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REVIEW ARTICLE

Review Series: Primary Immunodeficiency and Related Diseases

Wiskott-Aldrich Syndrome; An X-Linked Primary Immunodeficiency Disease with Unique and Characteristic Features

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ABSTRACT

Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency disease with unique and characteristic features. In 1994, the responsible gene for WAS, the *WASP* gene on X-chromosome, was identified. Since then, renewed clinical and basic researches of WAS have started and remarkably developed. I will comment on recent progress in the clinical and basic researches of WAS, including some topics reported by our and other groups.

KEY WORDS

gene therapy, hematopoietic stem cell transplantation, immunodeficiency, WASP, Wiskott-Aldrich syndrome

INTRODUCTION

Wiskott-Aldrich syndrome (WAS) is an X-linked primary immunodeficiency disease (PID) characterized clinically with the triad; immunodeficiency, bleeding tendency with microthrombocytopenia and severe eczema. An increased risk for autoimmune disorders and lymphoid malignancies is another characteristic clinical features. In 1937, Wiskott A., a German pediatrician, originally reported three brothers who presented shortly after birth with thrombocytopenia, bloody diarrhea, eczema and recurrent ear infections.[1] All the three brothers died less than 2 years old of intestinal bleeding and sepsis. In 1954, Aldrich R.A. and others reported six generations of the family members, 16/40 males but no females, died with similar clinical features that described by Wiskott, indicating the disease inherited as X-linked trait.[2] Clinical features and severity of WAS patients are highly varied, resulting in difficult to make diagnosis in early infancy especially for milder cases. Indeed, only one third of the patients show all the triad. A breakthrough in clinical and basic researches of WAS has been started since 1994, when the responsible gene for WAS, the *WASP* gene, was identified.[3] *WASP* consists of 12 exons, encodes a 502 amino-acid protein expressing only in hematopoietic lineage cells, and contains several unique domains corresponding to actin polymerization and signal transduction. Since then, the definite diagnosis of WAS has been possible for suspected cases, and researches of molecular pathogenesis for WAS have started and remarkably developed. In this review, I will comment on recent progress in the clinical and basic researches of WAS, including some topics reported by our and other groups.

WAS IN PID CLASSIFICATION AND WAS PHENOTYPES

WAS belongs to the category of "well defined syndromes with immunodeficiency" in the latest classification in the report from the international union of immunological societies expert committee for primary immunodeficiencies.[4] In the category, six sub-

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Table 1 Clinical phenotypes and associated with mutations of *WASP*

	WAS	XLT	IXLT	XLN
Phenotypes				
Clinical scores	3, 4, 5A/M	1, 2, (5A/M)†	<1	0
Thrombocytopenia	+	+	-/+	-
Small platelets	+	+	+	-
Eczema	+	-/+	-	-
Immunodeficiency	+	-/(+)	-	+‡
Autoimmunity	Frequent	Possible†	-	-
Malignancy	Frequent	Possible†	-	-
WASP mutation	Nonsense, Missense, Frame shift caused by small deletion/insertion, Splicing defects	Missense, Inframe small deletions/insertions, Splicing defects	Missense	Specific missense disrupting autoinhibition; L270P, S272P and I294T
WASP expression	Absent or truncated	Reduced	Normal quantity	Normal quantity

† XLT patients may progress to clinical score 1 or 2 into 5 (A: autoimmunity, M: malignancy).

‡ Immunodeficiency in XLN patients results from severe neutoropenia, different from immunodeficiency in WAS.

classified groups are included with common key words such as DNA repair defects, thymic defects and so on. WAS is the only disease in one subclassified group without the key words, thus, WAS is a unique PID.

