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Review article

The role of WASp in T cells and B cells

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ABSTRACT

Wiskott-Aldrich syndrome (WAS) is a form of primary immunodeficiency (PIDs) resulting from mutations of the gene that encodes Wiskott-Aldrich syndrome protein (WASp). WASp is the first identified and most widely studied protein belonging to the actin nucleation-promoting factor family and plays significant role in integrating and transforming signals from critical receptors on the cell surface to actin remodeling. WASp functions in immune defense and homeostasis through the regulation of actin cytoskeleton-dependent cellular processes as well as processes uncoupled with actin polymerization like nuclear transcription programs. In this article, we review the mechanisms of WASp activation through an understanding of its structure. We further discuss the role of WASp in adaptive immunity, paying special attention to some recent findings on the crucial role of WASp in the formation of immunological synapse, the regulation of T follicular helper (Tfh) cells and in the prevention of autoimmunity.

1. Introduction

WASp is the first identified and most widely studied protein belonging to the actin nucleation-promoting factor family and plays significant role in integrating and transforming signals from critical receptors on the cell surface to actin remodeling. WASp mediates the signaling from membrane receptors to initiate actin polymerization by binding to actin-related protein 2/3 (Arp2/3) in its active conformation [1]. In the resting state, WASp exists in an auto-inhibited conformation by intramolecular interaction, preventing its activation. The C-terminal of WASp mediates the interaction between Arp2/3 and actin monomers following the binding of GTP-bound cell division control protein 42 homolog (Cdc42) and phosphatidylinositol-4,5-bisphosphate (PIP2), which then results in actin remodeling and changes in cell morphology and function [2].

The actin cytoskeleton is a complex network of actin filaments which are constantly polymerizing and depolymerizing in dynamic response to extrinsic signals. In addition to maintaining the cellular scaffold, remodeling of the actin cytoskeleton is critical in the process of signal transduction [3]. Actin remodeling enables the cell to reorganize the signal effectors critical for the abrogation of external signals to specific internal sites. This process further enables the cell to change shape, mobility and polarity in response to external stimuli. Loss of integrity of actin cytoskeleton causes defects in almost every aspect of the innate and adaptive immune response, by impairing cell migration, adhesion, proliferation and phagocytosis of invading pathogens [4].

Patients and murine models of WAS have major T cell defects that contribute to the pathophysiological mechanisms underlying immunodeficiency and immune dysregulation [5–7]. The number of T cells is reduced, and they manifest gross abnormalities in morphology and T-cell receptor (TCR)-induced actin polymerization [8,9]. Abnormal proliferative responses to allogeneic, antigenic, and mitogenic stimuli have also been found in WASp-deficient T cells [10–12]. These T-cell abnormalities are closely associated with the cellular process implicated with WASp and these aberrant functions are the main cause of immunodeficiency found in WAS patients (See Table 1).

Although humoral immune defects are well characterized in WAS, how B cell dysfunction relates to the pathology of WAS is not well understood. Studies focus on B-cell functions in the development of WAS found results from various aspects and led to different interpretation. The lack of an antibody response to polysaccharide antigens is found in B cells from WAS patients especially when unconjugated

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Table 1
The table compares the phenotype of T and B cells with WASp deficiency in critical cellular processes with elucidation of the underlying mechanisms. The similar manifestations in development and migration of WAS T and B cells are mediated by the same molecular mechanism. Although defects in WAS T-cell activation is evident and widely studied, inconsistent changes of BCR-evoked events have been reported in different studies, making it a controversial issue. During differentiation, WAS T cells display prominent Th2 dominance due to reduced IL-2 whose normal transcription is dependent on nuclear WASp. Reduced TI memory B cells and increased CD19hiCD21low B cells have been found and the latter is suspected to play a role in the triggering of autoimmunity in WAS patients. In the aspect of antibody production, recent finding suggests only the elevated IgM in WAS patients is B-cell intrinsic. Insufficient Treg and Breg cells along with increased autoreactive naive B cells contribute to occurrence of autoimmunity. Existing relevant studies together provides possible explanations to reduction and disability of Treg cells and enhanced positive selection in both cellular and molecular level.

Cellular Process	T cell		B cell	
	phenotype	cellular/molecular mechanism	phenotype	cellular/molecular mechanism
Development Migration Activation by TCR/BCR	normal defective defective	the compensatory role of N-WASp lack of activated Arp2/3 in lamellipodia inability to form "foci"; reduced internalization of TCR	normal defective controversial	the compensatory role of N-WASp lack of activated Arp2/3 in lamellipodia different requirement for WASp or different extent of compensatory effect
Differentiation	Th2 dominance	aberrant histone methylation at TBX21 promoter; significantly reduced IL-2 production	reduced TI memory B cells; increased CD19hiCD21low B cells	reduced B1 cells due to defective migration; elusive
Cytokine Secretion	reduced TCR-evoked IL-2 production	impaired binding of NF-AT and AP-1 to the promoter region of IL-2 gene		
Antibody Production			different phenotypes observed in patients, WKO mice and B/WckO mice(see in the main body)	elevated IgA and IgE may be due to abnormal function of Treg cells and DCs; mechanism of B-cell intrinsic elevation of IgM is obscure
Autoimmunity	decreased frequency and impaired function of Treg cells	defective migration due to lack of chemokine receptors: unclear mechanism irrelevant to defective actin remodeling	increased autoreactive naïve B cells; reduced Breg cells	dual engagement of BCR and TLR with hyperreactivity; abnormal development due to defective migration and adhesion

pneumococcal antigens are used [8]. This might be due to the defects in signal transduction cascades [13]. Humoral defects might also be the result of morphological change and reduced adhesion, migration and homing of WAS B cells. Additionally, the reduction in germinal center (GC) has been confirmed in WAS-deficient murine B cell. It can be speculated that B-cell intrinsic defects, rather than the reduction of T-cell help lead to these defects [14]. However, it's probably not the lack of T-cell help, but the intrinsic B-cell defects in functions that contribute to these observed defects. To be specific, study using WASP−/− chimeras with specific WASp deficiency in B cells reveals that the defects in B-cell intrinsic function rather than dysfunction of regulatory T cells (Treg) is the primary triggering factor of autoimmunity which is widely seen in WAS patients [15]. Impaired B-cell tolerance has also been found in WAS patients. Deficiency of WASp and neural Wiskott-Aldrich-syndrome protein (*N*-WASP) of B cells perturbs the humoral homeostasis [16], suggesting their role in the regulation of autoimmunity. However, the in-depth mechanism of many WASp's functions in B cells is still unclear and the role of WASp in B-cell maturation, development and other functions is somewhat controversial [17]. Studies on the typical manifestations of WASp-deficient B cells provide us with insight into the activation, development and signaling process of B cells, thus help us understand B cells function in immune responses and the possible role of WASp in B cells (See Table 1).

