Biochemical and Biophysical Research Communications 458 (2015) 28–33

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Anti-WASP intrabodies inhibit inflammatory responses induced by Toll-like receptors 3, 7, and 9, in macrophages

Chisato Sakuma [a], Mitsuru Sato [a, *], Takuma Oshima [b], Takato Takenouchi [a], Joe Chiba [b], Hiroshi Kitani [a]

[a] Animal Immune and Cell Biology Research Unit, National Institute of Agrobiological Sciences, 1-2 Ohwashi, Tsukuba, Ibaraki, 305-8634, Japan
[b] Department of Biological Science and Technology, Graduate School of Faculty of Industrial Science and Technology, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan

ARTICLE INFO

Article history:
Received 29 December 2014
Available online 26 January 2015

Keywords:
Wiskott-Aldrich syndrome protein (WASP)
Bruton's tyrosine kinase (Btk)
Toll-like receptors (TLRs)
Macrophage
Intracellular expressed antibody (intrabody)

ABSTRACT

Wiskott-Aldrich syndrome protein (WASP) is an adaptor molecule in immune cells. Recently, we showed that the WASP N-terminal domain interacted with the SH3 domain of Bruton's tyrosine kinase (Btk), and that the complex formed by WASP and Btk was important for TLR2 and TLR4 signaling in macrophages. Several other studies have shown that Btk played important roles in modulating innate immune responses through TLRs in immune cells. Here, we evaluated the significance of the interaction between WASP and Btk in TLR3, TLR7, and TLR9 signaling. We established bone marrow–derived macrophage cell lines from transgenic (Tg) mice that expressed intracellular antibodies (intrabodies) that specifically targeted the WASP N-terminal domain. One intrabody comprised the single-chain variable fragment and the other comprised the light-chain variable region single domain of an anti-WASP N-terminal monoclonal antibody. Both intrabodies inhibited the specific interaction between WASP and Btk, which impaired the expression of TNF-α, IL-6, and IL-1β in response to TLR3, TLR7, or TLR9 stimulation. Furthermore, the intrabodies inhibited the phosphorylation of both nuclear factor (NF)-κB and WASP in response to TLR3, TLR7, or TLR9 stimulation, in the Tg bone marrow-derived macrophages. These results suggested that WASP plays important roles in TLR3, TLR7, and TLR9 signaling by associating with Btk in macrophages.

1. Introduction

Wiskott-Aldrich syndrome (WAS) protein (WASP) is the gene product responsible for X-linked immunodeficiency [1]. WASP is mainly expressed in the cytosol of hematopoietic cells and acts as an adaptor protein that regulates immune responses such as actin filament reorganization and intracellular signal transduction. Patients with WAS have macrophages, dendritic cells, and monocytes that show impaired responses to inflammatory chemokines, including defects in polarization, migration, and formation of the actin-rich phagocytic cup [2–4].

The majority of gene mutations in patients with WAS have been mapped to the WASP N-terminal region, including the EVH1 domain [5], which suggested that this domain is important for WASP functions. To elucidate the functions of the WASP N-terminal domain in innate immune responses, we developed transgenic (Tg) mice that overexpressed exon 1–5 of the WASP gene, which encode the N-terminus (amino acids 1–171, designated WASP15) [6]. Bone marrow–derived macrophages (BMDMs) from WASP15 Tg mice showed impaired in lipopolysaccharide (LPS)-induced inflammatory responses, including nuclear factor (NF)-κB activation and subsequent gene expression of TNF-α, IL-6, and IL-1β. Thus, the overexpressed WASP15 construct showed dominant-negative effects [7]. The functions of the WASP N-terminal domain were confirmed in two types of Tg mice that expressed an intracellular antibody (intrabody) that targeted the WASP-N-terminus; one intrabody comprised the single-chain variable fragment (scFv), and the other intrabody comprised only the light-chain variable region (V_L), single-domain (SD), of an anti-WASP-N-terminus monoclonal antibody. Both intrabodies could specifically bind to the WASP-N-

* Corresponding author. Fax: +81 29 838 6028.
E-mail address: mitsuru.sato@affrc.go.jp (M. Sato).

http://dx.doi.org/10.1016/j.bbrc.2015.01.049
0006-291X/

terminus domain. BMDMs derived from scFv Tg mice and V_L SD Tg mice were impaired in inflammatory cytokine gene expression upon LPS stimulation [8,9].

We previously found that the WASP N-terminal domain could associate with the Src homology (SH) 3 domain of Bruton's tyrosine kinase (Btk). This association was strongly inhibited by overexpression of WASP15, scFv intrabodies, and V_L SD intrabodies, in the respective Tg BMDM cell lines [7–9]. Furthermore, the inflammatory responses induced by Toll-like receptor-2 (TLR2) ligands, Pam3CSK4 and heat-killed *Listeria monocytogenes*, were impaired in BMDMs isolated from scFv Tg mice and V_L SD Tg mice [9]. These observations strongly suggested that the interaction between the WASP N-terminal domain and Btk played an important role in the TLR2- and TLR4-signaling cascades in macrophages.