Clinical features and severity of WAS patients are highly varied resulting from the type of mutations in *WASP* gene. X-linked thrombocytopenia (XLT), having been presumed a variant disease of WAS with isolated thrombocytopenia sometimes associated mild eczema, was turned out to be caused by mutation in *WASP*, confirming that XLT and WAS are allelic diseases.[5] The scoring system (0 to 5) to define clinical phenotype associated with *WASP* gene mutations was proposed.[6] Based on the score, we can predict the prognosis, thus provide an appropriate therapy for each patient.[7] Recently, X-linked severe congenital neutropenia (XLN), totally different clinical features from WAS/XLT, was revealed being caused by gain of function-mutations of WASP.[8] Then, XLN was added to the scoring system as "0", although it looks incongruity presenting there. Clinical phenotypes associated with mutations of *WASP* gene are shown in Table 1.

WASP; STRUCTURE, FUNCTION AND MUTATIONS

WASP is a member of a family of proteins involved in signaling and cytoskeletal organization that includes N-WASP and Scar/WAVE.[9,10] WASP plays a critical role in linking cellular signals that activate Cdc42 to the actin cytoskeleton. WASP possesses an Ena-Vasp homology domain 1 (EVH1), also referred to as a WASP homology domain 1 (WH1), a short basic region (BR), a Cdc42/Rac GTPase binding domain (GBD), a proline-rich region (PRR), a G-actin-binding verprolin homology (V) domain, a cofilin homology (C) domain and a C-terminal acidic (A) segment (Fig. 1a). In resting cells, WASP exists in an autoinhibited inactive state and WASP interacting protein (WIP) binds to the EVH1 domain and stabilizes WASP.[11] Many signals that activate WASP to relieve this autoinhibition: these include binding of Cdc42 to the GBD domain, phosphorylation, and binding of SH3 domains containing proteins (such as Nck) to the proline-rich region. Disruption of the autoinhibited conformation enables actin related protein (Arp) 2/3 binding and actin polymerization (Fig. 1b).

Approximately 300 different mutations in *WASP* have been reported. Those mutations are distributed throughout all area in the gene, but clustering in the first 4 exons and exons 7 and 10. Then, analyzing a large number of patients with WAS/XLA/XLN has made us possible to establish a strong association of genotype-phenotype relation.[12-15] Mutations for WAS are various, but patients with WAS show lack or sparse of WASP in the cells. Common mutations for XLT are missense mutations in exons 1-2 or splice site mutations, those result in reducing but remaining WASP level. In contrast, mutations for XLN are unique; thus far, only three missense WASP mutations; L270p, S272P and I294T have been identified.[16] All the three mutations are gain-of function types, which lead to disrupt the hydrophobic fold, loss of the auto-inhibited structure of inactive WASP, and constitutive availability of the carboxy-terminal VCA-segment for actin-polymerization.

CLINICAL FEATURES

The triad of WAS, susceptibility to infection due to various immunodeficiency, bleeding tendency with micro-thrombocytopenia and severe eczema, is famous clinical features. However, it would be necessary to notice that only one third of WAS patients rep-

