Regulation of WASP activity through interaction and posttranslational modification

A proline-rich region in exon 10 shared by WASP and WASP family verprolin–homologous protein proteins is required for optimal actin polymerization activity and has been shown to be indispensable for effective recruitment of WASP to the immunologic synapse in T cells.[45-48] This sequence provides binding sites for multiple SH3 domain–containing proteins, including the adapter proteins Nck[49,50] and Grb2,[51] and several protein tyrosine kinases, including Fyn[52] and Btk,[53,54] which might directly activate WASP by means of tyrosine phosphorylation. Conversely, binding of the adapter protein proline-serine-threonine phosphatase–interacting protein 1 to the same region is associated with inhibition of actin polymerization.[55,56] Several proteins involved in endocytotic processes, including syndapin and intersectin, also contain SH3 domains that bind WASP–N-WASP, which might therefore participate in localization to sites of membrane remodeling.[57-62] Other interactions with this region include binding to the actin-binding protein profilin; vasodilator-stimulated phosphoprotein, which is known to promote the formation of filopodia (finger-like protrusive actin structures); and Cdc42-interacting protein 4 (CIP4), which might mediate interaction of WASP with the microtubule cytoskeleton.[46,63-65]

One of the key, although probably not indispensable, mechanisms of WASP activation is specific interaction with guanosine triphosphate (GTP)–loaded Cdc42, which is mediated through a Cdc42- and Rac-interactive binding (CRIB) domain.[66-70] Cdc42 is a member of the Rho family of GTPases, which regulates the formation of filopodia and cell-substrate adhesions and controls cell polarity and chemotaxis.[71] In the resting or inactive state, WASP adopts an autoinhibited conformation in which the VCA domain forms a hydrophobic interaction with the CRIB domain.[72] This closed structure is thought to prevent successful interaction with (or activation of) the Arp2/3 complex (Fig 2). Disturbance of the autoinhibited conformation by GTP-bound Cdc42 and other factors, some cooperatively, presumably facilitates activation of the Arp2/3 complex. A highly basic region N-terminal of the CRIB domain contributes to autoinhibition and might also direct the interaction with Cdc42 through formation of favorable electrostatic steering forces.[73] In a physiologic context GTP-bound Cdc42 alone is insufficient for activation. Instead, an additional factor, transducer of Cdc42-dependent actin assembly (Toca-1), is essential for Cdc42 and phosphatidylinositol (4,5) bisphosphate (PIP_2)-induced actin polymerization and binds to both Cdc42 and a WASP-interacting protein (WIP)-WASP complex through the SH3 domain of Toca-1 (shown experimentally for N-WASP, Fig 2).[74] It is not clear whether Toca-1 can interact directly with WIP or whether the WIP-WASP complex dissociates after binding. Other factors have also been shown to influence this process. For example, SH3 domains of the adaptor molecule Nck markedly enhance actin nucleation by N-WASP in the presence of Arp2/3 complex *in vitro*, suggesting the existence of a Cdc42-independent mechanism.[50] Similarly, SH3 domains from WASP-interacting SH3 protein activate N-WASP in the absence of Cdc42.[75]

The basic region of N-WASP, N-terminal to the CRIB domain, has been shown to bind PIP_2, which at least *in vitro* synergizes with Cdc42 to fully activate N-WASP by destabilizing the autoinhibited conformation through a highly sensitive threshold-dependent mechanism.[76-78] This might be important for localization of activity to highly specific sites or membrane compartments of the cell. It remains unclear whether this region, which is poorly conserved,