It's worth mentioning that the role of WASp in proliferation, differentiation and survival is relatively minimal in B cells. In studies focused on the somatic mosaicism phenomenon, revertant T cells can be readily detected with selective advantage in vivo while revertant B cells cannot in mosaic patients [18,19], which confirm the minimally WASp-dependent selective advantage of B cells compared with that of T cells.

2. WASp structure and activation

The understanding of the structure of WASp helps us identify the specific function of each domain. In the cytoplasm, WASp exists in an auto-inhibited conformation in which the GBD domain conceals the VCA domain, the binding site of Arp2/3 [2,4].

Upon cellular activation such as the activation mediated by the stimulated T cell receptor (TCR) and B cell receptor (BCR), the recruitment of WASp to signaling site on the membrane where adaptors containing specific domains exist occurs. The recruitment of WASp is mediated by the binding of adaptor molecules to the PPP domain, and/or the interaction of Nck or CrkL with WASp-interacting protein (WIP) [1,2,20–22]. Transformation of WASp into active conformation is dependent on the binding of Cdc42-GTP to the GBD domain, PIP_2 to the BR domain, binding of Toca-1 to PPP domain and Cdc42-GTP or binding of a series of adaptor molecules to PPP domain and phosphorylation of Tyr291 within GBD domain. This is followed by unfolding of the WASp molecule, allowing the VCA domain to be exposed. Actin monomers are recruited and they polymerize to form actin filament (See Fig. 1) [2,23,24]. Additionally, phosphorylation of Y291 in the GBD domain is considered to direct WASp for degradation by calpain and proteasome proteolysis [25,26]. During the elongation and branching of actin filaments, the homology domain (V) binds to the monomeric actin and couples it with Arp2/3 via the central-hydrophobic-region (C) and acidic-region (A) domains [24,27,28]. Thus, the activation of WASp serves as a link between signaling from the cell surface receptor and cytoskeleton rearrangement with G-actin and it can never work alone. Besides, by in vitro reconstitution, recent study has shown WASp-family proteins on the membrane have potent polymerase activity accelerating elongation of uncapped actin filaments, which has not found in previous studies performed with soluble NPFs [28,29]. Thus the roles activated WASp plays in the assembly of the cytoskeleton network are expanded including the dendritic nucleation, the link of actin to the membrane and acceleration of filament elongation.

We may further conclude that recruitment of WASp to the internal

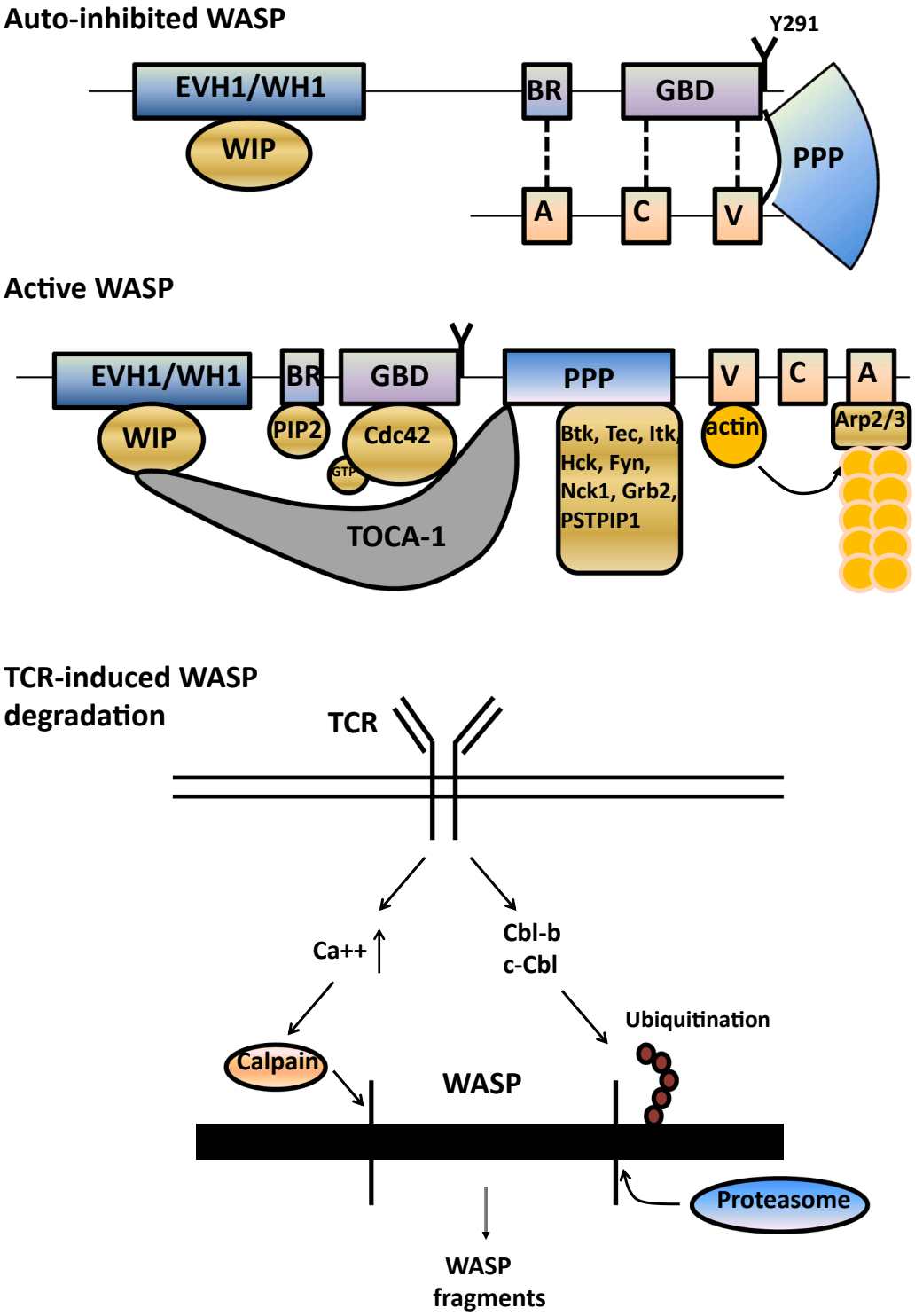

Fig. 1. Activation and TCR-induced degradation of WASp. EVH1, Ena-VASP homology domain 1; WH1, WASP homology 1; BR, basic region; GBD, GTPase-binding domain; PPP, polyproline domain; V, verprolin homology domain; C, cofilin homology or central domain; A, acidic domain; WIP, WASP-interacting protein; PIP2, phophatidylinositol(4,5)-biphosphate; Cdc42, cell division control protein 42 homolog; TOCA-1, CDC42-dependent actin assembly-1; Btk, Bruton's tyrosine kinase; Tec, tyrosine kinase expressed in hepatocellular carcinoma; Itk, IL-2-inducible T cell kinase; Hck, haematopoietic cell kinase; Nck 1, non-catalytic region of tyrosine kinase 1; Grb 2, growth factor receptor-bound protein 2; PSTPIP1, proline-serine-threonine phosphatase-interacting protein 1; Arp2/3, actin-related protein. WASp is degraded by Ca++ dependent calpain and proteasome followed by Cbl-mediated ubiquitination after TCR ligation.