It has been shown that TLR3, TLR7, and TLR9 recognize double-stranded RNA, single-stranded RNA, and CpG-DNA, respectively [14]. Moreover, Btk was shown to play important roles in TLR3, TLR7, and TLR9 signaling in innate immune cells [10–12]. However, it remains unknown how the association of WASP with Btk might be involved in TLR3, TLR7, and TLR9 signaling pathways.

Here, we investigated the possible roles of the WASP-Btk complex in TLR3, TLR7, and TLR9 signaling by evaluating inflammatory responses to TLR stimulation in BMDMs isolated from Tg mice that expressed scFv and V_L SD anti-WASP intrabodies. We stimulated these BMDMs with the TLR3 ligand, poly(I:C): the TLR7 ligand, imiquimod: and the TLR9 ligand, CpG ODN. We found that the expression of scFv and V_L SD intrabodies interfered with the specific interaction between WASP and Btk, which resulted in impaired inflammatory responses to TLR3, TLR7, and TLR9 stimulation.

2. Materials and methods

2.1. Establishment of bone marrow–derived macrophage cell lines

We established cell lines from BMDM cells derived from wild-type mice and from Tg mice that expressed either scFv or V_L SD anti-WASP intrabodies. Cells were cultured as described previously [7–9].

2.2. Western blot analysis

BMDMs were activated with poly(I:C) (10 μg/mL), imiquimod R837 (1 μg/mL), or CpG ODN (1 μM) (all obtained from InvivoGen, San Diego, CA, USA) at 37 °C for different time intervals. The activated cells were washed with PBS, lysed with SDS sample buffer, and boiled for 10 min. The cell lysates were separated on 12% SDS-PAGE gels and the separated proteins were transferred to a polyvinylidene difluoride membrane (Bio-Rad, Hercules, CA, USA). The membrane was blocked with Blocking One (Nacalai Tesque, Kyoto, Japan) and probed with the following primary antibodies: anti-TLR7, anti-TLR9, anti-phospho-NF-κB p65 (Ser-536), anti-NF-κB p65 (Cell Signaling Technology, Danvers, MA, USA), anti-TLR3, anti-phospho-WASP (Abcam, Cambridge, UK), anti-WASP (Upstate, Lake Placid, NY, USA), anti-phospho-Btk (Cell Signaling Technology), anti-Btk (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and anti-β-actin (Cell Signaling Technology). The secondary antibody was HRP-conjugated anti-rabbit IgG (Dakocytomation, Glostrup, Denmark). Immunoreactive proteins were detected with Chemi-Lumi One L or Chemi-Lumi One Super (Nacalai Tesque).

2.3. Quantitative real-time PCR

BMDMs were cultured in the absence (controls) or presence of poly(I:C) (10 μg/mL), imiquimod R837 (1 μg/mL), or CpG ODN (1 μM), for 5 h at 37 °C. BMDMs were lysed with the RealTime Ready Cell Lysis Kit (Roche Diagnostics, Basel, Switzerland), and total RNA was isolated. RNA was reverse-transcribed to produce cDNA with the Transcriptor Universal cDNA Master (Roche Diagnostics), according to the manufacturer's instructions. The cDNA served as template in quantitative real-time PCR assays to determine gene expression levels. The primer sequences and procedure for real-time PCR are described in Supplementary Table 1.

2.4. Statistical analysis

Statistical significance was assessed with GraphPad Prism 6 (GraphPad Software, La Jolla, CA, USA). Sample pairs were analyzed with the Student's *t*-test. Multiple samples were evaluated with one-way ANOVA. Differences were considered significant when $p < 0.05$.

3. Results

3.1. Expression of TLR3, TLR7, and TLR9 in BMDMs isolated from wild-type, scFv Tg, and V_L SD Tg mice

Cell lines were established from BMDMs isolated from wild-type mice and from Tg mice that expressed either scFv or V_L SD anti-WASP intrabodies. All mice had a C57BL/6 strain background [7–9]. Western blot analysis showed that both WASP and Btk proteins were equivalently expressed in wild-type, scFv Tg, and V_L SD Tg BMDMs (Fig. 1). Our previous study demonstrated that expression of anti-WASP scFv and V_L SD intrabodies diminished the specific binding between WASP and Btk in BMDMs isolated from scFv Tg and V_L SD Tg mice [9].

