Recent advances in understanding the pathophysiology of Wiskott-Aldrich syndrome

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Wiskott-Aldrich syndrome (WAS) is a severe X-linked immunodeficiency caused by mutations in the gene encoding for WASP, a key regulator of signaling and cytoskeletal reorganization in hematopoietic cells. Mutations in WASP result in a wide spectrum of clinical manifestations ranging from the relatively mild X-linked thrombocytopenia to the classic full-blown WAS phenotype characterized by thrombocytopenia, immunodeficiency, eczema, and high susceptibility to developing tumors and autoimmune manifestations. The life expectancy of patients affected by severe WAS is reduced, unless they are successfully cured by bone marrow transplantation from related identical or matched unrelated donors. Because many patients lack a compatible bone marrow donor, the administration of WAS gene–corrected autologous hematopoietic stem cells could represent an alternative therapeutic approach. In the present review, we focus on recent progress in understanding the molecular and cellular mechanisms contributing to the pathophysiology of WAS. Although molecular and cellular studies have extensively analyzed the mechanisms leading to defects in T, B, and dendritic cells, the basis of autoimmunity and thrombocytopenia still remains poorly understood. A full understanding of these mechanisms is still needed to further implement new therapeutic strategies for this peculiar immunodeficiency. (Blood. 2009;113:6288-6295)

Introduction

Wiskott-Aldrich syndrome (WAS, OMIM 301000) is a complex and severe X-linked disorder characterized by microthrombocytopenia, eczema, immunodeficiency, and increased risk in developing autoimmunity and lymphomas. WAS affects 1 to 10 of every 1 million male newborns; life expectancy is approximately 15 years for patients lacking WAS protein (WASP) expression.1,2 The protein encoded by the WAS gene (WASP) is a hematopoietic specific regulator of actin nucleation in response to signals arising at the cell membrane.3,4 Mutations impairing but not abolishing WASP expression can cause X-linked thrombocytopenia (XLT). This disease can be chronic5 or intermittent6 and is considered an attenuated form of WAS because it is characterized by low platelet counts with minimal or no immunodeficiency. Recently, gain-of-function mutations in the WAS gene, giving rise to a constitutively active protein, were found to cause a distinct pathology, X-linked neutropenia. X-linked neutropenia is characterized by low neutrophil counts and predisposition to myelodysplasia in the absence of thrombocytopenia and T-cell immunodeficiency.7,8

The wide spectrum of clinical manifestations highlights the complex role of WASP in various cellular mechanisms.

Clinical manifestations in WAS

Microthrombocytopenia

Among clinical manifestations, hemorrhages are frequent (> 80% incidence) in WAS and XLT patients and range from non–life-threatening (epistaxis, petechiae, purpura, oral bleeding) to severe manifestations, such as intestinal and intracranial bleeding.9 Death of WAS patients is caused, in 21% of the cases, by hemorrhages.9,10 Bleeding is the result of severe thrombocytopenia with reduced platelet size, which is the most common finding in WAS and XLT patients (100% incidence). Thrombocytopenia occurs irrespectively of the severity of the mutation and is possibly caused by instability of mutated WASP in platelets.11 Despite intensive research, the mechanisms underlying WASP-related thrombocytopenia and hemorrhages are not completely understood. Megakaryocyte numbers have been reported to be normal in the majority of WAS patients,12-14 whereas proplatelet formation depending on actin polymerization and formation of branching structures is conserved when tested in vitro and ex vivo cultures.12 Peripheral destruction of platelets in the spleen is thought to play an important role in thrombocytopenia because a substantial correction of the platelet count and size after splenectomy has been reported.15 The accelerated destruction could be caused by an intrinsic defect of WASP-deficient platelets, showing an increased surface exposure of phosphatidylserine, or could be mediated by autoimmune reaction because of the presence of antiplatelet antibodies reported in patients and in the murine knockout model,13 although the latter hypothesis is still a matter of controversy in the field. Finally, defects in filopodia and podosomes could play an additional role in migration of megakaryocytes from the endosteeal to the perivascular niche within the bone marrow and during proplatelet formation. Indeed, ectopic release of platelets within the bone marrow of Was−/− mice has been recently demonstrated.16 Overall, these findings indicate that a full comprehension of the mechanisms causing thrombocytopenia still needs to be achieved.
Eczema in WAS