site of actin polymerization is indispensable for its normal function of signal integration and transformation and the binding of WASp to various domains on the membrane mediate different down-stream activities.

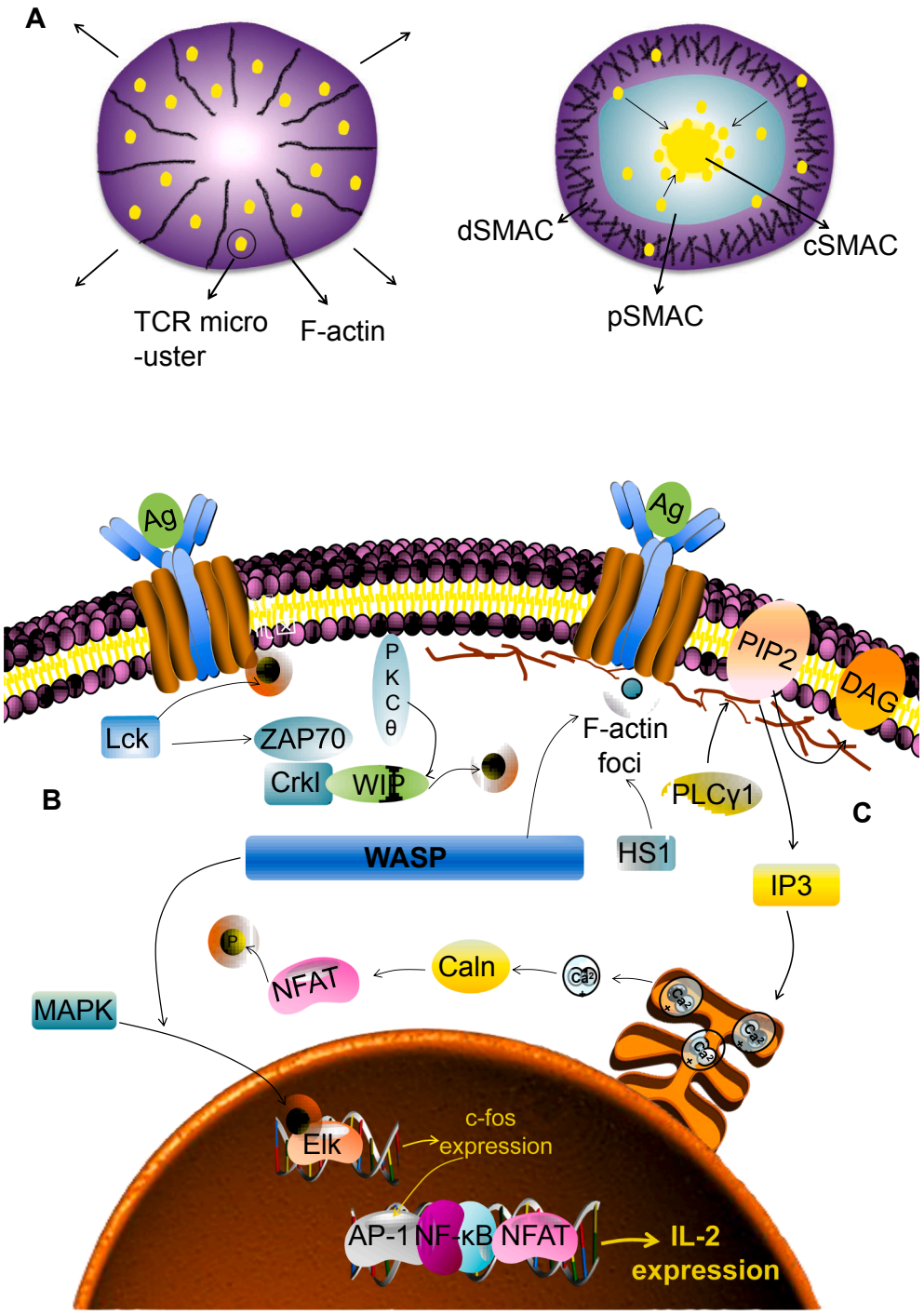

Fig. 2. The role of WASp in IS formation and IL-2 secretion. (2.A) The association between IS formation and F-actin dynamics. T-cell spreading over the surface of APC is driven by the actin polymerization at the edge of the contact zone along with the formation of TCR microcluster. During IS maturation, TCR microclusters move centripetally driven by the retrograde flow of F-actin while F-actin form dSMAC by its remodeling in the peripheral ring. (2.B) One pathway of WASp recruitment. Lck, lymphocyte-specific protein tyrosine kinase; ZAP70, Zeta-chain-associated protein kinase 70;Crkl, Crk-like protein; PKCθ, protein kinase C theta; WIP, WASp-interacting protein. (2.C) The molecular mechanism for regulation of Ca++ flux and further of the IL-2 expression via F-actin foci formation. HS1, hematopoietic cell lineage-specific protein 1; PLCγ1, phospholipase Cγ1; PIP2, phosphatidylinositol 4,5-bisphosphate; DAG, diacylglycerol; IP3, inositol 1,4,5-trisphosphate; Caln, Calcineurin; MAPK, mitogen-activating protein kinase; Elk, ETS domain-containing protein kinase; NFAT, nuclear factor of activating T cells; AP-1, activating protein; NF-κB, nuclear factor kappa-B.

3. The role of wasp in T cells

3.1. WASp regulates F-actin-dependent T-cell function by facilitating efficient actin nucleation

T cells undergo cytoskeletal organization after encountering antigen-presenting cells (APC), and this process involves both the formation of lamellipodia and microtubule-organizing center (MTOC) reorientation to the immunological synapse (IS) [30,31]. Nucleation is the process in which a new actin microfilament branches to form or to modulate the cytoskeleton. The Arp2/3 complex is one of the best studied nucleators in T cells [32,33].