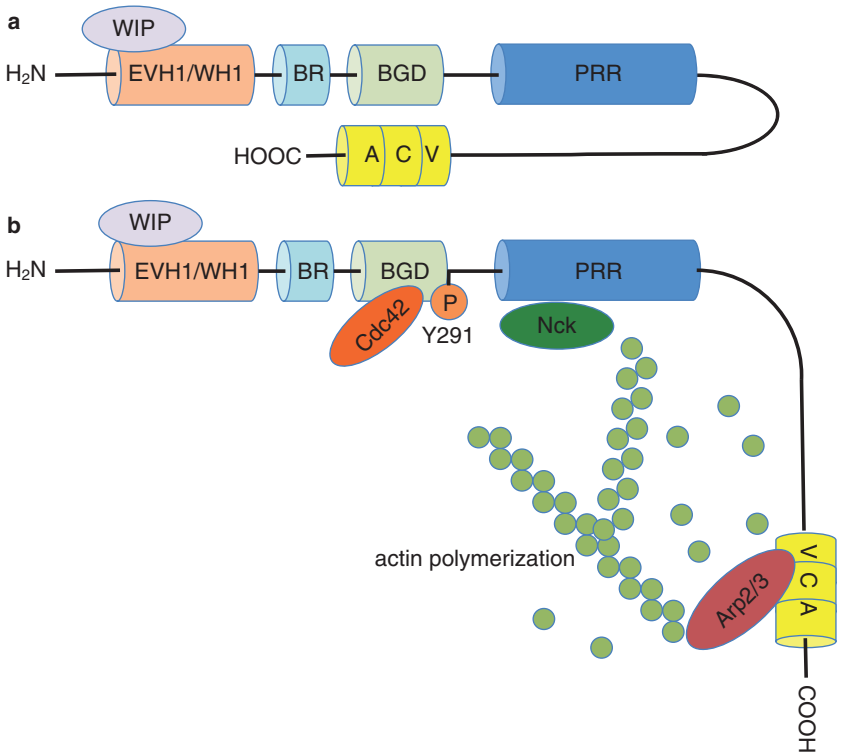

Fig. 1 WASP structure and function. **a.** Inactive form of WASP. **b.** Active form of WASP. The VCA domain is bound to the BGD to form an autoinhibitory conformation. Activation of WASP by CDC42 or phosphorylation of tylosin291 relieves the inactive to the activate form. Disruption of the autoinhibited conformation enables Arp 2/3 binding and actin polymerization. EVH1, Ena-Vasp homology domain 1; WH1, WASP homology domain 1; BR, basic region; GBD, a Cdc42/Rac GTPase binding domain; PRR, a proline-rich region; V, verprolin homology domain; C, cofilin homology domain; A, acidic segment; WIP, WASP interacting protein.

resent the triad especially during their infancy. Older WAS patients reveal an increased risk for autoimmune disorders and lymphoid malignancies, that is another characteristic clinical features.

DEFECTS OF IMMUNE SYSTEM

WAS patients with the clinical score more than 3 show high susceptibility to various pathogens. Main pathogens involved are herpes viruses, some bacteria including capsule forming such pneumococcus, fungus, pneumocystis jeroveci and others. Defects of immune system in WAS are complicated and various. Since WASP is expressed exclusively in hematopoietic cells, and is a key molecule of the actin polymerization and actin-regulating signals, defective immunological function is observed in most immune cell lineages including T cells, B cells, monocytes/macrophages/dendritic cells (DCs), and granulocytes. The defects are based on impaired cell migration and cytoskeletal rearrangement, those affects immune cell effector function and immunological synapse formation. Some typical cases of WAS show low IgM, high IgA and IgE, albeit exceptional cases are also observed. Antibody responses to polysaccharide antigen would be reduced in some cases including low isohemagglutinin titers. Peripheral blood lymphocyte number often decreases over time, especially CD4+ and CD8+ T cells due to accelerated cell death due to spontaneous apoptosis resulting from attenuated expression of Bcl-2.[17] Poor proliferation responses to immobilized anti-CD3, mimicking stimulation through T cell receptors, are commonly observed.

PLATELET ABNORMALITIES

Bleeding such as bloody diarrhea, epistaxis, purpura, intracranial hemorrhage due to thrombocytopenia is most commonly observed clinical manifestations. Because platelet associated IgG is detected in some cases, such WAS patients are sometimes misdiagnosed and mistreated as immune thrombocytopenic purpura (ITP). In this sense, evaluation of platelet volume would be important for the correct diagnosis of WAS; microthrombocyte in WAS versus large to normal size thrombocyte in ITP. Although an intrinsic