TLR3, TLR7, and TLR9 recognize poly(I:C), imiquimod, and CpG ODN, respectively. The binding of these ligands to their cognate TLRs on BMDMs leads to innate immune responses, such as the production of inflammatory cytokines [13,14]. To compare the

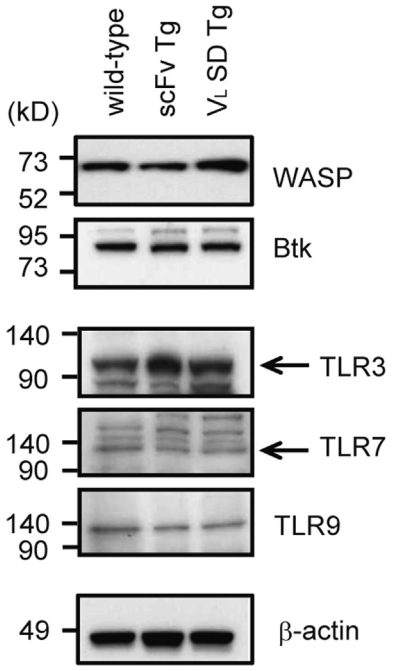

Fig. 1. Expression of WASP, Btk, TLR3, TLR7, and TLR9 in bone marrow-derived macrophages (BMDMs). Cell lysates of wild-type, scFv Tg, and V_L SD Tg BMDMs were analyzed on Western blots probed with the following specific antibodies: anti-WASP, anti-Btk, anti-TLR3, anti-TLR7, anti-TLR9, and anti-β-actin. The immunoblots are representative of three independent experiments.

expression levels of TLR3, TR7, and TLR9 among wild-type, scFv Tg, and V_L SD Tg BMDMs, we performed a Western blot analysis with anti-TLR3, TLR7, and TLR9 antibodies. Similar levels of TLR3, TLR7, and TLR9 proteins were detected in all three types of BMDMs (Fig. 1). The protein loaded in each lane was evaluated by probing the blot with anti-β-actin antibody (Fig. 1). These results suggested that the anti-WASP intrabodies did not affect expression of TLR3, TR7, and TLR9 in BMDMs.

3.2. Impairment of cytokine production in scFv Tg and V_L SD Tg BMDMs upon stimulation with poly(I:C), imiquimod, or CpG ODN

Macrophages stimulated with poly(I:C), imiquimod, or CpG ODN produce a variety of inflammatory cytokines [15–17]. We created two independent BMDM clones (#1 and #2) from each mouse studies: wild-type, scFv Tg, and V_L SD Tg mice. To assess the effects of anti-WASP intrabodies on TLR3, TLR7, or TLR9 signaling, we stimulated the BMDM clones with poly(I:C), imiquimod, or CpG ODN. Then, we isolated the RNA and performed quantitative real-time PCR to evaluate the levels of mRNA that encoded inflammatory cytokines. In wild-type BMDMs, poly(I:C), imiquimod, or CpG ODN stimulation caused marked transcription of TNF-α, IL-β, and IL-6 cytokines. In contrast, the scFv Tg and V_L SD Tg BMDMs expressed significantly lower levels of these inflammatory cytokines (Fig. 2). In the absence of TLR stimulation, no basal gene transcription of these cytokines was detectable in any of the BMDM lines (unpublished data). These results suggested that the WASP N-terminal region was important for WASP function in the production of inflammatory cytokines following stimulation with poly(I:C), imiquimod, and CpG ODN in BMDMs.

In a previous study, we investigated the effect of anti-WASP scFv and V_L SD intrabodies on the NOD1-signaling pathway, which was not associated with Btk. NOD1 is an intracellular sensor for microbial components derived from bacterial peptidoglycans. When bacterial γ-D-glutamyl-meso- diaminopimelic acid (iE-DAP) activates NOD1, it results in NF-κB activation and the production of inflammatory cytokines [18]. We found that the inflammatory cytokine expression profiles in response to C12-iE-DAP stimulation were similar among wild-type, scFv Tg, and V_L SD Tg BMDMs [9].

Taken together, the results suggested that anti-WASP scFv and V_L SD intrabodies could specifically inhibit TLR2, TLR3, TLR4, TLR7, and TLR9 signaling. Moreover, this inhibition was achieved by blocking the specific interaction between the WASP N-terminal domain and Btk.