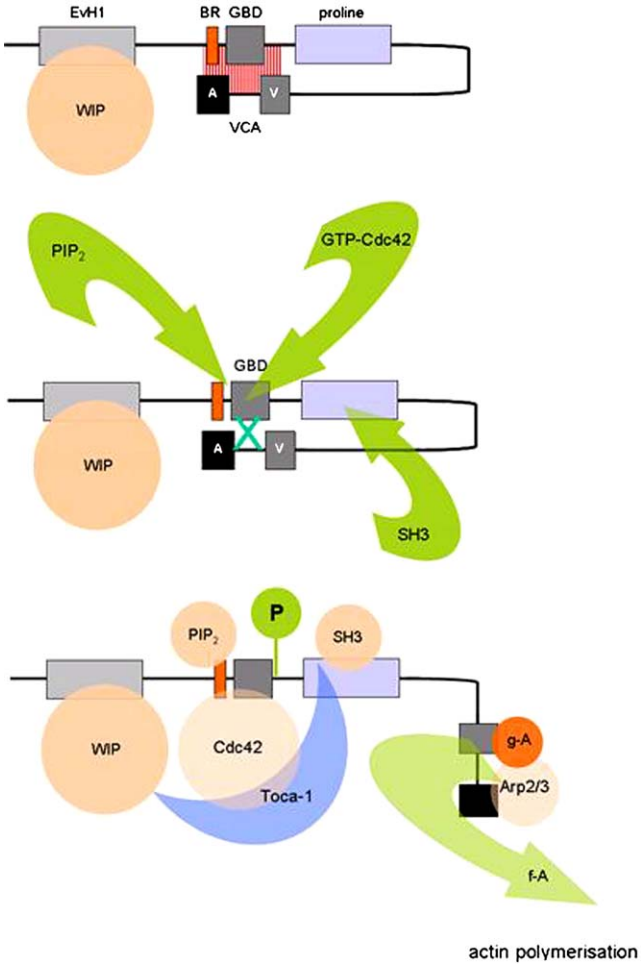

FIG 2. Model depicting mechanisms for activation of WASP. In the inactive state WASP adopts an autoinhibited configuration in which the C region of the verpoline cofilin homology domains/acidic region module interacts with the GTPase-binding domain and adjacent sequences. In this configuration either Arp2/3 complex cannot be bound or is inactive. Activation after cell stimulation is initiated by several factors, including GTP-bound CdC42, PIP_2, and many SH3 domain–containing proteins that in some cases are responsible for phosphorylation of Y291. These individual factors might operate cooperatively but might also be redundant for activation, depending on the context. *EVH1*, Ena/VASP homology 1 domain; *BR*, basic region; *GBD*, GTPase-binding domain; *VCA*, verpoline cofilin homology domains/acidic region.

operates as a PIP_2-binding site in WASP, and the physiologic role of PIP_2 therefore remains somewhat uncertain.

The N-terminus of WASP–N-WASP contains an Ena/vasodilator-stimulated phosphoprotein homologue 1, which binds the widely expressed verprolin homologue WIP (WASP-interacting protein, Fig 2).[79-81] The majority of WASP in cells is complexed with WIP, which is crucial for integrating N-WASP activity in a vaccinia-based actin motility system[82] and to recruit WASP to the immune synapse (IS) after T-cell receptor ligation.[83] WIP-WASP interaction is important for WASP activation by Cdc42 through Toca-1, although the precise role of WIP during regulation of WASP activity is unclear.[74]

Phosphorylation has emerged as an important physiologic regulator of WASP activity. A single WASP tyrosine phosphorylation site, Y291 (Y256 in N-WASP), is a target for nonreceptor kinases, such as Btk and Fyn, and the protein tyrosine phosphatase–PEST through binding of the adapter proline-serine-threonine phosphatase-interacting protein 1.[53,55,84] Phosphorylation of this residue activates WASP *in vitro* and *in vivo*, presumably through disruption of the autoinhibited conformation.[84,85] Tyrosine phosphorylation might also facilitate activation by SH2 domain binding.[85]

Phosphorylation at Y291 is critical for multiple WASP effector functions downstream of the T-cell receptor, including efficient actin polymerization and IS formation.[55] Although some evidence points to cooperative signals for phosphorylation and activation of WASP–N-WASP, this can also occur independently of binding to GTP-bound Cdc42.[55,85,86] In addition, phosphorylation of WASP is absolutely required for normal T-cell activation.[55] WASP and N-WASP are also phosphorylated on serine residues that lie at the junction of their C and A

domains.[87] This is mediated by the protein kinase CK2 and is required for optimal WASP activity.