WASp family protein mediates the binding of actin filaments to the Arp2/3 complex, and is a prerequisite to nucleation activity and triggers the branching of the actin filaments [34]. The structure of different members of WASp family varies, but the C-terminal VCA domain is conserved, suggesting the crucial role of the VCA domain during Arp2/3 binding. The depletion of Arp2/3 in T cells leads to defective lamellipodia formation, and impairs their conjugation with APCs [29,35].

WASp binds to WASp-interacting protein (WIP), the critical molecule for the stability of WASp through its EVH1 domain in the resting cell [36,37]. WIP can inhibit WASp degradation by calpain [25,38] and is the bridge of WASp to other molecules. A recent study by Erin Janssen et al. finds the guanine exchange factor (GEF) activity of

dedicator of cytokinesis 8 (DOCK8) is indispensable for WASp activation driven by TCR ligation and that WIP serves as the bridge between DOCK8 and WASp [39]. Additionally, the direct binding of DOCK8 and WIP is a critical step of the actin assembly mediated by DOCK8. This study explains the similar clinical manifestations and cellular behavior in actin-dependent processes of DOCK8 deficiency and WASp deficiency [40,41].

3.2. The role of WASp in the assembly of immunological synapse

To achieve efficient cell-cell interaction, T cells contact antigen-presenting cells through the stochastic repertoire scanning [42,43], during which T cells undergo changes in morphology and search for antigen epitope. Once antigen recognition achieved by the engagement of T cell receptors (TCR), T cell will experience a period of stable interaction (the phase of its maturation) with APCs. During this efficient interaction, T-cell antigen receptors and signaling components binding with them assemble into spatially segregated supramolecular activation clusters (SMACs) at the cell-cell contacting area [44,45]. The signaling molecules include Syk and src family kinases, PKCθ and Cdc42-GTP as well as adaptor proteins such as Nck, SLP-76 and Fyb. However, mechanism of the assembly of SMACs remains unclear. The immunological synapse (IS) is a symmetric structure with three kinds of SMACs—the distal SMAC (dSMAC), the central SMAC (cSMAC) and the peripheral SMAC (pSMAC) [44,46].

During TCR signaling, TCR microclusters initially form in dSMAC and eventually translocate to cSMAC, where they coalesce [47]. The docking of centrosome behind cSMAC with accumulation of actin in dSMAC is the mark of a mature synapse. During the maturation process, the movement of newly formed TCR microclusters to cSMAC to be incorporated continuously occurs [48,49]. This stereotyped centripetal movement has been shown to be driven by actin retrograde flow for T cells treated with inhibitors of actin polymerization show discontinued transportation of TCR microclusters and suspended formation of signaling complex [47,50]. WASp, WASp family verprolin homologous protein 2 (WAVE2) and hematopoietic lineage cell-specific (HS1) may be the proteins to polymerize F-actin with Arp2/3, which triggers the centripetal movement toward cSMAC poor of F-actin. And depolymerization is thought to occur in cSMAC eventually [51,52].

Previous study shows that WASp serves as the force of F-actin localization to IS and it is imagined to be activated by Cdc42-GTP after the interaction with the exchange factor Vav [53]. In the study by Narayanaswamy Ramesh et al, it was shown that TCR ligation results in the formation of a ZAP-70-CrkL-WIP-WASP complex. Zeta-chain associated protein kinase-70 (ZAP70) is essential for WASp to be recruited to IS via TCR-driven signaling pathway. CrkL, the newly found interactive molecule binds directly to WIP and PKCθ-mediated phosphorylation of WIP results in the release of WASp from its inhibition. Then WASp is recruited to IS and lipid rafts together with this complex, which is the prerequisite for T cell activation (Fig. 2) [54]. The recruitment of WASp is dependent on the binding of its PPP to SH3 domain of Nck, the component of scaffold complex. Biochemical analysis has shown that many of the proteins linking actin polymerization to TCR ligation such as SLP-76, Vav1, Itk and WASp are presented in large complexes, which makes it difficult to figure out how they actually function together [55–57]. Additionally, there are multiple interactive pathways coupling TCR activation and cytoskeleton remodeling via IS, making it a hard work to figure out the specific function of every molecules involved [58].

However, reports have shown that WASp is dispensable for the formation of IS under certain condition [52,59]. It seems that the importance of WASp for IS formation depends on the TCR-signaling strength. WASp is necessary for low-affinity-receptor T cells or those interacted with low-density pMHC. However, WASp seems to be superfluous when TCR with high affinity or abundant pMHC exists [59].

Normal total F-actin and intact Smac organization in IS have been observed in WASp−/− T cells in both mice and human [46,60–62]. The early TCR signaling triggered by MHC complex is also intact while the downstream calcium ion signal transduction is apparently impaired [63]. A recent study identifying the specific subclass of F-actin dependent on WASp regulation makes an explanation to the calcium response defect [64]. Dynamic change of cytoskeleton is originally regarded as all or none response of F-actin neglecting different roles of different subclass of F-actin. The WASp-dependent F-actin network called F-actin 'loci' in IS is focusedly formed [63] and is critical for phosphorylation of PLC-γ1 which further influence the elevation of calcium ion in T cell. However, detailed mechanism of the formation of F-actin foci remains unclear, the study of which is definitely challenging as the F-actin subclasses already known all consist of the same kind of G-actin. In addition, the growth of foci exerts force on membrane [65,66], which may be related to the mechanism of the stabilizing effect of WASp on IS.

As has been mentioned above, WASp is needed for the maintenance of IS and WASp−/− T cells display normal IS formation but defective reformation unless PKCθ is inhibited [46]. Thus the function of WASp in amplifying TCR signaling and stabilizing contacting state rather than its role in mediating F-actin accumulation seems to be critical for IS formation.

3.3. WASp is indispensable for TCR endocytic process

After TCR engagement, the TCR-complex endocytosis mediated by clathrin is triggered, which is regarded as a significant part in the modulation of T-cell activation, attenuation of responsiveness of T cells as well as the thymocyte selection and T cell lineage decisions [67–69]. Ligand-evoked TCR internalization has been found significantly decreased in WASp-deficient T cells [60]. Intersectin-2 is an endocytic adaptor protein to function in clathrin-dependent endocytosis which contains Dbl homology (DH) [70]. Co-localization of intersectin-2, WASp and Cdc42 in Cos-7 cells confirm the interaction of these proteins after TCR engagement. The overexpression of intersectin-2 in Jurkat T cells induces a remarkable increase in ligand-induced TCR endocytosis [71]. Previous studies have confirmed that actin cytoskeleton is necessary for receptor-mediated endocytosis [72,73]. Altogether, these findings indicate the indispensable role of WASp in the induction of TCR endocytosis by colocalizing with intersectin-2 and bridging the endocytic process to actin rearrangement as well.