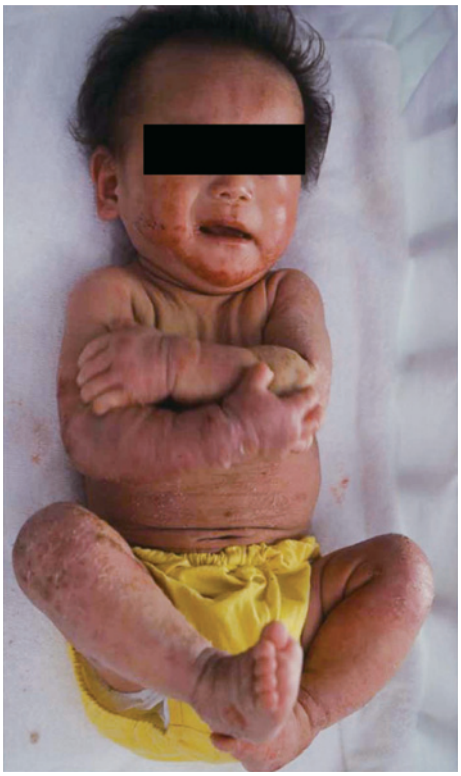

Fig. 2 Severe atopic like eczema in a patient with WAS. Male infant with WAS representing severe atopic like dermatitis with petechial hemorrhage.

platelet defect related to abnormal WASP, such as abnormal thrombopoietin (TPO) signaling, is proposed,[18] production of platelets is normal in most WAS patients. Destruction of platelets in spleen is considered as major mechanism of thrombocytopenia in WAS, because WAS patients receiving splenectomy show correction of platelet number and size.[19]

ECZEMA

Eczema develops in 80% of WAS patients (Fig. 2).[12] Eczema varies in severity and persistence, and sometimes shows poor response to the standard therapy for eczema. Topical tacrolimus would be effective in some WAS patients with severe eczema.[20] The pathogenesis of eczema in WAS remains unknown, however, the similar basis for atopic dermatitis could be considered, because high level of IgE and imbalanced cytokine production patterns (Th1 < Th2) are generally observed in patients with WAS.[21]

AUTOIMMUNE DISEASES

WAS patients frequently (40-70%) develop autoimmune diseases.[22,23] The onset age is less than 2 years in most cases, however, some autoimmune manifestations may develop later. The most common complications reported are autoimmune hemolytic anemia, neutropenia and thrombocytopenia (autoimmune type), cutaneous vasculitis, arthritis, nephritis[24] and inflammatory bowel diseases. Although less frequently, XLT patients also develop the similar autoimmune complications.[7] In those cases of XLT, the clinical score of 1 or 2 will progress into 5(A). The precise mechanism of autoimmunity has not fully been understood. Since WASP is committed to various immune cell (T cells, B cells, NK cells, DCs) functions, dysfunction of WASP may destabilize pivotal mechanisms participating in the maintenance of immune tolerance. Recent studies indicated that WAS patients have the defective peripheral tolerance due to dysfunctional WASP-defective regulatory T cells (Tregs).[25]

MALIGNANCIES

Malignant diseases, mainly lymphoreticular type such as lymphoma, leukemia, myelodysplasia, are observed in 10-20% of WAS patients.[12,23] We also reported a fatal case of Epstein-Barr virus associated lymphoproliferative disorder (EBV-LPD).[26] Malignancies can develop during early in childhood, but after their adolescence in most cases. Immunodeficiency is prone to malignant diseases, however, the basis for high incidence of malignancy in WAS has not yet been clarified. Impaired function of cytotoxic T cells and NK cells, observed in WAS patients may in part contribute to high incidence of malignancies. The incidence of malignancies in XLT patients is lower than that of WAS patients, but still significant. Furthermore, it is to note that patients with some specific *WASP* mutation [the introns 6 (+5), g to a], although their immune defects seemed to be mild, showed high incidence (44%) of lymphoma.[27] Thus, the mechanisms of oncogenesis in WAS patients are thought to be complex and multi-factorial.

