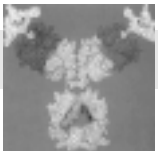

The Wiskott-Aldrich Syndrome

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The Wiskott-Aldrich syndrome, first described in 1937, is an X-linked recessive disorder characterized by immunodeficiency, eczema, thrombocytopenia and increased risk of autoimmune disorders and malignancies [1–3]. X-linked thrombocytopenia, typified by thrombocytopenia and small platelets but without the other findings associated with WAS, is caused by mutations of the same gene [4,5]. The gene responsible for WAS/XLT, the Wiskott-Aldrich syndrome protein *(WASP)*, has been identified by positional cloning [6], and a large number of unique mutations of WASP has been described in affected families worldwide [6,7]. The identification of the *WASP* gene has provided an effective tool to confirm the diagnosis, especially of mild cases, to identify carrier females, and to facilitate prenatal diagnosis.

WASP contains several domains, with unique functions suggesting a critical role of the protein in signal transduction and cytoskeletal reorganization [8]. This review describes the clinical features of WAS/XLT, discusses the abnormal laboratory findings, and explores the function of WASP.

Clinical manifestations

Infections and immunodeficiency

Because of the defective immune system, recurrent infections are a frequent complication in patients with classic WAS and may become life threatening. Bacterial infections due to common organisms consist of otitis media, sinusitis, meningitis, sepsis and pneumonia [3]. Severe viral infections include recurrent herpes simplex infections, observed in 12% of WAS patients; varicella with systemic complications often requiring treatment with acyclovir and high dose intravenous immunoglobulin or varicella-zoster immune globulin; and *Pneumocystis carinii* pneumonia reported in 9% of WAS patients [3]. Fungal infections consist chiefly of *Candida* infections and are observed in 10% of WAS patients. XLT patients often lack a history of severe and frequent infections [9]; however, if splenectomized, WAS/XLT patients are highly susceptible to overwhelming sepsis.

Both the cellular and humoral immune system is affected, although the severity of the immune deficiency may vary from family to family. Serum IgG levels are often close to normal, IgM levels are moderately depressed, and IgA and IgE concentrations are elevated. Low isohemagglutinin titers are a persistent finding and antibody responses to many protein antigens are depressed [3,10].

Abnormal T cell function is suggested by diminished but not absent lymphocyte responses to mitogens and by depressed proliferative responses to allogenic cells and immobilized anti-CD3 monoclonal antibody [11]. Anti-CD3 monoclonal antibody-activated T cells have been shown to secrete less interleukin-2 than do normal cells. Lymphopenia due to a net loss of T lymphocytes is a common finding [10] and may be due to accelerated apoptosis, which has been observed in WAS lymphocytes [12].

Defective actin remodeling has been suggested by the morphologic abnormalities observed in WAS T cells. The surface of peripheral blood lymphocytes from WAS patients is void of projections when compared with normal lymphocytes. T cell lines derived from WAS patients, if activated with an anti-CD3 monoclonal antibody, fail to polymerize and reorganize actin [13]. WASP, if activated by the Rho family GTPase Cdc42, stimulates actin polymerization through the Arp2/3 complex; the lack of function of WASP has been shown to lead to defective cell membrane capping, cytoplasmic signaling and cell motility [8]. WASP is also required for optimal efficiency of IgG-mediated phagocytosis [14]. Recently, Leverrier and co-workers [15] demonstrated that clearance of apoptotic cells is accompanied by recruitment of WASP to the phagocytic cup and that failure to do so results in delayed phagocytosis in WASP knockout mice, both *in vitro* and *in vivo*. This mechanism may contribute to the immune dysregulation of WAS and the high rate of autoimmune disorders [15]. The actin polymerization of T cells toward antigen-presenting cells [16] suggests that the abnormal antibody responses observed in WAS patients are a direct consequence of defective T/B cell interaction, resulting in failure to develop memory cells and to isotype switch [10].

WAS = Wiskott-Aldrich syndrome
XLT = X-linked thrombocytopenia
WASP = WAS protein

Ig = immunoglobulin

Although the number of circulating neutrophils and monocytes is normal, *in vitro* chemotaxis of WAS monocytes is deficient [17] and Fcγ-receptor-mediated phagocytosis is impaired [14]. Dendritic cells derived from peripheral blood of WAS patients failed to develop polarized morphologies or to extend dendritic processes [18]. It has been hypothesized that the defects observed in monocytes and dendritic cells are likely to be the direct consequence of the observed disorganization of the actin cytoskeleton. The resulting defect in immune cell trafficking may play a major role in the complex immune defects seen in WAS patients [19].