3.4. Migration defects in WASp-deficient T cells

The first step of cell migration is to extend protrusions such as lamellipodia and filopodia. In lammellipodia, Arp2/3 which is activated by WASp helps to form the actin meshwork via its junction between microfilaments [74]. The vital role WASp plays in actin remodeling and podosome formation accounts for the the defects in cell migration observed in WASp-deficient cells both in patients and mice.

After a series of developmental processes, T cells egress from the thymus to peripheral lymphoid tissues. Decrease in the number and percentage of CD4+ and CD8+ T cells has been shown both in WAS patients and WAS knockout murine model [5,75]. Early studies attribute the T-cell lymphopenia to abnormal proliferation after TCR ligation and increased apoptosis of WAS T cells [5,76]. A recent study by Wenyan Li et al. displays defects in thymic output which is dependent on cell migration by using TCR excision circles (TRECs) as a molecular marker for thymic output cells [77]. In addition, WASp does not seem to be indispensable for T cell development for the compensatory role of *N*-WASp, the member of WASp family sharing more than 50% homology with WASp. Study using cells devoid of both WASp and *N*-WASp [double knockout (DKO)], wild-type (WT) mice, WASp KO mice (WKO), *N*-WASp KO mice (NWKO) and mice specifically lacking both WASP and *N*-WASP only in T cells [conditional DKO (cDKO)] has shown that only T-cell development in DKO and cDKO mice was significantly disrupted and the block seems to happen at the DN3-to-DN4

stage, which indicates the overlapping function of WASp and *N*-WASp during T-cell development [78]. Therefore, the decreased thymic output is most likely due to the defects in migration of WAS T cells. One feasible explanation is about the defects in intrathymic migration which prevents cells to reach certain location to receive stimulating signals for thymic output [79]. However, the precise mechanism underlying the relation of altered cell migration and defective thymic output remains elusive.

During immunological confrontation against foreign microbes and pathogens, migratory and adhesive capacity of lymphocytes plays a pivotal role. Lymphocyte homing is a special form of lymphocyte migration based on the interaction of lymphocyte homing receptor (LHR) on lymphocytes and the corresponding vascular addressin on endothelial cells [80]. Homing of T cell to sites of inflammation is a critical step in the elimination of pathogens. T cells migrate to inflamed tissues by rolling which decreases T-cell velocity in bloodstream, adhesion and chemotaxis mediated by selectin, integrin and chemokine respectively. Deficiency of WASp does not interfere with the process of rolling and adhesion in vitro [81]. However, rearrangement of the actin cytoskeleton is critical for T-cell chemotaxis [82]. WASp-deficient T cells manifest impaired migratory capacity both in human and mice model [81]. Previous study has found that WASp is critical for chemotaxis of T cells stimulated by stromal cell–derived factor (SDF)-1 [83], which is critical for homing of lymphocytes via its ligation with CXC chemokine receptor 4 (CXCR4). It has been shown that WASp interacts with Cas, Nck and Fak after being tyrosine phosphorylated, followed by interaction with Cdc42 and actin polymerization [83,84]. Recent study identified a unique internal region with 30 amino acid resides in WASp which may play indispensable role in chemotaxis of Jurkat-T cells. Besides, this region cannot be found in *N*-WASp, which may account for the failure of *N*-WASp to functionally compensate for WASp in chemotaxis process [85]. The remarkable reduction of chemotaxis of T cells in WAS patients which is also found in WASp KO mice to a lesser extent, might be an important part of the pathogenesis of immunodeficiency both in human and murine.

3.5. WASp regulates cytoskeleton-independent cellular processes

In WASp-deficient T cells, normal F-actin formation and/or polarization to the immunological synapse has been reported in several reports [59,86], which can be explained by the compensatory role of other NPFs. However, WAS T cells still display functional deficits contributing to clinical manifestations indicating the imbalance of Th1-Th2 immunity like hyper immunoglobulin E and autoimmune colitis [87], which implies the nonnegligible role of WASp in cytoskeleton-independent cellular processes.

It has been shown that WKO T cells have reduced IL-2 production both in human and mice model and that the role WASp plays in this process is independent of its role in the IS formation [59]. Additionally, both CD4+ and CD8+ T cells with WASp deficiency have reduced secretion of cytokines controlling cellular immune process [88,89]. However, WAS CD4+ T cells have relatively intact capability to produce and secrete IL-4, IL-5, IL-10 and the Th2 cytokines, thus leading to abnormal Th2 dominance [90].

Portion of WASp has been found to translocate to nucleu of T cells and to be associated with histone H3K4 trimethylase and H3K9/H3K36 tridemethylase activity in vitro and their enzymatic activity is required for TBX21 in Th1-skewed cells [59,88]. More recently, the fact that the role of WASp in supporting the enzymatic activity and then the TBX21-driven transcriptional process at chromatin level is independent on its VCA domain and the Arp2/3-binding functional characteristic [91]. In addition, the portion of WASp is found in necleu to be recruited to the promoter locus of TBX21 rather than GATA3, the regulator gene of Th2-cell development, and RORγ, the gene promoting Th17-cell differentiation [88,90]. Accordingly, the presence of WASp in Th2 cells is much less and its loss doesn't result in impaired transcriptional regulation [91].

A recent study by Nikolai V. Kuznetsov et al. identifies 15 WASp-enriched genes among which more attention is paid to the gene encoding T cell factor (TCF) 12. The interaction of WASp with TCF12 and TCF1 both of which are significant for T cell development has been explicitly displayed [92]. However, the interaction partners, the specific binding sites and function of WASp in nucleu need to be further excavated.

3.6. The role of WASp in the homeostasis and function of Tfh cells

The cytokine secretion in Th1 and Th2 are both impaired in T cells from WAS patients, which can be partly explained by the defective WASp function in transcriptional events [90,93]. A study by Gerben Bouma et al. displays decreased regulatory T cells (Treg) and increased Th17 cells, which is contributory to the development of autoimmunity [94]. However, the role of WASp in Follicular helper T cells (Tfh), a CD4+ T-cell subset broadly studied in recent years is just appreciated.