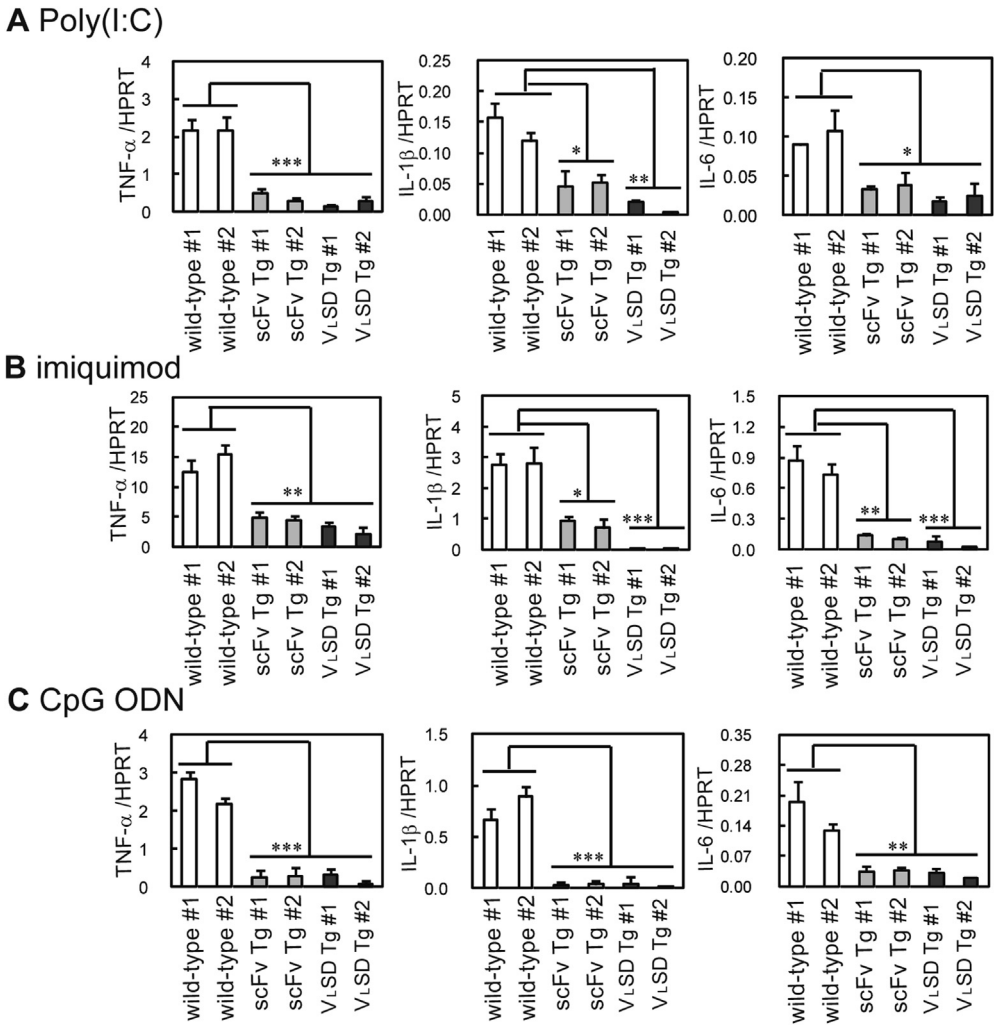

Fig. 2. Impaired cytokine expression upon stimulation of BMDM cell lines with TLR-specific agonists, poly(I:C), imiquimod, or CpG ODN. BMDMs derived from wild-type, scFv Tg, and V_L SD Tg mice were stimulated with (A) poly(I:C), (B) imiquimod, or (C) CpG ODN. Then, RNA was isolated, and quantitative real-time PCR was performed. Expression levels were calculated relative to expression of the control gene *HPRT*. Clones #1 and #2 were independently isolated from wild-type, scFv Tg, and V_L SD Tg mice. Values represent the mean ± standard error of the mean (SEs) of triplicate assays. The profiles are typical examples of at least three independent experiments performed with three independent biological samples. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$.

3.3. Activation of NF-κB induced in BMDMs by poly(I:C), imiquimod, or CpG ODN

In macrophages, NF-κB activation is essential for the production of inflammatory cytokines upon stimulation of TLR3, TLR7, or TLR9 [17,19,20]. We next assessed the effects of anti-WASP scFv or V_L SD intrabodies on TLR3, TLR7, or TLR9-induced activation of the NF-κB–signaling pathway. We determined the extent of NF-κB phosphorylation upon stimulation with poly(I:C), imiquimod, or CpG ODN in wild-type, BMDMs compared to that in scFv Tg and V_L SD Tg BMDMs. Western blot analysis showed that phosphorylation of NF-κB p65 (Ser-536) was rapidly induced after poly(I:C), imiquimod, or CpG ODN stimulation in wild-type BMDMs (Fig. 3). In contrast, lower levels of NF-κB p65 phosphorylation were observed in scFv Tg and V_L SD Tg BMDMs (Fig. 3). The expression levels of total NF-κB p65 protein were comparable among wild-type, scFv Tg, and V_L SD Tg BMDMs (Fig. 3) and similar amounts of total cellular protein were loaded in each lane (β-actin; Fig. 3). These results suggested that anti-WASP scFv and V_L SD intrabodies inhibited NF-κB activation mediated by TLR3, TLR7, and TLR9 stimulation.

In addition, stimulation of TLR3, TLR7, or TLR9 induced the phosphorylation of MAPK family members, including p38 MAPK [15,21,22]. However, we found that the p38 MAPK phosphorylation profiles upon poly(I:C), imiquimod, or CpG ODN stimulation were similar among wild-type, scFv Tg, and V_L SD Tg BMDMs (Supplementary Fig. 1). These findings suggested that anti-WASP intrabodies affected the activation of NF-κB, but not p38 MAPK, in response to poly(I:C), imiquimod, or CpG ODN stimulation in BMDMs.