Platelet abnormalities

The platelet defect, characteristically presenting as thrombocytopenia and small platelet volume, is a consistent and until recently a pathognomonic finding in patients with mutations of the *WASP* gene. Within individual WAS/XLT patients, the platelet count may be as low as 3,000/mm^3 or as high as 70,000/mm^3. The exception are two families with missense mutations of WASP in exon 2 and exon 11, respectively, who had intermittent thrombocytopenia with platelet counts usually between 100,000 and 250,000/mm^3; however, their platelets occasionally drop to values of 30,000/mm^3 and they develop petechiae [20]. The mean platelet volume in most WAS/XLT patients is approximately half that of normal control subjects [10]. Following splenectomy, platelet counts and platelet volume often increase but are still lower than in normal controls [21]. Platelet counts usually fail to increase in response to prednisone or high dose intravenous immunoglobulin.

WAS platelets have many functional and morphologic abnormalities, including lack of platelet-specific α-granules, reduced numbers of cytoplasmic organelles, and defective aggregation following exposure to epinephrine, adenosine diphosphate or collagen. A markedly reduced survival of defective platelets [22] and ineffective thrombocytopoiesis [10] have been suggested. Miki et al. [23] reported that megakaryocyte differentiation and microvesicle formation is dependent upon the interaction of WASP with actin filaments, a process requiring tyrosine phosphorylation of Shc, suggesting that WASP controls the assembly of actin filaments that are essential for microvesicle and pro-platelet formation.

Clinical manifestations of thrombocytopenia include petechiae and bruises, hematemesis and melena, epistaxis and oral bleeding. Life-threatening hemorrhage, including severe oral bleeding, gastrointestinal bleeding, and intracranial hemorrhage has occurred at least once in 30% of WAS/XLT patients [3] and may require platelet transfusions.

Eczema and other allergic manifestations

Eczema is one of the typical findings that differentiate WAS from idiopathic thrombocytopenia [1]. However, XLT patients have, by definition, either mild and transient eczema or none at all. In the most severe cases, eczema is resistant to therapy and may persist into adulthood. *Molluscum contagiosum*, herpex simplex, or bacterial infections may develop in areas of the skin with eczema, posing a therapeutic challenge.

Autoimmune manifestations

Autoimmune diseases have been reported in 40% of WAS patients [3]. XLT patients without apparent immune deficiency may develop autoimmune disorders, although less frequently than do classic WAS patients. The most common autoimmune manifestations include hemolytic anemia, non-specific vasculitis, Henoch-Schönlein purpura-like symptoms, inflammatory polyarthritis and inflammatory bowel disease. IgA nephropathy with or without the association with Henoch-Schönlein purpura may cause chronic renal failure, which requires dialysis or renal transplantation (Shigeaki Nonoyama, personal communication).

Malignancies

Malignancies can occur during childhood but are more frequent in adolescents and young adults with classic WAS phenotype. In a large study population, malignancies were reported in 13% [3]. In that series, the average age at onset of malignancies was 9.5 years. The most frequent malignancy reported is lymphoma, especially Epstein-Barr virus-positive B cell lymphoma, suggesting a direct relationship with the defective immune system. The true incidence of malignancies in patients with the XLT phenotype is unknown but is less than in classic WAS; a few cases of malignant lymphomas have been reported in XLT.

Diagnosis of WAS/XLT

The spectrum of WAS/XLT

Owing to the wide spectrum of the clinical presentation, the diagnosis of WAS/XLT should be considered in any male with congenital or early-onset (or even transient) thrombocytopenia and small platelets. A history or the presence of mild or severe eczema supports the diagnosis. Infections and immunologic abnormalities are characteristic for WAS but may be absent or mild in patients with the XLT phenotype. Autoimmune diseases and malignancies may develop in patients with WAS or XLT. A definitive diagnosis of WAS/XLT is based on congenital thrombocytopenia and small platelets (mean platelet volume <5.0 fl in most patients) in a male with a mutation in the *WASP* gene, or with absent WASP mRNA or protein in peripheral blood mononuclear cells. A positive family history of WAS or XLT is not always present.

Because XLT patients are frequently misdiagnosed as having chronic idiopathic thrombocytopenia, any male patient with chronic ITP and a family history of thrombocytopenia should be examined for WASP mutations.