Follicular helper T cells, also known as follicular B helper T cells are subset of antigen-experienced CD4 T cells identified by the expression of CXC chemokine receptor 5 (CXCR5), which is vital for their migration into B-cell follicles [95] and is induced by Bcl6 [96]. The differentiation of Tfh cells is a complicated process which is orchestrated by various cytokines and transcription factors, including IL-6, IL-21, IL-2 and Bcl6 in the mouse model [97,98]. Along with the coordination of different interleukins, inducible costimulator (ICOS) serve as a critical early signal molecule to induce the transcription factor Bcl6 which then induce CXCR5 [97]. Tfh cell is specialized B-cell help subset of T cells and is indispensable for the generation and maintenance of protective immunity. In addition to the role of Tfh cells in B-cell maturation and immunoglobulin production in GCs, Tfh cells are the major component of T-cell memory [99], which is of significance for vaccine design to improve vaccine-induced protective immunity.

Although the number of circulating CXCR5+ CD4+ T cells of patients with autoimmunity is found increased, in the study by Xuan Zhang et al. a reduced frequency of these cells is been shown [100]. Line with the decrease in number of Tfh cells in blood of WAS patients and WAS KO mice, the expression of BCL6, the critical transcription factor for Tfh-cell development is apparently decreased in WAS patients [101]. However, increase in ICOS expression has been shown in Tfh cells of both WAS patients and WAS KO mice, which has been confirmed cell extrinsic by measurement of chimera mice. Along with the developmental defects of Tfh cells in WAS patients, the reduced memory response of these cells in blood and spleen which is demonstrated by decrease in number of memory Tfh cells plays a role in pathogenesis of immunodeficiency in WAS. Additionally, Th1-like Tfh cells in peripheral blood is significantly reduced which may imply the defective development of protective antibody production from memory B cells [102,103].

These new findings beg the question—how does WASp deficiency result in decrease in circulating CXCR5+ CD4+ T cells and memory Tfh cells? The decreased number of Tfh cells is most probably attributed to the abnormal expression of ICOS and Bcl6 expression, which may be explained by function of WASp mediated by translocating to nucleu. However, the mechanism underlying defective memory response of Tfh cells requires more study. Furthermore, Tfh cells with high ICOS expression display increased apoptosis and reduced proliferation for which in depth study is of significance given the nonnegligible role of these cells in the development of autoimmunity [104].

4. The role of WASp in B cells

4.1. WASp-deficient B cells manifest defective migration, adhesion and immunoglobulin production

In secondary lymphoid organs where efficient immune response

occur, dendritic cells (DC) present antigens to naive T and B cells. Chemokine gradients contribute to the formation of B-cell/T-cell boundary, where B and T cells meet and T helper cells activate B cells via interleukins and direct CD40-CD40L interaction. After activation, B cells proliferate and form GCs [105,106]. Reduced polarization response to IL-4 and CD40L of B cells found in both WAS patients and WAS KO mice, accompanied by reduced migration response to chemokines of human WASp-deficient B cells substantiates the drastical decrease of migration capability resulted from WASp deficiency [14,107,108]. The defective homing of B cells in both WAS patients and mice resulted from extensive defects in motility provides an explanation for the abnormal morphology of spleen and lymph nodes along with reduced T and B cells in the blood [14]. It's likely that the mechanism underlying the defective migration of B cell runs in the same groove as that of T cells which has been introduced in Section 3.4. In addition, abnormal shape of long protrusions has been found in WASp-deficient B cells, which is contributory to the defective GC formation, homotypic adhesion and immunoglobulin (Ig) class switch given their role in contact inhibition and Ig switch and secretion [107].

Impaired antibody response, especially to TI-antigen is consistently found in WAS B cells [75,109,110]. Early studies have reported elevation in IgA and IgE level in WAS patients [8,111] while Mike Recher et al. found elevated IgA, IgE and IgM in WKO mice and elevated IgM in B/WcKO mice by using Cre-Lox technology [110]. Altogether, these findings may suggest that B-cell intrinsic deficiency only results in the the elevation of IgM while the increased IgA and IgE is attributable to abnormal function of Treg and DCs.

It's worth mentioning that application of site-specific recombinase technology has made it possible to distinguish the specific contribution of B cells to the pathogenesis of WAS-associated immunodeficiency, which can also elucidate the role of cell-extrinsic factors in the formation of B-cell phenotypic discrepancies found in WAS patients and mice. And possible mechanisms underlying maifestations resulted from B-cell intrinsic deficiency are based on logistical analysis of various results of these studies (See in Fig. 3).

4.2. The role of WASp in BCR signaling

The engagement of B cell receptor (BCR) results in antigen processing and presentation via specific signaling cascades. Activation of BCR triggers adaptive immune responses and determines the development of B cells under the influence of microenvironment. Previous studies have shown that surface BCRs aggregate and associate with lipid rafts after antigen encounter [112]. To achieve optimal signaling in response to membrane antigen, B cell experiences spreading and then contraction in the level of morphology with the coordinated lateral motility of BCR [113,114]. During the centripetal movement of BCR to form central clusters [115], B cells expands their contact with antigen through the actin polymerization at the edge of the contact zone with BCR clustering in the periphery [116]. The retrograde flow of F-actin along with the BCR movement is followed by the degradation of F-actin in the center of contact zone resulting in B-cell contraction subsequently [114,117]. All these observations establish critical role of actin remodeling in BCR clustering and signaling.

Actin remodeling driven by BCR is triggered by various molecules including protein kinases, adaptor protein and actin-binding protein [112,118]. After dephosphorylation of ezrin mediating the detachment of cortical actin network from lipid raft [113], the constraint to BCR movement is released, followed by BCR clustering and subsequent coalesce in central cluster. A study has identified the negative regulator Bruton's tyrosine kinase (Btk) and positive regulator SH2-containing inositol-5 phosphatase-1 (SHIP-1) in BCR-driven actin remodeling through the regulation of WASp [114]. Btk, the multifunctional molecule in BCR signaling activates Cdc42 and Rac by phosphorylation of Vav and mediates the enhance in PIP2 production by activating phosphatidylinositol-5 kinase (PI5K), which further activates WASp through conformational change [119]. While SHIP-1 suppresses WASp activation via its inhibitory effect to Btk. Activated WASp localizes to BCR microclusters and the edge of the contact zone, which is in accordance with F-actin [114]. Both the aggregation and internalization of BCR is impaired in WAS KO mice, which indicates WASp regulates the amplification and attenuation in sequence by its role in mediating actin binding to filaments. However, the modest deficiency of BCR-driven behavior observed in WAS KO mice [15,114] is likely due to the concurrent effect of other effector molecules activated by Btk like *N*-WASp, which exerts negative impact on BCR signaling [120]. The role for WASp in B-cell activation has always been a controversial issue as the downstream events including B-cell proliferation and elevated $Ca++$ flux differs in different reports [75,121,122]. However, the defects in the growth of BCR clustering and B-cell spreading of WAS memory B cells which mirrors the defective activation of these cells have been reported in recent studies [123,124]. Furthermore, prominent and exclusive decrease in both recruitment and transcription level of CD19, the critical molecule for early activation of B cells has been found, which indicates the role of WASp in the transcriptional regulation of CD19 gene [123]. In addition, the relatively normal BCR clustering and B-cell spreading found in both $WASp-/-$ and $DOCK8-/-$ B cells may implies the actin cytoskeleton-associated downstream events of BCR is mediated by various molecules and they can compensate for each other [124].