Abnormalities of cell locomotion, trafficking, and localization

The development of an organized immunologic system for host defense and maintenance of tolerance is dependent on the ability of immune cells to respond to growth, differentiation, and localization signals. The effects of *WASP* gene mutations on these processes has therefore become of considerable interest, particularly because the actin cytoskeleton plays such a prominent role in the basic mechanisms of cell adhesion and migration.

Migration through tissues and endothelial barriers is a complex series of events requiring a highly regulated cycle of cell protrusion, retraction, adhesion and detachment, and major dynamic rearrangements of the actin cytoskeleton. Macrophages and DCs from patients with WAS and from WASP-deficient mice have been shown to be defective in their migratory behavior in several ways. Chemotaxis of mutant macrophages in response to colony-stimulating factor 1, N-formyl-methionyl-leucyl-phenylalanine, monocyte chemoattractant protein 1, and macrophage inflammatory protein 1α has been shown to be abrogated *in vitro*.[88,89] WASP-deficient DCs exhibit similar abnormalities of cytoarchitecture, chemotaxis, and quality of migration.[90,91] The response of DCs to physiologically relevant stimuli has also been studied. For example, normal DC homing to lymph nodes from the skin and migration within lymphoid tissue are known to involve the upregulation of chemokine receptor CCR7, which might therefore alter responsiveness to the CCR7 ligands CCL21 and CCL19. CCL21 is expressed on high endothelial venules, by stromal cells within T cell zones, and on lymphatic endothelium and is therefore critical for orchestrating this process.[92] WASP-deficient murine DCs exhibit multiple defects of trafficking *in vivo* after stimulation, including the emigration of Langerhans cells from the skin, the migration to secondary lymphoid tissue, and also the correct localization of DCs within T-cell areas.[93,94] These findings might well be directly linked to the fact that the migratory response of DCs to chemokines, such as CCL19 and CCL21, are markedly defective, as demonstrated *in vitro*.[93,94] It is therefore possible that DC trafficking abnormalities contribute in a significant way to the immune dysregulation observed in WAS. For example, the failure of DCs to localize appropriately within secondary lymphoid tissue in the context of normal activation, cytokine production, or upregulation of cell surface ligands might be sufficient to initiate uncontrolled T-cell responses. Similarly, trapping of DCs (eg, Langerhans cells in the skin) at peripheral sites after activation might result in the formation of ectopic inflammatory foci and the initiation of eczema.

Although there is no doubt that cell migration is compromised in the absence of WASP, the cell biologic events that contribute to these defects have not been absolutely determined. It is possible that abnormalities of cell polarity and chemotaxis relate to a failure to produce normal protrusions, such as filopodia, as observed experimentally in macrophages microinjected with a dominant negative Cdc42 mutant.[95] Similarly, efficient adhesion and detachment from surfaces is tightly linked to cell motility. WASP-deficient macrophages and immature DCs lack specialized adhesion structures known as podosomes.[90,91,96] In the hematopoietic system these are found particularly in large motile cells, including macrophages and immature DCs, and are formed on the ventral surface behind the leading edges. In these cell types podosomes concentrate β_2-integrins around an actin core and, although their function has not been clearly identified, are responsible for tight adhesion of cells to intercellular adhesion molecule 1 and possibly junctional adhesion molecule-A (JAM-A).[28,29] Furthermore, they are highly dynamic, with a turnover of minutes providing a migrating cell with a mechanism for rapid attachment and detachment and with localized anchorage points that could facilitate diapedesis. Interestingly, the sealing zone of bone-resorbing osteoclasts is also defined by circular clusters of podosomes that associate with αvβ3 integrins.[97-101] The absence of podosomes in WASP-deficient murine osteoclasts has been shown to result in partially defective bone resorption, although the relevance to human disease has not been characterized.[102]