The typical skin lesions in WAS and XLT patients resemble acute or chronic eczema in appearance and distribution. Eczema develops in 80% of the patients and is heterogeneous in severity and persistence. Indeed, in its most severe form, eczema is resistant to therapy, persists into adulthood, and facilitates opportunistic skin infections (Molluscum contagiosum, herpes simplex, or bacteria). The incidence and severity of eczema are significantly lower in patients with residual WASP expression. The causes of eczema in WAS patients are currently unknown. WAS patients often have elevated IgE levels and develop allergies, therefore suggesting an atopic origin. Recently, an imbalance in cytokine production toward the Th2 type has been described in WAS patients' T-cell lines and might contribute to the pathogenesis of eczema and allergy. Abnormal priming of antigen-specific T cells in the skin caused by defective chemotaxis of dendritic cells (DCs) and Langerhans cells may also play a role.

Autoimmune manifestations

WAS-associated autoimmune complications are frequently observed. The incidence of autoimmunity in classic WAS is high in the US and European populations (40%-72%), whereas a lower incidence was reported in Japan (22%). The most common manifestations are autoimmune hemolytic anemia, cutaneous vasculitis, arthritis, and nephropathy. Less common autoimmune manifestations include inflammatory bowel disease, idiopathic thrombocytopenic purpura, and neutropenia. Patients frequently have multiple autoimmune manifestations at the same time. Development of autoimmunity can have a prognostic value. Indeed, it has been reported that WAS patients who develop autoimmune hemolytic anemia or autoimmune thrombocytopenia early (<180 days) after splenectomy have a poor prognosis.

Moreover, autoimmunity is associated with a higher risk of a later development of tumors and an increased risk of mortality.

Until now, the mechanisms of WAS-associated autoimmunity have not been clarified. It has been proposed that autoimmunity could be the result of a bystander tissue damage originating from the chronic inflammatory state that is established after incomplete pathogen clearance. Another possible cause is the loss of central or peripheral tolerance to self-antigens. Indeed, several groups, including ours, have recently described a defective localization and function of naturally occurring CD4+CD25+FOXP3+ regulatory T cells (Tregs) in the absence of WASP.

Tumors in WAS

Two distinct surveys report a tumor incidence of 13% and 22% in WAS patients. Tumors can arise during childhood (especially myelodysplasia) but are more frequent in adolescents and young adults. WAS-associated tumors are mainly lymphoreticular malignancies, with leukemia, myelodysplasia, and lymphoma (often Epstein-Barr virus [EBV]-positive) resulting in up to 90% of the cases. WAS-associated malignancies have a poor prognosis because less than 5% of patients survive 2 years after diagnosis, and result in up to 25% of death cases. However, current chemotheraphy protocols, including anti-CD20 monoclonal antibody, can result in higher cure rates. Immune deficiency can contribute to the genesis of tumors. Indeed, defective NK-cell functions, as well as other alterations of immune surveillance, may play a key role in the susceptibility to tumor development. In addition, an activating mutation in the Cdc42-binding site of WASP, resulting in X-linked neutropenia, reveals a role of WASP in regulation of cytokinesis and genomic stability in human cells, leading to the hypothesis that WASP mutations may directly alter cellular homeostasis.

WAS-XLT clinical scoring system

The severity of WAS-associated symptoms can be estimated through a scoring system originally developed by Zhu et al. and slightly refined in subsequent work. A score of 0.5 or 1, assigned to patients with intermittent or chronic thrombocytopenia and small platelets, and a score of 2, assigned to patients with additional findings of mild, transient eczema or minor infections, identify XLT patients. Those with treatment-resistant eczema and recurrent infections despite optimal treatment receive a score of 3 (mild WAS) or 4 (severe WAS). Regardless of the original score, if a patient develops autoimmune disease or malignancy, a score of 5 is attributed. Scores 5A and 5M indicate a score of 5 with autoimmune disease (A) or malignancy (M), respectively. For a schematic summary of the scoring system, refer to Table 1.