Scoring system for WAS/XLT

The scoring system, described in Table 1, is based on the postulate that patients with WAS/XLT have in common thrombocytopenia and small sized platelets and that most develop some form of immunodeficiency, although to a different degree [9]. The extent of eczema may be difficult to assess. Lack of a history of eczema, or the presence of mild transient eczema that responds well to treatment, indicated by (+) in Table 1, and mild infrequent infections not resulting in sequalae indicated as (+), are consistent

ITP = idiopathic thrombocytopenia

Table 1. Scoring system to define the phenotypes of WASP mutations

	TXLT	XLT		WAS classic			XLN
Score	<1	1	2	3	4	5*	0
Thrombocytopenia	(+)	+	+	+	+	+	_
Small platelets	+	+	+	+	+	+	_
Eczema	_	_	(+)	+	++	(+)/+/++	_
Immunodeficiency	_	-/(+)	(+)	+	+	(+)/+	_
Infections	_	_	(+)	+	+/++	(+)/+/++	_
Autoimmunity and/or malignancy	_	_	_	_	_	+	_
Congenital neutropenia	_	_	_	_	_	_	+

TXLT = transient X-linked thrombocytopenia, XLN = X-linked neutropenia.
-/(+) Absent or mild
(+) Mild transient eczema, or mild infrequent infections, not resulting in sequalae
++ Eczema that is difficult to control, and severe life-threatening infections
* Since patients with XLT may develop autoimmune disorders or lymphoma, albeit at a lower rate than those with classic WAS, a progression from a score of 1 or 2 to a score of 5 is possible for XLT.

with XLT (score 1 or 2). Severe, treatment-resistant eczema, recurrent infections in spite of optimal therapy, autoimmune diseases, and malignancies are characteristic for classic WAS and are scored as mild (score of 3), moderate (score of 4), or severe (score of 5). If a patient who was classified as XLT (score 1 or 2) develops autoimmune disease or lymphoma, he moves from a score of 1 or 2 to a score of 5.

Using these criteria, patients with missense mutations within exons 1, 2 and 3 usually have mild disease. Other mutations observed in patients with mild disease include splice anomalies resulting in multiple splicing products. As a rule, mutations associated with a mild phenotype have in common the expression of a normal sized or truncated protein usually in decreased quantity [9]. Most of the mutations resulting in the lack of protein, e.g., nonsense mutations, insertions and deletions and most splice site mutations, are associated with the classic WAS phenotype.

Carrier detection and prenatal diagnosis

X-inactivation studies in WAS carrier females have shown that the normal X chromosome is preferentially used as the active X chromosome in all hematopoietic cell lineages, including $CD34^+$ cells [24]. An exception are families with a very mild form of XLT in which X chromosome inactivation is random (Ochs, unpublished). If in a given family the WASP mutation is known, carrier females can be identified by mutation analysis. Similarly, prenatal diagnosis of a male fetus at risk for WAS/XLT can be accomplished by DNA analysis using chorionic villi biopsies, or cultured ammniocytes as the DNA source.

Treatment and prognosis

In case of an infection, prompt and effective antimicrobial therapy is essential and requires an extensive search for a bacterial, viral or fungal etiology. Because of the abnormal antibody responses to multiple antigens, patients with the classic WAS phenotype are candidates for prophylactic treatment with IVIg. Eczema, if severe, requires complex therapies including steroid ointments and short-term systemic use of steroids. Whether the newly approved FK506-containing ointment will be effective in the treatment of WAS-associated eczema remains to be seen. Food allergies have to be considered and an appropriate diet designed based on food exclusion. Platelet transfusions should be avoided unless bleeding is serious, in order to prevent sensitization. All blood products need to be irradiated and should be negative for cytomegalovirus. Central nervous system hemorrhages require immediate platelet transfusions. If autoimmune phenomena develop, high doses of IVIg and systemic steroids may correct the problem. Aspirin, which interferes with platelet function, is contraindicated in WAS/XLT patients. Splenectomy may decrease the bleeding tendency by increasing platelet number but will increase the risk of septicemia. WAS as well as XLT patients who have undergone splenectomy require life-long prophylactic antibiotic therapy.