Defects of migration, anchorage, and localization have more recently been defined for other cell lineages, including T and B lymphocytes, neutrophils, and HSCs. T lymphocytes from patients with WAS respond less well than normal cells *in vitro* to stromal cell–derived factor 1 (SDF-1) and CCL19 and demonstrate abrogated homing to secondary lymphoid tissue after adoptive transfer *in vivo*.[94,103,104] Interestingly, cells that are deficient for both WASP and WIP exhibit much more profound deficiencies than either alone, indicating that there is some redundancy.[104] Similarly, WASP-deficient B lymphocytes have been shown to have marked morphologic abnormalities, defective migration, and adhesion *in vitro* and impaired homing *in vivo*.[105] This is likely to contribute to the observed deficiencies of humoral responses to both T-dependent and T-independent antigens and to the marked deficiency of marginal zone B cells in both murine and human spleens.[21,105] A further example of defective trafficking *in vivo* originates from the observation that carrier female subjects for classic WAS almost universally exhibit nonrandom X-inactivation patterns in $CD34^+$ bone marrow progenitors.[37] This implies that WASP is functional within the progenitor compartment and is consistent with evidence for WASP expression in this cell type in adults and in very primitive human embryonic hematopoietic cells.[106,107] Serial stem cell transplantation and competitive repopulation studies in mice have confirmed a selective homing and engraftment advantage for normal HSCs.[108] Furthermore, hematopoiesis established by means of engraftment of chimeric fetal liver populations results in nonrandom dominance of normal cells over WASP-deficient cells,[108] suggesting that throughout development, there might be preferential establishment of hematopoiesis by normal rather than

mutant HSCs in carrier female subjects caused by an intrinsic homing advantage. This observation might have relevance for gene therapy of WAS.

Defective cell signaling and cell-cell interaction

Reorganization of the actin cytoskeleton has emerged as a critical feature of normal assembly of receptor signaling complexes at the cell surface and of effective signal transduction. WASP-deficient T cells characteristically exhibit defective proliferation and actin rearrangement in response to CD3 coreceptor ligation.[20,109] The fact that WASP is critical for cytoskeletal remodeling downstream of the T-cell receptor is highlighted by its role in formation of the IS, which in the absence of WASP is at least partially defective.[48,110,111] WASP-deficient T cells have lower levels of the glycosphingolipid raft marker GM1 at the cell surface during activation, which additionally fails to cluster normally.[110] Actin polymerization at the IS after T-cell receptor ligation is compromised and accompanied by failure to recruit molecules such as protein kinase Cθ, defective calcium influx, decreased production of IL-2 (which might be in part independent of normal IS formation), and abnormal proliferative responses.[48,110,111] In addition, WASP-deficient $CD4^+$ cells have been shown to be unable to polarize cytokine secretion toward antigen-specific target cells, whereas chemokine secretion appears to be unaffected, indicating that the respective secretory pathways are distinct and differentially dependent on WASP.[112] The regulation of WASP activity during T-cell activation is unclear. However, phosphorylation of Y291 by Fyn and dephosphorylation by the protein tyrosine phosphatase–PEST might be particularly important and independent of Cdc42 binding.[55] Similarly, localization of WASP to the IS is dependent on interaction with SH3 domain–containing proteins and not Cdc42.[45] WASP might also be recruited to lipid rafts in a complex with ZAP70-CrkL-WIP, which is activated at the IS by protein kinase Cθ-mediated phosphorylation and dissociation of WIP.[83]

Recently, it has been shown that WASP is necessary for antigen-presenting cell cytoskeletal remodeling during formation of the DC-NK immunostimulatory synapse and for subsequent DC induction of NK cell IFN-γ production and killing.[113] Similarly, impaired actin polymerization and perforin accumulation at the NK target contact point has been shown to result in partially reduced NK cytolytic activity.[22] WASP appears to be necessary for integration of signals leading to nuclear translocation of nuclear factor of activated T cells 2 and nuclear factor κB (RelA) during cell-cell contact, which might be independent of its direct role in cytoskeletal rearrangement.[114] The importance of WASP for normal B-cell signaling is less well established. WASP deficiency results in abnormalities of morphology and impaired B-cell receptor capping.[21,105,115-117] However, purified murine cells respond relatively normally to antigen receptor stimulation.[118] Processing of particulate antigens and chemotactic responses are significantly disturbed, although the signaling mechanisms have not been investigated.[105,117]