Cellular defects in WAS

Hematopoietic stem cells

Early studies conducted on human samples have demonstrated that WAS RNA is transcribed already at the stage of CD34+ hematopoietic stem cells (HSCs), and its expression is maintained throughout hematopoietic differentiation. Nonetheless, the cytologic appearance of bone marrow of WAS patients is often normal, and CD34+ cells isolated from a WAS patient can differentiate into normal numbers of myeloid colonies in vitro. Therefore, WASP might be dispensable in early hematopoiesis.

The study of female carriers of a mutated WAS allele shed light on the function of WASP in HSCs. Indeed, a nonrandom X-inactivation in CD34+ cells and in mature hematopoietic cells was observed in these persons, leading to the hypothesis that WASP may play a role in the lyonization process. However, a direct role for WASP in X-chromosome methylation has never been demonstrated. Alternatively, nonrandom X-inactivation in the bone marrow could be explained by a migratory defect of...
WASP-null HSCs. Indeed, competitive transplantation experiments indicated a role of WASP in the migration of HSCs from the fetal liver to the bone marrow, and for their engraftment. However, these results have been significantly challenged, at least in the murine model, by 2 reports showing lack of advantage for WASP+ hematopoietic progenitor cells in Was−/− female mice. In addition, contrast to the skewed pattern observed in WAS female carriers, a random pattern of X-inactivation was detected in carriers of XLT. The information deriving from gene therapy studies will be crucial to define whether human WASP+ HSCs show a significant engraftment advantage over WASP-null cells.

**T-cell defect in WAS**

T-cell defects, hampering both effector and helper functions, are thought to play a crucial role in WAS-associated immunodeficiency. WASP plays a key role in T-cell activation and actin cytoskeleton remodelling after the engagement of the T cell receptor (TCR), and the costimulatory molecules CD28 and CD2. T-cell activation is regulated by the formation of the immunologic synapse (IS), a polarized cluster of TCR, costimulatory molecules, signaling molecules, and integrins at the T cell–antigen-presenting cell interface. The IS is a symmetric structure organized in concentric rings, with the TCR, the TCR-associated molecules, and costimulatory molecules residing in the center, whereas integrins are localized in the outer ring. Larger molecules, such as CD45 and CD43, which may interfere with synapse assembly through steric hindrance, are actively excluded from the IS. To promote their lateral movement on the plasma membrane, the molecules being recruited to the IS are associated with specific cholesterol-enriched membrane microdomains, called lipid rafts. After TCR engagement, WASP is promptly recruited to the lipid rafts at the IS through WIP, and is activated in situ by GTP-Cdc42. In addition, WASP recruitment to the IS could be mediated by CD2 through the adaptor molecules CD2AP and PSTPIP1.

In the absence of WASP, IS can be formed only after strong TCR stimulation. In addition, lipid raft dynamics during IS formation and IS stability are compromised. Another level of regulation of T-cell activation is achieved by prompt internalization of the TCR and CD28 costimulatory molecule after specific engagement, functions that are defective in WASP-deficient cells. As a consequence of impaired signaling through the TCR and costimulatory molecules, T cells from WAS patients and Was−/− mice show defective proliferation as well as impaired secretion of interleukin-2 and Th1 cytokines. These defects are associated with delayed nuclear factor of activated T cell (NFAT) nuclear translocation and defective T-bet induction. In addition to its role in T-cell activation, WASP is also critical for T-cell chemotaxis in vitro in response to stromal cell–derived factor-1α and in vivo homing to secondary lymphoid organs. A reduction in the number of circulating naïve CD4+ and CD8+ T cells may be present in WAS patients, especially at a young age, contributing to the immunodeficiency. On the other hand, recent studies have highlighted that WASP is dispensable for thymic generation of T cells in mice. Indeed, Was−/− mice have a relatively normal thymic development, but abrogation of both WASP and N-WASP function through a dominant-negative portion of WASP, or simultaneous knockout of N-WASP, caused the block of thymocyte maturation at the DN3 stage. Thus, N-WASP can complement WASP deficiency to promote the generation of normal numbers of T cells. In addition, the observation that the TCR Vβ repertoire is normal in young WAS patients suggests that WASP absence does not impair thymopoiesis qualitatively. In the same study, it was observed that the TCR Vβ repertoire of WAS patients becomes skewed after 15 years of age. This finding supports the hypothesis of defective T-cell survival in the periphery. Indeed, T lymphocytes isolated from the blood of WAS patients are abnormally prone to spontaneous in vitro apoptosis because of decreased Bcl-2 or increased Fas levels. Despite the above information, the precise relationship between T-cell abnormalities and WAS-associated immune deficiency, autoimmunity, and cancer remains to be elucidated.