Bone marrow transplantation or the use of cord blood as a source of stem cells are the only curative therapy available for WAS patients. The most widely used conditioning regimen includes cyclophosphamide and busulfan. Fully matched siblings are preferred doners, possibly leading to successful bone marrow transplantation even in older patients. Matched unrelated donors can be used successfully in young boys with WAS, but after the age of 5–8 years the success rate decreases and matched unrelated donor transplants are not recommended in older children [25]. Cord blood stem cells, fully or partially matched, are excellent alternatives for young WAS patients (weighing <25 kg) if a matched donor is not available. The use of haploidentical bone marrow transplantation has been disappointing.

The life expectancy of patients with classic WAS, originally reported to be 3.5 years, is now over 11 years. A considerable proportion of WAS/XLT patients are over the age of 20. However, the incidence of malignancies, most often lymphomas, increases substantially during the third decade of life in patients with classic WAS. The cause of their death has remained similar over the years: infections (44%), bleeding (23%), and malignancies (26%). Patients with XLT have a more favorable prognosis, with most reaching adulthood and several being older than 60 years of age. Patients with successful bone marrow transplantation are cured of infections, bleeding and autoimmune diseases; no excessive rate of malignancies has been reported.

The molecular basis of WAS/XLT

WASP family of genes

The *WASP* gene was identified by positional cloning (gene bank accession number U12707) [6]. The gene consists of 12 exons

IVIg = intravenous immunoglobulin

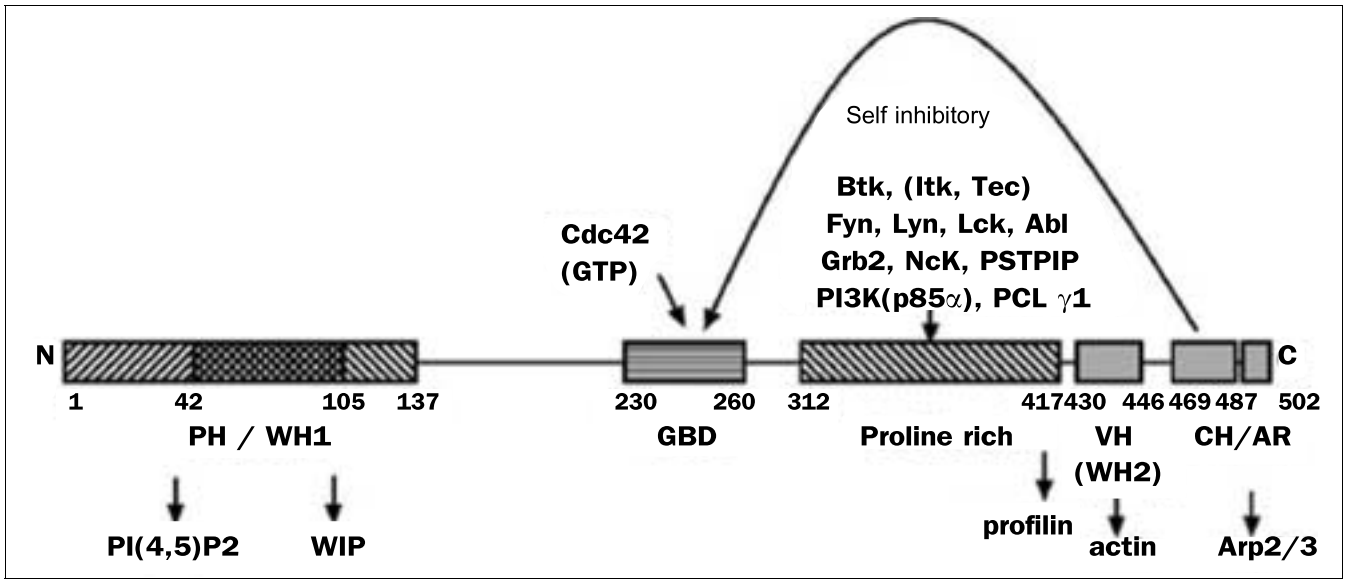

Figure 1. Structure and the major functional domains of WAS protein. PH = pleckstrin homology, WH1 = WASP homology domain 1, GBD = GTPase binding domain (or CRIB motif), VH = verprolin homology domain, CH/AR = cofilin homology domain/acidic region, WIP = WASP interactive protein, Arp 2/3 = actin-related protein 2/3. The proline rich region in exon 10 contains SH3 binding domains that interact with cytosolic adapter proteins and Tec family cytoplasmic tyrosine kinases, as indicated. Listed are exons and nucleotide numbers of exon-intron borders.