4.3. WASp is crucial for homeostasis of B cells

As research of the contribution of B cells in pathogenesis of immunodeficiency in WAS moves along, defective B-cell development in many aspects has been displayed. A decrease in both mature and immature B cells along with redundant peripheral B cells is found in WAS patients [125,126]. Reduced mature B cells in BM can be explained by defective recirculation of B cells resulted from impaired migration as has been elucidated in 4.1. However, the loss of immature B cells is most likely due to reduced sense of developing B cells in BM for retention signal. CXCR4 on B-cell surface has been found to specifically drive the chemotasis of B-cell precursors in BM to SDF-1 in the early study [127], which is further substantiated by the reduced B-cell precursor in BM and overpresentation of immature B cells in periphery [128,129]. In view of the role of WASp in CXCR4 signaling observed in T and NK cells [84,130], the defective function of WASp in SDF-1 pathway is likely to be the underlying mechanism for the abnormal immature B-cell counts in BM and periphery.

In the spleen, B cells derived from bone marrow differentiate into either marginal zone (MZ) B cells or follicular B cells, which lies on BCR signaling [131]. Remarkably reduced B-cell counts in spleen has been found in WAS patients and mice, which results in prominent decrease in both follicular and MZ B cells [110,125]. Nevertheless, reduced follicular B cells is rarely observed in other lymphoid organs and the decrease in spleen ban be reversed by recovering WASp which may indicate the critical role of BM output and follicular B-cell development in the spleen [110]. In the contrast, the significant reduce of MZ B cells which results in impaired primary immune response [132] can be explained by several mechanisms. The decrease in MZ B cells is likely due to defective migration and adhesive capacity of WAS B cells resulting in insufficient location and retention. WASp-deficient spleen B cells display inability to respond to sphingosine-1-phosphate [133] and S1P [134] to localize in MZ and the general defects in adhesive response to ICAM-1 and VCAM-1 may explain the insufficient retention of MZ B cells [14]. Additionally, a recent study has observed enhanced BCR responsiveness in WAS mice, which further increase the output of splenic MZ [135].

Normal frequency of classical memory B cell from GC and significantly reduced TI memory B cells which may be attributable to the reduction of B1 cells have been found in different studies of WAS patients [125,126]. This can explain the relatively normal response to TI-

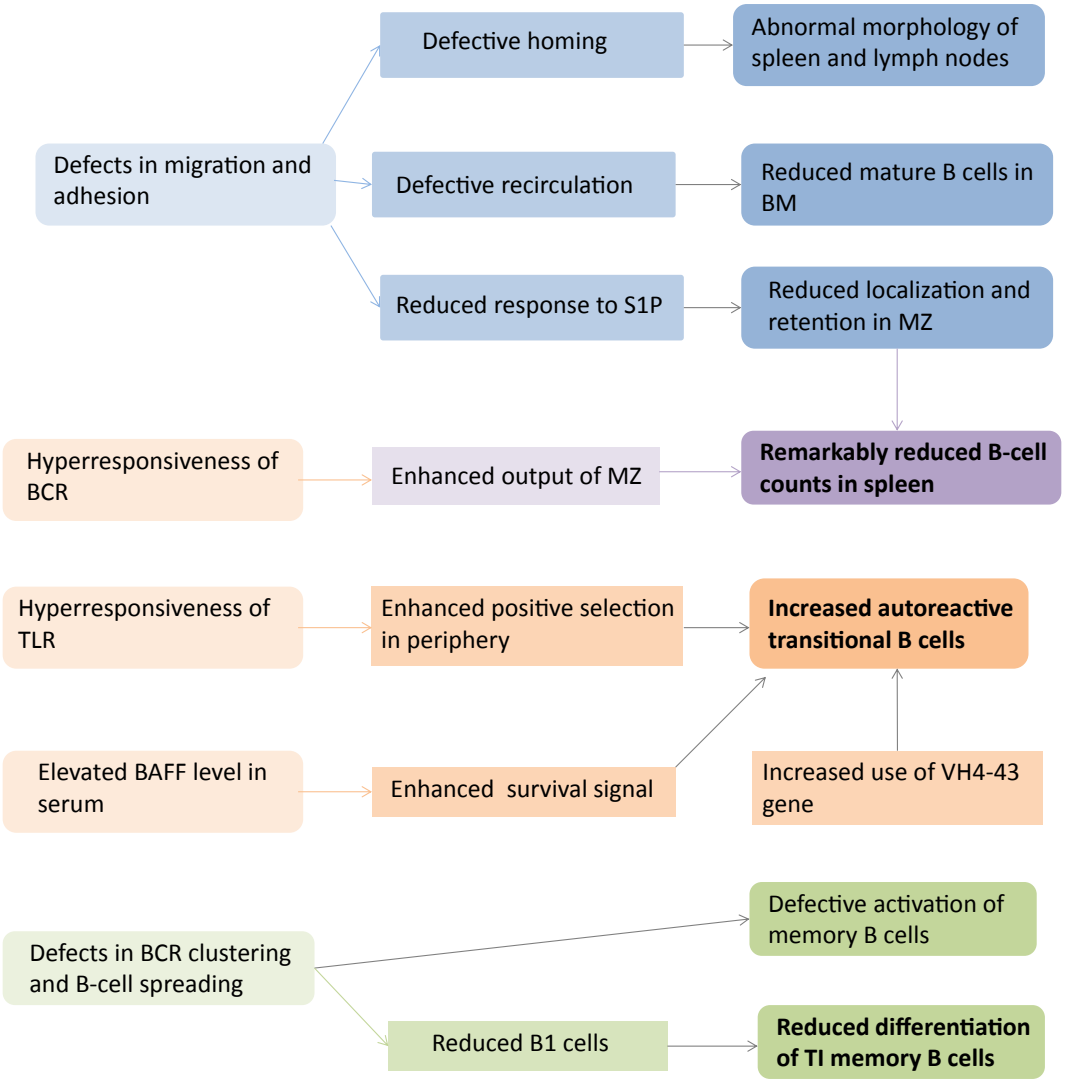

Fig. 3. Overview of the speculated cellular mechanisms of WAS B-cell related defects. The structure diagram summarizes how the WASp deficiency in B cells leads to significantly reduced B-cell amount in MZ, decreased TI memory B cells and triggering of autoimmunity based on analysis of reported results.