**Naturally occurring T regulatory cells and the pathogenesis of autoimmunity**

Autoimmunity is a serious and frequent complication in WAS patients and could be caused by defective peripheral tolerance because of alterations in generation or function of T regulatory cells. Tregs play a key role in suppressing immune responses and in maintaining immunologic homeostasis. Tregs have been shown to prevent autoimmune diseases, to down-modulate immune response to allergens, pathogens, and cancer cells, and to mediate peripheral transplantation tolerance. The best characterized subset of Tregs are the CD4+CD25+FOXP3+ natural Tregs (nTregs), whose differentiation, peripheral survival, and function are controlled by TCR engagement, CD28 engagement, FOXP3, and interleukin-2. nTregs are generated in the Hassall corpuscles within the thymic medulla, where autoreactive T cells may interact with thymic stromal lymphopoietin-producing DCs and acquire a regulatory phenotype, instead of undergoing negative selection. By this mechanism, nTregs express a broad repertoire of high-affinity TCR, recognizing self-antigens, tumor-associated antigens, and pathogen-derived antigens. The mechanisms by which nTregs are activated and mediate suppression of effector CD4+ and CD8+ T cells are still a matter of intense investigation. However, in some experimental settings, they mediate suppression through release of inhibitory cytokines, induction of cytosis, metabolic interference, and modulation of DC maturation and function. It is now well established that quantitative and qualitative defects in Tregs may result in the pathology. Indeed, there is evidence that skewing of antigen-specific T cells toward a regulatory instead of a Th1 or Th2 phenotype plays a key role in the maintenance of homeostasis and prevention of autoimmunity and allergy. Therefore, it is possible that defects in nTreg generation and function could be associated with the development of autoimmunity and the unbalanced Th2 response in WAS patients. To determine whether the absence of WASP is associated with nTreg cell dysfunction, we and others have characterized nTreg cells isolated from Was−/− mice and WAS patients. WASP appears to be dispensable for thymic development and steady-state distribution of nTregs to the periphery, although selective advantage for WASP expressing nTreg cells has been reported in competitive settings. Despite a relatively normal number of CD4+FOXP3+CD25+ cells in spleens of Was−/− mice and in peripheral blood of patients, nTregs show a marked defect in in vitro and in vivo suppressor function. Indeed, in vitro experiments showed that Was−/− nTregs had a significantly reduced capacity to suppress wt effector T-cell proliferation. After strong TCR stimulation, a residual suppressor activity of Was−/− nTregs on wt effector T cells could be detected suggesting that the in vitro dysfunction of Was−/− nTregs is mainly the result of an activation defect. Despite the
master role of WASP in immune synapse assembly, we showed that, in contrast to effector T cells, murine nTregs failed to polarize F-actin and concomitantly WASP to the site of contact with anti-CD3 monoclonal antibody-loaded antigen-presenting cells or beads coated with anti-CD3 and anti-CD28 monoclonal antibodies, therefore excluding a role of WASP in this process. On the other hand, it cannot be excluded that nTregs assemble a structurally different immunologic synapse or that its assembly follows different kinetics. It is also possible that the activation and functional defects of Was−/− nTregs are independent from immunologic synapse formation and are the result of defects in signaling downstream the TCR. Besides defective in vitro nTreg suppression activity in the mouse model and in patients, impaired in vivo suppressive function of Was−/− nTregs has also been demonstrated. Indeed, Was−/− nTregs display a defective in vivo suppressor activity toward colitis induced by transfer of wt CD45RBhi cells. In line with that, spontaneous genetic reversion of WAS mutations in nTregs of a WAS patient correlated with amelioration of autoimmunity. In addition to an intrinsic dysfunction in mediating suppression, WASP-deficient nTregs may also display impaired migration, survival, and/or proliferation in vivo. Indeed, we demonstrated that Was−/− nTregs transferred in wt recipients were unable to reach lymph nodes draining the site of OVA immunization and thus modulate an in vivo response to OVA. Consistently, WASP expression confers selective advantage to nTregs, in both the Was−/− murine model and in a revertant patient. The selective advantage for nTregs appeared to be stronger than that occurring in naive T lymphocytes. Given the key role played by these cells in maintaining peripheral tolerance, all these findings suggest that nTreg dysfunction might participate in the high susceptibility of WAS patients to developing allergies and multiple autoimmune disorders.