3.4. Impaired WASP phosphorylation upon poly(I:C), imiquimod, or CpG ODN stimulation in scFv Tg and V_L SD Tg BMDMs

We next assessed the effects of scFv and V_L SD intrabody expression on WASP tyrosine phosphorylation in response to stimulation with poly(I:C), imiquimod, or CpG ODN. Western blot analysis clearly showed WASP tyrosine phosphorylation in wild-type BMDMs upon poly(I:C), imiquimod, or CpG ODN stimulation. In contrast, WASP phosphorylation it was dramatically reduced in scFv Tg and V_L SD Tg BMDMs (Fig. 4). In contrast, Btk tyrosine phosphorylation in response to TLR stimulation was equivalent among all three BMDM cell lines (Fig. 4). Also, the total amounts of WASP and Btk proteins were equivalent among all three BMDM cell lines (Fig. 4). These results suggested that anti-WASP scFv and V_L SD intrabodies inhibited tyrosine phosphorylation of WASP by interfering with Btk binding to the WASP N-terminus. However, these intrabodies did not affect the activation of Btk upon stimulation with poly(I:C), imiquimod, or CpG ODN in macrophages.

4. Discussion

Our finding showed that BMDMs that expressed anti-WASP intrabodies exhibited impaired phosphorylation of WASP and NF-

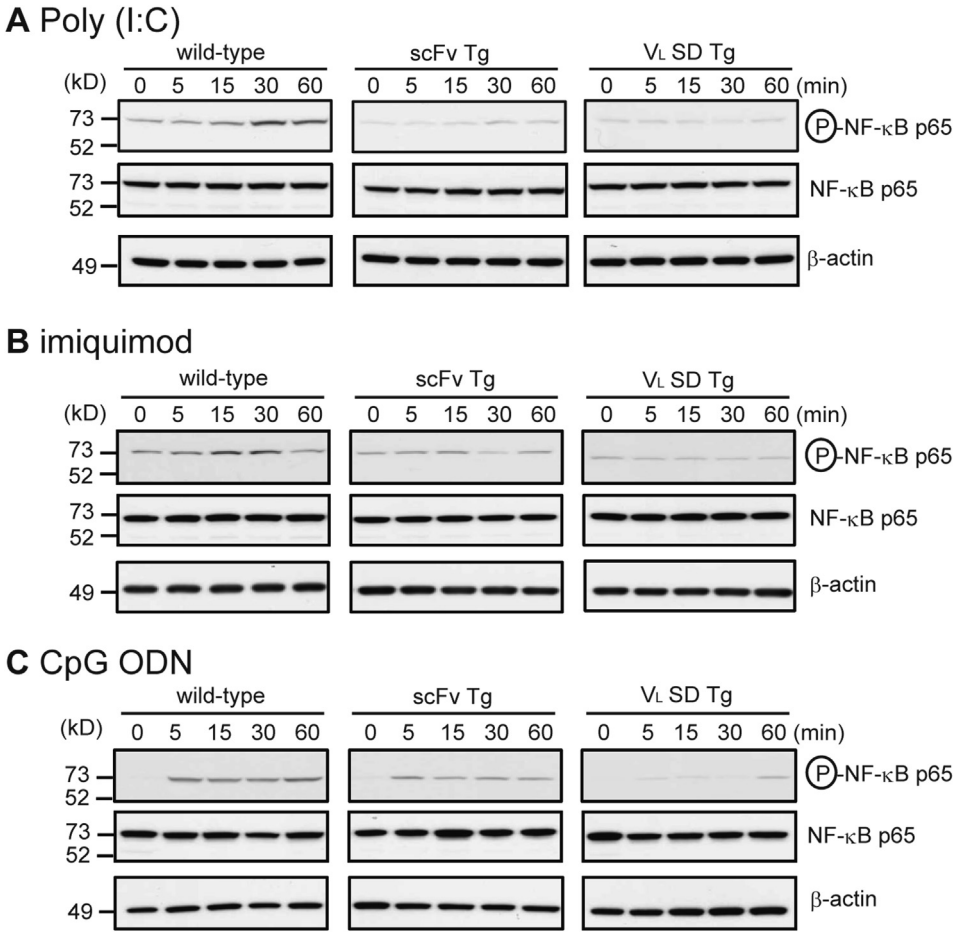

Fig. 3. Phosphorylation of NF-κB induced by stimulation with poly(I:C), imiquimod, or CpG ODN. Wild-type (clone #1), scFv Tg (clone #1), and V_L SD Tg (clone #1) BMDMs were stimulated with (A) poly(I:C), (B) imiquimod, or (C) CpG ODN for the times indicated. Cells were lysed, proteins were separated by SDS-PAGE, and separated proteins were transferred to immunoblot membranes. Immunoblots were probed with anti-phospho-NK-κB antibodies. Equivalent expression of total NK-κB protein was observed in wild-type, scFv Tg, and V_L SD Tg BMDMs. Anti-β-actin signals demonstrate equal protein loading. Immunoblots are representative of three independent experiments.