Disturbances of WASP regulation modeled by human disease

Multiple independent signals are integrated to ensure correct spatial and temporal regulation of WASP activity in response to specific cellular activation signals. The details of how these operate *in vivo* and how they interact with posttranslational modifications of WASP remain largely unclear. However, much can be learned from the study of human patients with naturally occurring mutations. Usually, molecular defects in the *WASP* gene result in diminished activity, either because of aborted protein production, intrinsic instability of the mutant mRNA or protein, or disturbance to key regulatory interactions. For example, a significant proportion of *WASP* gene defects result in expression of mutant protein with amino acid substitutions within the Ena/VAS homology 1 domain (exons 1-3, Fig 1). This would be predicted to disturb interaction with WIP to a variable extent, depending on the precise molecular abnormality.[80] Furthermore, clinically relevant mutations in this region that correspond to naturally occurring missense mutations of WASP have been shown to abolish *in vivo* proper N-WASP localization and actin polymerization.[82] In contrast, it has been demonstrated experimentally that a synthetic phosphomimicking mutant of WASP (Y291E), which introduces a negative charge into the hydrophobic core of the autoinhibited conformation, enhances actin polymerization both *in vitro* and *in vivo*, an indication that posttranslational modification in the form of phosphorylation of Y291 has a direct regulatory function.[84] Three unique human mutations in the *WASP* gene have now been shown to cause very similar effects in terms of enhanced actin polymerization (L270P, S272P, and I294T; unpublished observations).[7,29] Furthermore, all 3 missense mutations are located within the hydrophobic core (Fig 1) and are predicted to prevent autoinhibition by either disrupting the α-helical structure or through insertion of a polar residue (I294T) adjacent to Y291, behaving in a similar way to the synthetic phosphomimicking mutant Y291E (Fig 2). Interestingly, the phenotype of clinical disease arising from these mutations affecting the Cdc42-binding site is quite unlike that of classical WAS. The most prominent features in all affected patients are neutropenia and monocytopenia, which are suggestive of inhibited myelopoiesis. The mechanism by which unregulated actin polymerization induces these features is not clear, but the mutants might cause abnormalities of cytokinesis, which is dependent on the formation of a dynamic actomyosin ring structure before chromosomal separation and cell cleavage. This latter suggestion is further supported by the observation of acquired chromosomal defects in the bone marrow of 1 patient (unpublished observations).

MUTATION ANALYSIS

The cloning and sequencing of the gene responsible for WAS-XLT has not only provided new insight into the function of WASP but has lead to the discovery of