**DCs in WAS**

The complex immunodeficiency observed in WAS partially results from defective cellular trafficking and impaired antigen uptake. The effects of WAS gene mutation on DCs, which are antigen-presenting cells, result in severe alterations in migration, antigen presentation, cell adhesion, and T-cell priming. Indeed, the ability of DCs to migrate through tissues and endothelial vessels depends on complex mechanisms involving reorganization of the actin cytoskeleton in response to different stimuli. Because WASP has a crucial role in controlling actin architecture and rearrangement, many defects in macrophages and DCs have been reported. Indeed, the initial response to a stimulus consists of cellular polarization, lamellipodia and filopodia formation, and subsequent adhesion. In addition, DCs assemble podosomes, special adhesion structures showing a unique organization with actin filaments forming foci containing different elements, such as the actin nucleating factor Arp2/3 complex, and WASP surrounded by a ring of integrins and integrin-associated proteins. Many groups have investigated the role of WASP in the formation of podosomes in monocyte-derived DCs, in macrophages and osteoclasts. Analysis of these cells in WAS patients reveals a dramatic lack of podosomes. Interestingly, podosome defect results in alteration of β2 integrin localization, which remains dispersed with consequent decreased adhesion to ICAM-1, a ligand for β2 integrin. These findings suggest that WASP plays a crucial role in providing a platform for integrin organization at the cell membrane. Moreover, Was-deficient DCs and macrophages fail to form leading lamellipodia, resulting in a defective chemotactic response to different chemoattractants, such as FMLP, MCP-1, and macrophage inflammatory protein-1α. In particular, the migratory response of Was mutant DCs toward the CCR7 ligands, CCL21 and CCL19, which are highly expressed on high endothelial venules and lymphatic endothelium, is severely decreased. Similarly, chemotaxis of Was-deficient immature DCs to CCL3, important for mobilization to inflammatory sites, is significantly reduced compared with controls. In addition, Was−/− DCs do not spread normally and fail to form persistent leading edge, resulting in defective directional migration and inefficient homing of Was−/− DCs into draining lymph nodes. Finally, recent reports describe that, in addition to the impaired motility, Was−/− DCs show defective interaction with and activation of T cells in lymph nodes. Indeed, DCs from Was-deficient mice are impaired in presenting antigens to naive CD8+ T cells after immunization with DEC205, especially at low doses. These data highlight the relevance of WASP in DCs during the first phases of adaptive immune response and, together with altered migration of Was−/− DCs, could contribute to the abnormal function of these cells.

**Role of WASP in B cells**

The role of WASP in B cells has not been explored in detail until recent years. Initially, reports carried out primarily on Was−/− murine models did not evidence defects in terms of development, number, and functionality of WASP-deficient B cells. Nonetheless, an intrinsic B-cell defect appears evident because of the abnormal distribution of serum immunoglobulin classes and the inability of severe WAS patients to respond to T-independent antigens, such as polysaccharides, for which T-cell help in triggering immune response is not required.