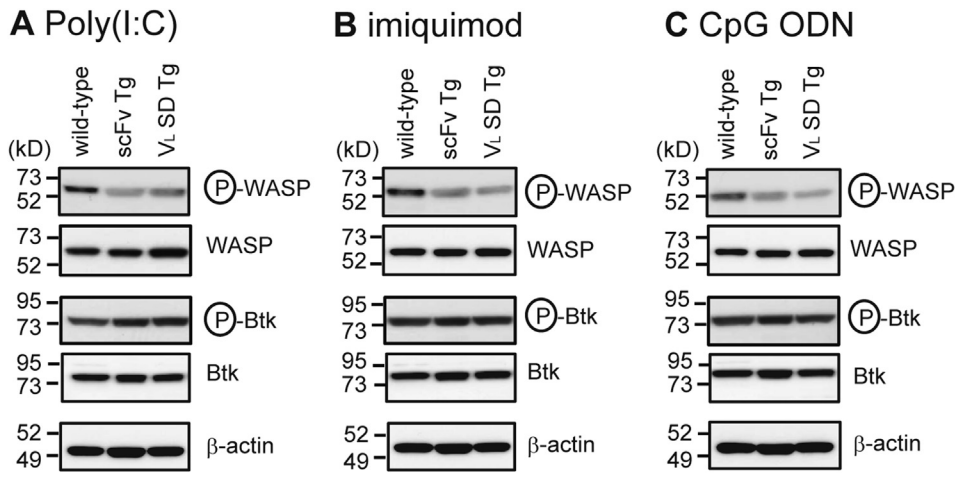

Fig. 4. Phosphorylation of WASP and Btk when BMDMs were stimulated with poly(I:C), imiquimod, or CpG ODN. BMDMs isolated from wild-type (clone #1), scFv Tg (clone #1), and V_L SD Tg (clone #1) were stimulated with poly(I:C), imiquimod, or CpG ODN. The lysate proteins were separated by SDS-PAGE, transferred to immunoblots, and probed with anti-phospho-WASP and anti-phospho-Btk antibodies. Equivalent expression of total WASP and Btk protein was observed in all three BMDM cultures. Anti-β-actin antibody demonstrated equal protein loading. Immunoblots are representative of three independent experiments.

κB which resulted in reduced expression of inflammatory cytokines. These results strongly suggested that WASP and Btk played important roles in TLR3, TLR7, and TLR9 signaling cascades.

Btk, a non-receptor tyrosine kinase, is a member of the Tec kinase family [23]. Recent studies have shown that Btk functions downstream of multiple TLRs to regulate inflammatory responses in innate immune cells. Upon TLR2 or TLR4 stimulation, Btk phosphorylates the Toll/IL-1 receptor domain-containing adaptor protein (TIRAP), an adaptor molecule required for recruitment of MyD88 to TLR2 and TLR4 [24–26]. This recruitment leads to the activation of TLR2– and TLR4–signaling pathways in THP-1 monocytic cells [27]. The TLR3 cytoplasmic domain is tyrosine phosphorylated by Btk upon ligand binding, which leads to downstream signaling in BMDMs [10].

Furthermore, the production of inflammatory cytokines induced by TLR7 stimulation was reduced in BMDMs derived from Btk-deficient mice [11]. In THP1 monocytic cells, Btk was activated by TLR7 and TLR9 stimulation, and this activation was required for NF-κB activation [12]. Although studies have shown that Btk was involved in the NF-κB signaling pathway through the TLR3, TLR7, and TLR9 activation, the mechanism of Btk activation of NF-κB is not fully understood. Based on our previous study, which demonstrated that the WASP-Btk interaction played an important role in TLR4 and TLR2 signaling in macrophages [7–9], we hypothesized that the WASP-Btk interaction may also be involved in modulating TLR3, TLR7, and TLR9 signal transduction. Indeed, the present study showed that the expression of scFv and V_L SD intrabodies reduced WASP phosphorylation, impaired inflammatory cytokine expression, and reduced NF-κB phosphorylation in response to TLR3, TLR7, or TLR9 stimulation. Thus, the WASP-Btk complex played important roles downstream of TLR3, TLR7, and TLR9 to modulate inflammatory responses in macrophages.

TIRAP is an adaptor molecule that bridges TLR2 or TLR4 signaling to MyD88 [24,25]. In response to TLR2 and TLR4 activation, Btk phosphorylates the TIRAP tyrosine, which ultimately leads to NF-κB activation [27,28]. Previously, we demonstrated that the formation of the WASP-Btk complex was important for TLR2- or TLR4-mediated TIRAP phosphorylation. However, TIRAP did not participate in TLR3, TLR7, or TLR9 signaling. Thus, the molecular mechanisms that link the WASP-Btk complex to the induction of TLR3, TLR7, and TLR9 signaling require clarification.