DIAGNOSIS

Since a clinical feature and severity of WAS patients are highly varied, the diagnosis of WAS for atypical cases has been clinically difficult. In 1994, the responsible gene for WAS; *WASP* gene, was identified.[3] Since then, the definite diagnosis of WAS has been possible by detection of mutations in the gene. We established the method to evaluate the intracellular WASP level using flow cytometory, and applied it for making diagnosis of WAS patients and carriers (Fig. 3).[28,29] It is to note that WAS-carriers usually show no WASP-negative lymphocytes, but their monocytes consist of a small portion of WASP-negative cells besides a large potion of WASP-positive cells, indicating that WASP-dependency for growth and development would be different among hematopoietic cell lineages. Some patients, especially male infants suspected to have immune thrombocytopenic purpura (ITP), should be considered the possible diagnosis of WAS. Because typical symptoms may not yet occur in WAS patients during infancy. We also reported that

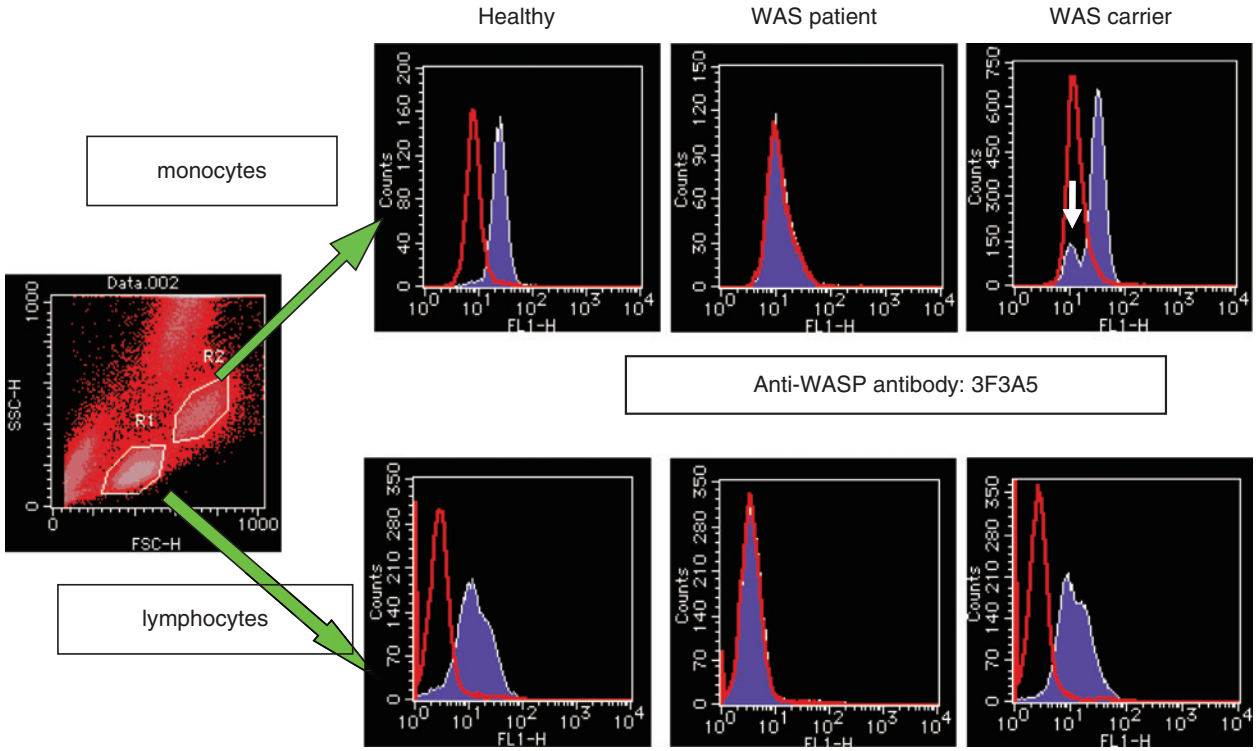

Fig. 3 Flow cytometric analysis of the intracellular WASP level. Intracellular WASP expression was analyzed by flow cytometory using anti-WASP antibody 3F3A5. Lymphocytes and monocytes populations were independently evaluated. No WASP-positive lymphocytes or monocytes was detected in the WAS patient. No WASP-negative lymphocytes, but small portion of WASP-negative monocytes (white arrow) were detected in the WAS carrier. Red line indicates results using a control antibody.