In contrast to that observed in T lymphocytes, activation of WASP-deficient B lymphocytes can occur normally. Indeed, measurement of calcium fluxes after B-cell receptor engagement highlighted a mild defect in WASP-deficient EBV-transformed B-cell lines, but not in primary B cells isolated from WAS patients. Moreover, B cells isolated from Was−/− mice did not show any activation defect. The description of B-cell anomalies was mainly focused on the defective cytoskeletal-dependent processes of WASP-deficient B cells, leading to decreased migratory ability, adhesion, and formation of long protrusions. In addition, defective cytoskeletal reorganization has been shown in EBV-transformed B-cell lines from WAS patients. Consequently, the decreased motility of WASP-deficient B cells has been considered the main cause of findings showing diminished B-cell number in peripheral blood and secondary lymphoid organs of WAS patients and later confirmed in the Was−/− murine model.

Recently, reports from Remold-O’Donnell’s group on a large number of WAS patients revealed an early deficit of B cells starting from infancy and indicating a defective cellular output. Their subsequent characterization of B-cell defect in WAS patients led to identification of a phenotypic perturbation with regard to complement receptors and CD27. Indeed, WAS patients’ B cells show a markedly reduced expression of CD21/CD35 receptors that may be responsible for impaired ability of antigen capture and presentation. In addition, compared with age-matched normal controls, WAS patients present a reduced amount of CD27+ postgerminal center B cells, indicating a defective differentiation, despite normal amount of class switching.
The underlying causes of these alterations are unknown, but recently, 2 reports performed in Was⁻/⁻ mice have clearly indicated a role for WASP in peripheral homeostasis of mature B-cell subsets.22,33 Indeed, WASP seems to be dispensable for early B-cell development, whereas its deficiency is detrimental for completion of B-cell maturation, starting from transitional stage and affecting in particular splenic marginal zone and peritoneal B1a cells. This phenotype appears to be caused by a defective homeostasis and/or retention of mature B cells, rather than increased apoptosis of WASP-deficient B lymphocytes.22,33

Importantly, B-cell involvement in the pathogenesis of autoimmune manifestations in WAS still needs to be addressed. A recent European study on the long-term outcome of bone marrow transplantation in WAS patients indicated mixed/split lymphocyte chimerism as a strong risk factor to develop autoimmunity.79 Indeed, in WAS patients, the development of autoimmune diseases is found to be associated with high levels of circulating IgM.80 Moreover, Humbert-Baron et al have recently shown that Was⁻/⁻ mice have circulating autoantibodies against single-strand DNA and double-strand DNA, providing the first evidence of an alteration of B-cell tolerance in Was⁻/⁻ mice.95 These alterations were confirmed in our unpublished observations in Was⁻/⁻ mice where we found an increased amount of serum immunoglobulins, in particular of IgG2a, a subclass that plays a critical role in the pathogenesis of humoral autoimmunity (M.B., manuscript in preparation).

Decreased complement receptor expression is intriguing evidence as it could predispose WAS patients to developing autoimmune manifestations.78 Several lines of evidence point toward the involvement of reduced CD21/CD35 in induction of autoimmunity. Indeed, CD21/CD35 expression is decreased in B cells of patients with systemic lupus erythematosus80 and also in the murine model of systemic lupus erythematosus before the development of clinical disease, suggesting a role of this alteration in break of self-tolerance.81,82

In conclusion, further comprehension of the intrinsic defects of Was⁻/⁻ B cells appears to be crucial to fully understand the pathophysiology of WAS and to develop more efficacious therapies.