spanning 9 Kb of genomic DNA. The cDNA (1,821 basepairs containing an open reading frame of 502 amino acids) generates a protein with a predicted molecular weight of 54 kDa. A neural tissue homologue of WASP (N-WASP) was cloned from bovine brain [26]. Whereas WASP is constitutively expressed only in hematopoietic stem cell-derived cell lineages [6], N-WASP is present in many non-hematopoietic tissue extracts. WAVE 1 (WASP-family verprolin-homologous protein 1), WAVE 2 AND WAVE 3 are newly identified WASP family proteins. These molecules are similar to WASP and N-WASP in their C terminal region, containing the verproline homology domain, the cofilin homology domain and an acidic region. Similar to WASP, they associate with the Arp2/3 complex and stimulate the nucleating activity of the Arp2/3 complex.

Structure of WASP

WASP consists of several functional domains [Figure 1], including WH1/PH (WASP-homology 1/pleckstrin homology) domain, a GBD (GTPase binding domain)/CRIB (cdc42 or Rac-interactive binding) motif, a proline-rich region and a verproline (V) homology domain, a cofilin (C) homology domain and acidic (A) region (VCA) [26,27]. Each of these domains plays a unique functional role.

The pleckstrin homology-like domain is located in the N terminal region of WASP and N-WASP and spans amino acids 6 to 105. Functionally, the PH domain is important for the localization of proteins through interaction with other proteins or lipids. Ligands for PH domains reported to date include PKC, the $\beta\gamma$ subunit of G proteins, and PIP2. Binding of PIP2 to the PH domain of N-WASP was demonstrated, with N-WASP regulating actin polymerization in a PIP2-dependent manner [26]. It is of interest that most of the missense mutations described to date are located in the PH domain of WASP.

A WASP interactive protein (WIP), which co-immunoprecipitates with WASP from lymphocyte extracts, was identified by the yeast two-hybrid system [28]. WIP binds to the WH1 domain of WASP *in vivo* and *in vitro*, and the WIP/WASP complex is known to support signal transduction and actin polymerization in hematopoietic cells [29]. The WIP gene encodes a 503 amino acid proline-rich protein with a calculated molecular mass of approximately 52 kDa. WIP contains the actin-binding KLKK motif and two APPPPP sequences, which have been shown to bind to profilin known to regulate actin polymerization. In addition, WIP has proline-rich SH3-binding motifs, which could provide a link with cellular signaling pathways.

A GTPase-binding domain (GBD), also referred to as CRIB motif, is located in exon 7 and 8 of the WASP gene. WASP, like other proteins containing a CRIB motif, recognizes the GTP but not the GDP-bound form of Cdc42. Cdc42, Rac, and other Rho-like GTPases are key elements in the dynamic organization of the actin cytoskeleton [30]. A recent report suggests an auto-inhibitory contact between the GTPase-binding domain and the carboxy-terminal region of WASP, which can be released by activated (GTP) Cdc42 [31]. This points to an indirect effect of Cdc42 on the interaction of the C-terminus of WASP with the Arp2/3 actin nucleating complex.

N-WASP = neural WASP
PH = pleckstrin homology

WIP = WASP interactive protein

A proline-rich region in exon 10 contains the PXXP binding consensus for SH3 domains. WASP was shown to interact with the SH3 domains of selected signaling molecules, including the cytosolic adapter proteins, Grb2, $P47^{nck}$, Fyn, cFgr, Lck, c-Src and p^{47phox}. WASP also interacts with the Tec family cytoplasmic tyrosine kinases, Btk, Tec, PLCγ1 and Itk. The data demonstrate that WASP, through its interaction with the SH3 domain of selected molecules, plays an important role in intracellular signaling of hematopoietic cells.

The verprolin/cofilin homology domains and acid region located in the C-terminus of WASP were shown to be important in the regulation of actin polymerization [26]. This function is mediated by Arp (actin-related protein), a recently identified group of proteins involved in the regulation of the actin cytoskeleton. Two of these proteins, Arp2 and Arp3, make up the actin-regulating complex. The Arp2/3 complex has been shown to interact directly with the verprolin/cofilin homology domain and acidic region of WASP [32]. This interaction is tightly controlled, since the carboxy-terminal region of WASP forms and auto-inhibitory contact with the GBD domain of WASP. This auto-inhibitory contact can be released by activated Cdc42 [31,33]. Thus, the carboxy terminal of WASP plays a dominant role in the regulation of the actin cytoskeleton and cell mobility.