Lorenzi et al. observed reduced phagocytosis in WASP-deficient monocytes and impaired actin-rich phagocytic cups formation in WASP-deficient macrophages [4]. In contrast, BMDMs from scFv Tg and V_L SD Tg mice showed normal phagocytic activity [9]. Therefore, knocking down the WASP N-terminal domain function by expressing anti-WASP intrabodies did not affect the phagocytic activity of macrophages, or the associated actin cytoskeletal rearrangement.

The results of the current investigation have clarified the details of signaling through TLRs. These findings represent great progress in understanding innate immune activation. TLRs recognize pathogen-associated molecules and products derived from damaged tissues, and they are linked to multiple diseases associated with inflammation, including atherosclerosis, asthma, rheumatoid arthritis, and inflammatory bowel disease [29]. Thus, targeting TLRs might be an important approach for treating several diseases. Other achievements that resulted from TLRs studies have been the clinical applications that utilize TLR ligands as vaccine adjuvants. Because TLR signaling represents a significant link between innate and adaptive immunity, an important aspect of vaccine adjuvants is the ability to activate TLRs [14,29]. Further identification of signaling molecules involved in TLR signaling will contribute to creating alternative strategies for curing multiple diseases, including infectious diseases and inflammatory diseases. The present study demonstrated that anti-WASP intrabodies could inhibit the innate immune responses induced by TLR stimulation in macrophages. Thus, future structural analyses of the intrabody binding sites may facilitate the design of new anti-inflammatory or immunosuppressive agents.

Conflict of interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This work was supported in part by the Animal Genome project of the Ministry of Agriculture, Forestry, and Fisheries of Japan.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.01.049.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.01.049.