Current therapeutic approaches and perspectives for gene therapy in WAS

Currently, the only curative therapeutic option for WAS patients is hematopoietic stem cell transplantation (HSCT). When a related human leukocyte antigen-identical donor is available, HSCT leads to more than 80% survival rate.83-86 On the other hand, transplantation using the bone marrow of a mismatched related donor results in a decreased survival rate.83-86 In addition, this type of transplantation is associated with an elevated risk of developing life-threatening EBV⁺ lymphoproliferative syndrome, infections, autoimmunity, and graft-versus-host disease.84 When a suitable related donor is not available, bone marrow or cord blood transplantation from a matched unrelated donor is a valid therapeutic option, leading to a 71% to 81% survival rate.23,84,85 A better outcome for matched unrelated donor and matched related donor HSCT in patients younger than 5 and 2 years of age, respectively, has been reported, suggesting that transplantations should be performed early in life.79,84,85 Successful HSCT with establishment of full chimerism leads to restoration of immune and hemostatic functions. In patients with stable mixed chimerism in both myeloid and lymphoid compartments, clinical conditions usually improve.73,87 Despite advances in HSCT, patients lacking a matched donor still need the development of alternative approaches. Therefore, the implementation of new therapeutic strategies, such as transplantation of autologous gene-corrected hematopoietic stem cells, is highly desirable because it will avoid rejection and graft-versus-host disease and could be applicable to all WAS patients lacking a suitable bone marrow donor, allowing the timely treatment of the disease. The rationale for gene therapy is also supported by the observation of frequent spontaneous somatic revertants conferring selective advantage to WASP-expressing cells.88 At present, retroviral vectors based on the murine Moloney leukemia virus have been used for the treatment of patients with SCID-X1, ADA-SCID, and X-CGD.99,101 In vitro studies on human WASP-deficient B- and T-cell lines transduced with oncoretroviral vectors have demonstrated the restoration of proliferative response to anti-CD3 and cytoketremodelling.90,94 Moreover, the availability of Was⁻/⁻ mice has allowed assessing the efficacy of WAS gene therapy in vivo. In early studies, Was⁻/⁻ bone marrow cells were transduced with murine WASP-encoding retroviral vectors and injected into lethally irradiated Rag2⁻/⁻ or Was⁻/⁻ recipient mice.94,95 After successful engraftment and multilineage differentiation of transduced cells, TCR-driven T-cell proliferation as well as cytokine production were improved.95,96 Klein et al demonstrated an attenuation of the colitogenic potential of Was⁻/⁻ T cells,95 and Strom et al reported the normalization of a secondary immune response to influenza virus after gene therapy.96 Very recently, a gene therapy trial for WAS was initiated in Germany using an MLV-derived retroviral vector encoding the full WASP cDNA. Preliminary data from the first 2 patients 18 months after gene therapy indicate amelioration of the clinical phenotype with correction of thrombocytopenia and resolution of eczema and autoimmunity.97 The use of Moloney leukemia virus vector in the context of WAS raises several concerns in view of the adverse events that occurred in X-SCID and X-CGD gene therapy trials.90,98,99 Because lentiviral vectors have been demonstrated to be less genotoxic than retroviral vectors,102 we and others have developed a gene transfer approach based on a lentiviral vector encoding the human WAS cDNA under the control of the human WAS endogenous promoter.101,102 We have demonstrated that our lentiviral vector encoding 1.6-kb fragment of the human WAS endogenous promoter (w1.6WLV) is able to successfully restore WAS expression in CD34⁺ HSCs, T cells, B cells, and DCs, and to correct TCR-driven activation in T-cell lines derived from WAS patients.29,103 In addition, in vivo studies performed in 2 different Was⁻/⁻ strains showed evidence of long-term multilineage WASP expression in hematopoietic cells and correction of T- and B-cell functions.104,105 Importantly, long-term observation of a large cohort of gene therapy–treated mice did not display any severe adverse event related to vector toxicity.105 All these findings represent a critical step in moving toward the implementation of a lentiviral vector-mediated gene therapy clinical trial.

In conclusion, WAS is a severe immunodeficiency with a characteristic variable clinical phenotype. The molecular bases of this primary immunodeficiency have been extensively explored, and many cellular defects have been reported as responsible of the immunologic phenotype, whereas pathogenesis of autoimmune immunity, malignancies, and thrombocytopenia still remain to be fully understood. Nonetheless, the recent advances
in the comprehension of molecular and cellular mechanisms have been crucial, not only in understanding the biology of this syndrome, but also in providing new and efficacious therapeutic tools.

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References

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