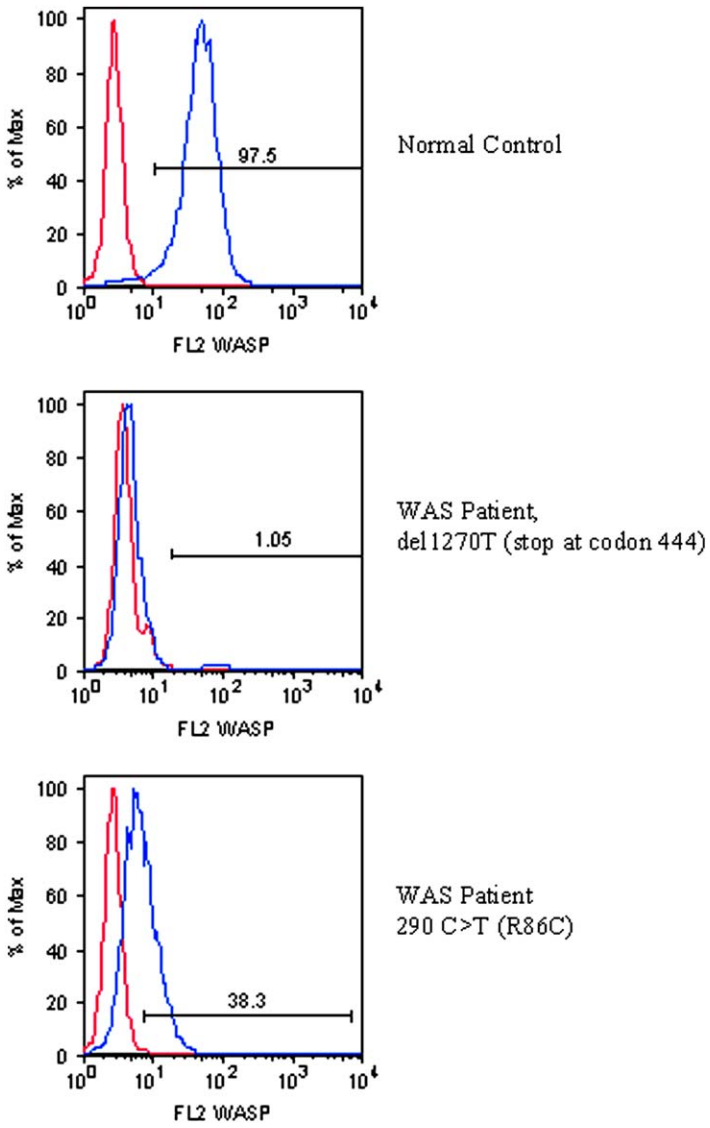

FIG 3. Flow cytometric analysis to assess WASP expression in peripheral blood lymphocytes. After permeabilization, as described by Yamada et al,[38] lymphocytes are stained with polyclonal antibody 503 or an isotype control. Cells are then incubated with a second (anti-rabbit) antibody. Shown are a healthy control subject *(top)*; a patient with a T deletion at position 1270, frame shift, and premature termination of transcription at codon 444 in the absence of WASP *(center)*; and a patient with the missense mutation Arg86Cys, resulting in reduced WASP expression *(bottom)*.

powerful tools to confirm molecularly the diagnosis in symptomatic male subjects, identify carrier female subjects, and diagnose WAS-XLT in at-risk fetuses. These new techniques include methods of screening lymphocytes for the presence or absence of WASP by means of flow cytometry (Fig 3) or Western blot analysis and sequencing cDNA, genomic DNA, or both for mutations in the *WASP* gene.

Spectrum of WASP mutations

Fig 1 summarizes the results of sequencing studies computed from single centers in the United States, Italy, and Japan. In this cohort of 270 unrelated families with patients with WAS-XLT, 158 unique *WASP* gene mutations were identified. The most common mutations observed were missense mutations (93 families), followed by splice-site mutations (n = 59), short deletions (n = 46), and nonsense mutations (n = 39). Insertions, complex mutations, and large deletions were less frequent (12%). Most deletions and insertions, typically involving less than 10 nucleotides, resulted in frame shift and early termination of transcription. The amino acid substitutions were typically located in exons 1 through 4. Splice-site mutations occurred predominantly in the downstream half of the *WASP* gene (introns 6-11). Mutations affecting variant splice sites resulted in multiple splicing products, which