screening by flow cytometry for WASP was recommended for boys with persistent thrombocytopenia of an unknown etiology for excluding and confirming the WAS diagnosis.[30]

REVERSION OF INHERITED MUTATIONS

There are various types of somatic mosaicism reported in patients with genetic and non-genetic diseases.[31] Among them, somatic mosaicism due to reversion to normal of inherited mutations sometimes occur in patients with various PID including adenosine deaminase (ADA) deficiency, X-linked severe combined immunodeficiency (X-SCID) and WAS, some of them show atypically mild clinical phenotypes. We also reported such cases with ADA deficiency[32] and WAS.[33,34] Basis for this phenomenon is thought that a cell making reversion to normal of inherited mutation acquires normal function and "selective advantage", thus expanding its population in vivo to be detected. Now it is well known that this phenomenon is commonly observed in WAS patients.

TREATMENT OF WAS/XLT

Supportive and prophylactic care for the complications including infectious diseases, bleeding, autoimmune diseases and malignancies, those are frequently observed in WAS/XLT patients, is very important. Those include intravenous immunoglobulin replacement, acyclovir for herpes virus, trimethoprim-sulfamethoxazole for Pneumocystis jiroveci, judicious platelet transfusion for bleeding episodes, and so on. Splenectomy to prevent serious bleeding complications is controversial, because it could be effective to increase platelet number, however, the risks of severe bacterial infections will be increased. To date, therefore, splenectomy is not recommended at least for patients planning to receive hematopoietic stem cell transplantation (HST).

Recently, several group reported the results of HST for patients with WAS, including our group.[35,36] Overall 5-year survival is good as approximately 80-90%, however, the results vary depending on recipient's conditions (clinical score, age, complication, etc.), types of donor source and conditioning regimens. Evaluation of the chimerism status after HST is important, because it is associated with an increased risk of incomplete reconstitution and post-HST autoimmunity.[35] In fact, we observed various mixed chimerism status in WAS patients after HST by flow cytometoric analysis, and found that the residual recipient cells were most consistently in the monocyte population.[37] To avoid the mixed chimerism status,

myeloablative conditioning is recommended for WAS patient in good condition at present, although less toxic conditioning should be considered in the near future. As for XLT patients, indication of HST is controversial, however, some cases received HST from suitable donor with good sequel. Considering autoimmune diseases or malignancies could be complicated in some XLT patients, one might consider the indication of HST for XLT more positively.

WAS has been considered as a good candidate for gene therapy, because we can use the same strategy of hematopoietic stem cell gene therapy that used for ADA deficiency and X-SCID. Furthermore, given many reversion cases of WAS as a presumptive evidence, gene introduced cells are expected to show selective growth advantage *in vivo*, which is a key condition for success of the gene therapy at present. Recently, stem cell gene therapy was successfully reported in two WAS patients,[38] however, it is noteworthy that a leukemic event occurred in one of the treated children, with LTR-mediated LMO2 gene transactivation as a consequence of insertion in that locus.[39] This serious adverse event was just the same as those observed in the stem cell gene therapy for X-SCID patients. Thus, safety stem cell gene therapy for WAS is still underway to be established.

CONCLUSION

In this review, I made a comment on the recent progress in the clinical and basic researches of WAS. Although the remarkable advance has been made in the fields of WAS researches, there are still various unsolved subjects to be confronted us.

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