References

[1] J.M. Derry, H.D. Ochs, U. Francke, Isolation of a novel gene mutation in Wiskott-Aldrich syndrome, Cell 78 (1994) 635–644.
[2] R. Badolato, S. Sozzani, F. Malacarne, S. Bresciani, M. Fiorini, A. Borsatti, A. Albertini, A. Mantovani, A.G. Ugazio, L.D. Notarangelo, Monocytes from Wiskott-Aldrich patients display reduced chemotaxis and lack of cell polarization in response to monocyte chemoattractant protein-1 and formyl-methionyl-leucyl phenylalanine, J. Immunol. 161 (1998) 1026–1033.
[3] D. Zicha, W.E. Allen, P.M. Brickell, C. Kinnon, G.A. Dunn, G.E. Jones, A.J. Thrasher, Chemotaxis of macrophages is abolished in the Wiskott-Aldrich syndrome, Br. J. Haematol. 101 (1998) 659–665.
[4] R. Lorenzi, P.M. Brickell, D.R. Katz, C. Kinnon, A.J. Thrasher, Wiskott-Aldrich syndrome protein is necessary for efficient IgG-mediated phagocytosis, Blood 95 (2000) 2943–2946.
[5] Y. Jin, C. Mazza, J.R. Christie, S. Giliani, M. Fiorini, P. Mella, F. Gandellini, D.M. Stewart, Q. Zhu, D.L. Nelson, L.D. Notarangelo, H.D. Ochs, Mutations of the Wiskott-Aldrich syndrome protein (WASP): hotspots, effect on transcription, and translation and phenotype/genotype correlation, Blood 104 (2004) 4010–4019.
[6] M. Sato, N.M. Tsuji, H. Gotoh, K. Yamashita, K. Hashimoto, N. Tadotsu, H. Yamanaka, K. Sekikawa, Y. Hashimoto, Overexpression of the Wiskott-Aldrich syndrome protein N-terminal domain in transgenic mice inhibits T cell proliferative response via TCR signaling without affecting cytoskeletal rearrangements, J. Immunol. 167 (2001) 4701–4709.
[7] C. Sakuma, M. Sato, T. Takenouchi, J. Chiba, H. Kitani, Critical roles of the WASP N-terminal domain and Btk in LPS-induced inflammatory response in macrophages, PLoS One 7 (2012) e30351.
[8] C. Sakuma, M. Sato, T. Takenouchi, J. Chiba, H. Kitani, Single-chain variable fragment intrabody impairs LPS-induced inflammatory responses by interfering with the interaction between the WASP N-terminal domain and Btk in macrophages, Biochem. Biophys. Res. Commun. 423 (2012) 164–169.
[9] C. Sakuma, M. Sato, T. Takenouchi, H. Kitani, Specific binding of the WASP N-terminal domain to Btk is critical for TLR2 signaling in macroophages, Mol. Immunol. 63 (2015) 328–336.
[10] K. Lee, S. Xu, Z. Kang, J. Huo, M. Huang, D. Liu, O. Takeuchi, S. Skira, K. Lam, Bruton's tyrosine kinase phosphorylates Toll-like receptor 3 to initiate antiviral responses, Proc. Natl. Acad. Sci. U S A 109 (2012) 5791–5796.
[11] J.C. Byrne, J.N. Gabhann, K.B. Stacey, B.M. Coffey, E. McCarthy, W. Thomas, C.A. Jefferies, Bruton's tyrosine kinase is required for apoptotic cell uptake via regulating the phosphorylation and localization of calreticulin, J. Immunol. 190 (2013) 5207–5215.
[12] S.L. Doyle, C.A. Jefferies, C. Feighery, L.A.J. O'Neill, Signaling by Toll-like receptors 8 and 9 requires Bruton's tyrosine kinase, J. Biol. Chem. 282 (2007) 36953–36960.
[13] T. Kawai, S. Akira, The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors, Nat. Immunol. 11 (2010) 373–384.
[14] L.A.J. O'Neill, D. Golenbock, A.G. Bowie, The history of Toll-like receptors – redefining innate immunity, Nat. Rev. Immunol. 13 (2013) 453–460.
[15] L. Alexopoulou, A.G. Holt, R. Medzhitov, R.A. Flavell, Recognition of double-strand RNA and activation of NF-κB by Toll-like receptor 3, Nature 413 (2001) 732–738.
[16] J. Lee, T. Chuang, V. Redecke, L. She, P.M. Pitha, D.A. Carson, E. Raz, H.B. Cottam, Molecular basis for the immunostimulatory activity of guanine nucleoside analogs: activation of Toll-like receptor 7, Proc. Natl. Acad. Sci. U S A 100 (2003) 6646–6651.
[17] S. Bauer, C.J. Kirschning, H. Häcker, V. Redecke, S. Hausmann, S. Akira, H. Wagner, G.B. Lipford, Human TLR9 confers responsiveness to bacterial DNA via species-specific CpG motif recognition, Proc. Natl. Acad. Sci. U S A 98 (2001) 9237–9242.
[18] J.H. Park, Y.G. Kim, C. McDonald, T.D. Kanneganti, M. Hasegawa, M. Body-Malapel, N. Inohara, G. Núñez, RICK/RIP2 mediates innate immune responses induced through Nod1 and Nod2 but not TLRs, J. Immunol. 178 (2007) 2380–2386.
[19] T. Kawai, S. Akira, Toll-like receptor and RIG-1-like receptor signaling, Ann. N Y Acad. Sci. 1143 (2008) 1–20.
[20] H. Hemmi, T. Kaisho, O. Takeuchi, S. Sato, H. Sanjo, K. Hoshino, T. Horiuchi, H. Tomizawa, K. Takeda, S. Akira, Small anti-viral compounds activate immune cells via the TLR7 MyD88-dependent signaling pathway, Nat. Immunol. 3 (2002) 196–200.
[21] H. Nagase, S. Okugawa, Y. Ota, M. Yamaguchi, H. Tomizawa, K. Matsushita, K. Ohta, K. Yamamoto, K. Hirai, Expression and function of Toll-like receptors in eosinophils: activation by Toll-like receptor 7 ligand, J. Immunol. 171 (2003) 3977–3982.
[22] A. Yi, J. Yoon, S. Yeo, S. Hong, B.K. English, A.M. Krieg, Role of mitogen-activated protein kinases in CpG DNA-mediated IL-10 and IL-12 production: central role of extracellular signaling-regulated kinase in the negative feedback loop of the CpG DNA-mediated Th1 response, J. Immunol. 168 (2002) 4711–4720.
[23] A.B. Satterthwaite, O.N. White, The role of Bruton's tyrosine kinase in B-cell development and function: a genetic perspective, Immunol. Rev. 175 (2000) 120–127.
[24] T. Horng, G.M. Barton, R. Medzhitov, TIRAP: an adaptor molecule in the Toll signaling pathway, Nat. Immunol. 2 (2001) 835–841.
[25] T. Horng, G.M. Barton, R.A. Flavell, R. Medzhitov, The adaptor molecule TIRAP provides signalling specificity for Toll-like receptors, Nature 420 (2002) 329–333.
[26] M. Yamamoto, S. Sato, H. Hemmi, H. Sanjo, S. Uematsu, T. Kaisho, K. Hoshino, O. Takeuchi, M. Kobayashi, T. Fujita, K. Takeda, S. Akira, Essential role for TIRAP in activation of the signalling cascade shared by TLR2 and TLR4, Nature 420 (2002) 324–329.
[27] P. Gray, A. Dunne, C. Brikos, C.A. Jefferies, A.L. Doyle, L.A.J. O'Neill, MyD88 adaptor-like (Mal) is phosphorylated by Bruton's tyrosine kinase during TLR2 and TLR4 signal transduction, J. Biol. Chem. 281 (2006) 10489–10495.
[28] L.A.J. O'Neill, A.G. Bowie, The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling, Nat. Rev. Immunol. 7 (2007) 353–364.
[29] L.A.J. O'Neill, C.E. Bryant, S.L. Doyle, Therapeutic targeting of Toll-like receptors for infectious and inflammatory diseases and cancer, Pharmacol. Rev. 61 (2009) 177–197.