often included small amounts of normal *WASP* cDNA. Six mutational hot spots, defined as occurring in 7 or more unrelated families (>2.5%), were identified in this cohort. Three hot spots represented point mutations within the coding regions, and the other 3 involved splice sites. The 168 C>T mutation, found in 10 families, results in the substitution of threonine with methionine at codon 45. Mutations at nucleotide position 290/291, observed in 23 unrelated families, result in the substitution of arginine at position 86 with several amino acids, depending on the nucleotide replacement. The 665C>T mutation, found in 10 families, converts Arg211 to a stop codon. IVS6+5g>n, present in 8 unrelated families, results in both abnormal and normal splicing. The IVS8+1g>n mutation, identified in 11 families, causes the deletion of exon 8, resulting in frame shift and premature termination. The IVS8+1 to +6 del gtga, resulting in the deletion of exon 8, frame shift, and early termination, was found in 7 unrelated families. These 6 hotspot mutations account for 25.6% of the cohort of 270 families. Three of these mutations (168C>T;290 C>N/291 G>N, and IVS6+5g>a) were consistently found in WASP-positive patients with a mild phenotype (XLT), whereas the 3 other mutations (665C>T, IVS8+1 g>n, and IVS8+1 to +6 del gtga) were predominantly WASP negative and had a high clinical score characteristic for WAS.

Genotype-phenotype correlation

Mutations of the *WASP* gene result in 3 distinct phenotypes: the classic WAS triad of thrombocytopenia–small platelets, recurrent infections, and eczema as first reported by Wiskott in 1937; the milder XLT variant,[5,119] which can be intermittent[6]; and congenital X-linked neutropenia without any of the clinical findings characteristic of WAS-XLT.[7,28,29] The most consistent phenotype-genotype correlation was observed when the patients were divided into 2 categories: WASP positive if the mutated protein was expressed and of normal size and WASP negative if the protein was absent or truncated.[11,12] Patients with mutations that allowed expression of normal-sized mutated protein, often in reduced quantity, had, with few exceptions, the XLT phenotype, whereas those patients whose lymphocytes could not express WASP or expressed only truncated WASP were more likely to have the classic WAS phenotype; this association was statistically significant ($P < .001$). Progression to a score of 5 caused by either autoimmune disease or malignancy was observed in both groups but was far more frequent in WASP-negative patients with an initial score of 3 to 4. There were, however, exceptions, making it difficult in individual cases to accurately predict the clinical course based solely on the type of mutations of the *WASP* gene.

Somatic reversion and mosaicism

A number of recent studies demonstrating somatic mosaicism (resulting from spontaneous reversion of an inherited disease-causing genetic mutation) have provided important information on the role of WASP in human T cells.[120-122] Reversion has also been detected molecularly in both T and B lymphocytes (presumably because of reversion in a common lymphoid precursor), although only revertant T cells were detected in significant numbers peripherally.[123] This demonstrates that a normal copy of the WASP allele confers clear survival or growth advantage to T cells, but not to B cells. Additionally, reversion events restore T-lymphocyte function with regard to CD3-stimulated proliferation and actin rearrangement and might result in significant improvement in clinical phenotype if a sufficient proportion and diversity of T cells express the reversion. More recently, mosaicism arising from somatic mutation has also been demonstrated in the NK cell lineage.[124]

ESTABLISHING THE DIAGNOSIS OF WAS-XLT

Because of the wide spectrum of clinical findings, the diagnosis of WAS-XLT should be considered in any male patient presenting with petechiae, bruises, and congenital or early-onset thrombocytopenia associated with small platelet size. The presence of transient, mild, or severe eczema supports the diagnosis of WAS-XLT. It is important to note that infections and immunologic abnormalities might be absent, mild, or severe, and autoimmune disease develops more often in patients with WAS than in patients with XLT. Lymphopenia, characteristic for classic WAS, might be present during infancy but will develop invariably during childhood. Screening for WASP mutations can be performed by means of flow cytometry[37] with a suitable anti-WASP antibody (Fig 3), but patients with expression of mutated WASP might be missed with this method. Mutation analysis of the *WASP* gene is essential for establishing a final diagnosis; it might also assist in estimating the severity of the disease (with caution) and in performing carrier detection and prenatal diagnosis.