WASP function

The elucidation of the biologic functions of this complex protein has provided new insight into the pathogenesis and clinical presentation of WAS/XLT. The interaction of WASP with SH3 domains of many cytoplasmic proteins suggests that WASP plays a central role in intracellular signaling of hematopoietic cells and that tyrosine phosphorylation contributes to this activity. Tyrosine phosphorylation by tyrosine kinases regulates signal transduction by connecting upstream cell surface receptors to downstream pathways. In an attempt to identify *in vivo* subtrates of Btk, Baba and colleagues [34] identified WASP as one of the major phosphoproteins associated with Btk and demonstrated that WASP is physically associated with Btk and can serve as a substrate for BTK. One of the seven tyrosines of WASP (Tyr291) serves as the major phosphorylation site. Tyr291 is also conserved in N-WASP and is the tyrosine adjacent to the Cdc42 binding site. It is intriguing to postulate that the phosphorylation of Tyr291 modulates the affinity of WASP for its target, Cdc42. Tyrosine phosphorylation of WASP has also been observed in platelets [35], where collagen induces a rapid increase in tyrosine phosphorylation of WASP.

Actin cytoskeleton signaling through the Arp2/3 complex has been associated with actin polymerization. WASP interacts directly with the Arp2/3 complex, leading to actin nucleation and formation of actin filaments [32]. The importance of WASP in the actin regulation by Arp2/3 has been illustrated by the finding that WAS macrophages fail to organize the Arp2/3 complex in podosomes. It was recently proposed that activated Cdc42 and phosphatidylinositol 4,5bi-phosphate induces confirmational changes in N-WASP, which allows the association of the Arp2/3 complex with the carboxyl-terminal of N-WASP [33]. In this model, N-WASP is present in two configurations. In the active form, the C-terminal of N-WASP is free to interact with the Arp2/3 complex. In the inactive form, the C-terminal of N-WASP forms an auto-inhibitory contact with a basic region of the GBD/CRIB domain. To revert to the active form, the auto-inhibitory contact is disrupted by the binding of the active (GTP) form of Cdc42 with the GTPase-binding domain, resulting in the release of the C-terminal region, which associates with the Arp2/3 complex. A similar reversible auto-inhibitory interaction has recently been shown for WASP [31]. The importance of this self-inhibitory mechanism is exemplified by a family with three generations of males with X-linked severe congenital neutropenia caused by a point mutation (L270P) within the CRIB domain of WASP [36].

The involvement of WASP in IgG-mediated phagocytosis has recently been demonstrated [14]. This FcγR-mediated process is impaired in WASP-deficient peripheral blood monocytes. In normal macrophages, WASP itself is actively recruited to the actin cup; in WASP-deficient macrophages, formation of the actin cup and local recruitment of tyrosine phosphorylated proteins is markedly reduced, suggesting that the cytoskeletal structure responsible for phagocytosis is dependent on WASP expression. Most recently it was shown that clearance of apoptotic cells by macrophages and dendritic cells requires recruitment of WASP to the phagocytic cup and that its absence results in delayed phagocytosis both *in vitro* and *in vivo* [15].

Finally, WASP plays a role in apoptosis itself, which may be relevant to the progressive cellular and humoral immunodeficiency. The progressive T cell depletion observed in WAS patients [10] may be a direct result of accelerated apoptosis caused by a defect in the cell death signaling pathway. An association has been suggested among actin cytoskeletal function, mutations of WASP and cell death. The morphologic changes that occur in apoptosis require actin cytoskeletal remodeling and are necessary for the execution of cell death. This may explain the accelerated cell death of lymphocytes *in vitro* seen in classic WAS, but not in XLT [12]. The precise role of WASP in the intracellular cell death pathways is not clearly defined. The downregulation of a cell survival pathway, or conversely, the upregulation of a cell death pathway in WAS lymphocytes has to be considered. Rawlings et al. [37] showed that Bcl-2 expression is reduced in WAS lymphocytes compared with controls. Conversely, increased expression of caspase-3 and of the cell death receptor CD95, or Fas, by WAS lymphocytes as compared with XLT and control lymphocytes has been reported [12]. Interestingly, ligation of the CD95 receptor results in the activation of the JNK kinase cascade that ultimately results in cell suicide. Cdc42 is believed to be a component of the JNK kinase cell death pathway. These observations suggest that mutations of WASP lead to accelerated apoptosis of lymphocytes, possibly due to the upregulation of cascapse-3 and CD95 and downreguation of Bc1-2.

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