antigen but prominently impaired response to TI-antigen, indicating the existence of compensatory mechanism in T but not B cells. The differentiation of TI memory B cells is dependent on sufficient primary B1 cells [136] of which the developmental mechanism has been controversial for decades [137,138]. Sufficient strength of BCR signaling and activation of NF-κB pathway are prerequisites for B-1 cell production. Considering the role of WASp in BCR signaling, the decrease in B-1 cells of WAS patients is likely due to the defective B-cell spreading and BCR clustering. Besides, a consistent increase in CD19hiCD21low B cells in children and adult has been reported [125,126]. Although the association between the overpresentation of these cells and autoimmunity is quite evident [139], neither the developmental processes nor the specific function of these cells has been figured out.

4.4. WASp plays a critical role in the prevention of autoimmunity

Autoimmune disorder is complicated by WAS in up to 70% patients among which autoimmune cytopenias are most commonly reported followed by arthritis, vasculitis, inflammatory bowel disease, IgA nephropathy and neutropenia [140,141]. The mechanism of autoimmunity in WAS has been extensively investigated but not fully understood yet. Early studies attribute the autoimmunity to defective development and function of WAS regulatory T (Treg) cells. Significant decrease of Treg cells in patients and mice model has been found in peripheral lymphoid tissues especially in inflammed tissues which may be attributable to the defective homing resulted from lack of chemokine receptors [142]. Besides, these cells manifest impaired ability to suppress proliferation of both B cells and effector T cells and to secret anti-inflammatory IL-10 [143–145]. Michel H. Maillard and her colleagues [145] finds that defects in immunosuppressive function of Tregs from WKO mice is, at least partly due to lack of exogenous IL-2. Furthermore, this defect is unlikely resulted from impaired WASp-associated actin polymerization as Treg cells from both WT and WAS KO mice display less efficient TCR-driven actin remodeling. However, whether the defective suppression of B cells is mainly resulted from the insufficient regulation by Treg or impaired T-cell help remains obscure.

Studies using mice with specific WASp deficiency in B cells have confirmed that intrinsic B-cell dysfunction is sufficient to establish autoimmunity [15,110]. Pala and her colleagues observe a decrease in autoreactive transitional B cells from BM and opposite alteration of mature B cells with autoreactivity in WAS patients [146], which mirrors a more rigid central tolerance with lower threshold but more relaxed periphery tolerance. In theory, the increase in autoreactive mature B cells can be explained by the reduced negative selection or enhanced positive selection. However, by assessment of Tg model and receptor editing [147], deficiency of WASp do exert no impact on negative

selection. Normal positive selection of transitional B cells is critical for the cell-debris clearance and conserved epitope recognition [148], during which period dual engagement of BCR and TLR activates autoreactive B cells. Superfluous signal of both BCR and TLR enhances positive selection of transitional B cells [147], which may indicate the association between hyperreactivity of BCR and TLR observed in both patients and mice model [15,146] and the abnormal periphery tolerance. The role of WASp in regulation of BCR internalization may partly explain the modestly increased reactivity. The regulatory role WASp plays in TLR signaling is not defined and is likely mediated by the adaptor molecule myeloid differentiation primary response protein 88 (MYD88) which is a common factor in various pathways of pathogenesis of autoimmunity [149]. In line with this speculation, deletion of MYD88 in WAS B cells results in evident decrease in proliferation and autoantibody production [15]. With respect to the survival, the elevated B-cell activating factors (BAFF) found in WAS-patient serum likely provide enhanced survival signal to transitional B cells with autoreactivity [125]. Besides, deep sequencing of mature naïve B cells of WAS mice model displays higher expression of VH4-43 [126,147], which in further study has been found to enhance survival signal to late transitional B cells via binding to self-antigen [147]. The increased autoreactive B cells then recruit helper T cells and result in formation of spontaneous GC (Spt-GC) [15] formation followed by autoantibody production and enhanced differentiation into class-switched plasmablasts [150]. Interleukin-10 (IL-10)-producing regulatory B cells (B10 cells), a newly found B cell subset assume many significant adaptive-immune functions including the peripheral-tolerance maintenance and the modulation of Treg-Th17 balance [151,152]. More recently, the contribution of reduced B10 cells found both in WAS patients and mice to autoimmunity and the role WASp plays in B10 has been studied [94,153]. It has been confirmed that the potent migratory and adhesive properties are the prerequisite for B10 cells to gain the ability to normally develop. Taking the defective BCR signaling of WAS B cells into account as well, it's reasonable to speculate WASp plays a nonnegligible role in the development of B10 cells [153,154].

5. Conclusion and some future perspectives

Although WAS is a rare monogenic disease in human, studies on WASp is a continuous evolving field. On one hand, aspiration to increase the accuracy of diagnosis and the effectiveness of treatment promotes studies performed on it. What's more, the critical role played by WASp in the process of signal integrating and transforming to actin remodeling along with the characteristic of its exclusive expression in hematopoietic cell establish the important place of WASp in adaptive immune process.

This review focuses on the role WASp plays in the cellular processes of T cells and B cells, based on the manifestations of these two lymphocytes and some molecular mechanisms that are already known. Although the functions of WASp in T cells are more thoroughly studied and the results are more pronounced and consistent comparing with these in B cells, the role WASp plays in humoral immunity is extensive and the mechanism of many WASp function in T cells is unclear. For example, defective assembly of LFA-1-cluster, the critical molecule for triggering of, and abnormal location of lytic-granule are found in WAS CTLs but how WASp functions in this change remains unknown. As for B cells with WASp deficiency, studies now mainly focus on the phenotypes of B cells from WAS patients and/or murine models and the association between alterations of various B cells. Thus mechanism underlying the clinical manifestations associated with B-cell defects is waiting for discovery. There are reasons to believe, as the research moves on, the understanding of autoimmunity and inflammation will be more in-depth and that the targeted therapies for this kind of disease will become more effective.

Conflict of interests

We declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

C. Liu and B. Ren organized the article. X. Sun and Y. Wei wrote the draft. P. P. Lee edited the language.

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