MANAGEMENT OF PATIENTS WITH WAS-XLT

Conventional treatment

Management of patients with WAS continues to present major challenges, particularly in attenuated phenotypes, where the natural history of disease progression is less predictable. Early diagnosis is most important for effective prophylaxis and treatment. If an infection is suspected, careful evaluation for bacterial, viral, or fungal causes followed by appropriate antimicrobial therapy is of crucial importance. Infants with WASP mutations and lymphopenia are candidates for *Pneumocystis carinii* prophylaxis. If antibody responses to protein or polysaccharide antigens are defective, which is most often the case in patients with the classic WAS phenotype, prophylactic intravenous immunoglobulin (IVIG) infusions at full therapeutic dose are recommended. Continuous antibiotic therapy should be considered if infections occur despite IVIG prophylaxis. Killed vaccines can be given; the response, however, might be insufficient and should be measured. Live

virus vaccines are not recommended. A high (extra) dose of IVIG (which has high antivericella antibody titers) and antiviral drugs are indicated after exposure to chicken pox.

Eczema, if severe, requires aggressive therapy, including local steroids and, if indicated, short-term systemic use of prednisone. Autoimmune manifestations might require more aggressive immunosuppresion and might be refractory to conventional modalities. Autoimmune blood dyscrasias might respond to mAbs targeting the CD20 antigen (rituximab), which might be a relatively safe therapy, particularly because these patients will be receiving general Ig replacement.

Cutaneous infections are common and might necessitate local or systemic antibiotics. Lymphadenopathy is frequently observed and is often the consequence of an infected eczema. Platelet transfusions are restricted to treat serious bleeding (eg, central nervous system hemorrhages or gastrointestinal bleeding). Blood products are best irradiated and should be cytomegalovirus negative. Aspirin, which interferes with platelet function, is contraindicated.

Splenectomy, sometimes recommended for patients with XLT, effectively stops the bleeding tendency by increasing the number of circulating platelets. However, splenectomy in patients with WAS-XLT of all ages increases markedly the risk of septicemia and, if performed, requires lifelong antibiotic prophylaxis.

Development of new therapeutic strategies

At present, the only curative therapy is HSC transplantation, with good results for patients with HLA-matched family or unrelated donors or partially matched cord blood donors but less satisfactory outcomes for other donor types.[125] Successful gene therapy of 2 forms of severe combined immunodeficiency and, more recently, chronic granulomatous disease has therefore encouraged the development of similar strategies for WAS.[126]

One particular challenge for treatment of WAS with gene therapy has been the recapitulation of normal gene expression in all hematopoietic lineages for complete phenotypic correction. Whether successful modification of T cells alone will be useful is debated, although, as discussed previously, studies in patients with WAS exhibiting somatic mosaicism have clearly demonstrated that a normal copy of the WASP allele confers a significant survival or growth advantage to T and NK cells.[120-124] Additionally, reversion events can partially restore T-lymphocyte function and might in some cases result in an attenuated clinical phenotype if a sufficient proportion and diversity of T cells, NK cells, or both express the reversion. Despite these findings, it is likely that all hematopoietic compartments will have to be successfully targeted for reliable long-term correction of the disease phenotype.

In preclinical studies multiple cellular defects, including macrophage migration, DC cytoskeletal arrangement, and T-cell proliferation, have been corrected in human and murine cells *in vitro* through gene transfer by using traditional gammaretroviral or more sophisticated lentiviral vectors.[27,127-129] In addition, gene transfer to HSCs in murine models of WASP deficiency has been shown to efficiently restore T-cell function[130,131] and myeloid cytoskeletal defects.[132] More recently, short segments of the natural regulatory elements of the *WASP* gene have been shown to confer near-physiologic gene expression, which might therefore be particularly useful in the context of both efficacy and safety.[129] Clinical studies for treatment of WAS by means of gene transfer to HSCs are currently in preparation.

CONCLUSION

WAS is a perfect example of the effect of molecular genetics on the understanding of complex diseases. WASP plays important roles in actin polymerization, development of hematopoietic cells, IS formation, cell signaling, and lymphocyte apoptosis. Mutations of WASP result in phenotypically unique diseases entities, depending on the effect of the mutation of WASP expression. Understanding the molecular basics has important implications for the diagnosis, treatment, and genetic counseling of patients with WAS.

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