Review

Adaptor Protein STAP-2 Modulates Cellular Signaling in Immune Systems

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Signal-transducing adaptor protein-2 (STAP-2) is a recently identified adaptor protein that contains a pleckstrin homology (PH), Src homology 2 (SH2)-like domains, and proline-rich regions in its C-terminal. STAP-2 belongs to a family of STAP adaptor proteins and plays a crucial role in a variety of cellular signal transduction pathways by interacting with signaling or transcriptional molecules. STAP-2, in particular, regulates both the innate and adaptive immune systems. STAP-2 functionally interacts with signal transducers and activators of transcription 3 (STAT3) and STAT5 in cytokine signaling pathways. In addition, STAP-2 also binds to myeloid differentiation factor 88 (MyD88) and inhibitor (I) κ B kinase α/β (IKK- α/β) in Toll-like receptor4 (TLR4) signaling, and enhances the production of inflammatory cytokines in macrophages. More importantly, experiments using STAP-2 deficient mice show that STAP-2 modulates several T-cell functions such as cell motility, survival and death. It is also reported that STAP-2 controls the immunoglobulin E (IgE)-mediated allergy response. This accumulated evidence indicates that adaptor protein STAP-2 is an important modulator of both the innate and adaptive immune systems.

Key words signal-transducing adaptor protein-2 (STAP-2); adaptor protein; cytokine; T-cell; macrophage; signal transduction

1. INTRODUCTION

Intracellular signaling triggered by extracellular ligands such as cytokines, hormones, and growth factors is transduced and regulated by numerous molecules. It has been well documented that the activation of receptor tyrosine kinases is mediated by oligomerization and subsequent autophosphorylation after the ligand ligation, thereby activating a downstream signaling cascade that includes kinases, transcription factors, and adaptor molecules.

In these cellular signaling pathways, adaptor proteins play an important role in modulating signal transduction. Although adaptor proteins themselves do not contain any enzymatic function, they can convey signals to their targets by interacting with other molecules through their unique domain structures. Adaptor proteins have a variety of functional domains which mediate specific protein-protein and protein-lipid interactions.^{1–5)} For example, Src homology 2 (SH2) and phosphotyrosine binding (PTB) domains can bind to phosphotyrosine containing motifs.^{6–9)} Src homology 3 (SH3) domains recognize and bind to proline-rich sequences in their respective binding partners.¹⁰⁾ PH domains are lipid interacting modules which bind to specific phosphoinositides and play a role in recruiting proteins to defined regions of the plasma membrane.¹¹⁾

This review focuses on a novel adaptor protein, signal-transducing adaptor protein-2 (STAP-2), which has recently been shown to have pleiotropic functions in cellular signaling pathways by interacting with numerous molecules through its functional domains (Table 1).

2. STAP FAMILY, STRUCTURE, EXPRESSION AND LOCALIZATION

In 2000, the human STAP-2 gene was initially isolated as *bks*, a substrate of breast tumor kinase (Brk), from a yeast two-hybrid screen from a human normal breast cDNA library, aiming to identify proteins that interact with Brk non-receptor tyrosine kinase.¹²⁾ However, the physiological functions of STAP-2/Bks had not been investigated. Three years later, the mouse homologue of STAP-2 was cloned as c-fms interacting protein by yeast two-hybrid screening of a fetal liver cDNA library, and STAP-2 gene-disrupted mice were generated to elucidate its functions.¹³⁾

STAP-2 shows high sequence and structural similarities to STAP-1, which was cloned as a c-kit-interacting protein from a hematopoietic stem cell library.¹⁴⁾ STAP-1 was also identified as a Tec-interacting protein, which is tyrosine phosphorylated in response to B-cell receptor (BCR) stimulation and termed as BCR Downstream Signaling 1 (BRDG1).¹⁵⁾ STAP-1 and STAP-2 share conserved domains, *i.e.*, an N-terminal PH domain and a region distantly related to the SH2 domain (referred to as an “SH2-like” domain in Fig. 1), and their overall amino acid sequence identity is 33%. Sequence similarity between STAP-1 and STAP-2 is observed as follows: N-terminal PH domain is 36% identical (and 58% similar) and SH2-like domain located in the central region of the molecule is 40% identical. The SH2-like domain in STAP-2 also shares sequence similarity with phospholipase C γ (PLC γ) SH2 domain (amino acid identity is 29%).¹³⁾ In contrast to STAP-1, STAP-2 conserves a proline-rich domain and a YXXQ motif in its C-terminus: the former is required for the binding to SH3 domain-containing proteins and the latter is conserved in the cytoplasmic region of certain types of cytokine receptors and is known to serve as the docking module to bind to the SH2 domain in signal transducer and activator of transcription 3

Table 1. STAP-2 Interacting Proteins

Interacting molecule	Domain or region	Functions	References
BRK	PH (SH2-like)	Brk/STAT3/STAT5 signaling ↑	12, 82, 83, 84
c-Fms	PH (SH2-like)	M-CSF/c-Fms signaling ↓	13, 36, 37
STAT3	YXXQ motif (SH2-like)	STAT3 phosphorylation and activation ↑	13, 83
STAT5a, 5b	PH, SH2-like	STAT5 phosphorylation and activation ↓	16
MyD88	SH2-like	LPS/TLR4 signaling ↑	27
IKK α , β	SH2-like	LPS/TLR4 signaling ↑	27
FAK	SH2-like	T-cell adhesion ↓	17
Cbl	PH, SH2-like	T-cell adhesion ↓	17, 18
Vav1	SH2-like, C-terminal	T-cell migration ↑	52
Rac1	n.d.	T-cell migration ↑	52
Fas/CD95	n.d.	T-cell apoptosis ↑	64
Caspase-8	SH2-like	T-cell apoptosis ↑	64
PLC γ 1, 2	SH2-like, C-terminal	IgE/Fc ϵ R1 signaling ↓	76
LMP1	PH, SH2-like	LMP1/NF- κ B signaling ↑	81
TRAF1	n.d.	LMP1/NF- κ B signaling ↑	81
TRAF3	PH, SH2-like	LMP1/NF- κ B signaling ↑	81
BCR-ABL	SH2-like	BCR-ABL/STAT5, ERK signaling ↑	85

This table summarizes the STAP-2-interacting proteins. The interacting domains and the functions of STAP-2 are also shown. n.d., not determined

Fig. 1. Structural Comparison of STAP Family Proteins

The domain structures of human STAP family proteins are schematically shown. Four predicted tyrosine phosphorylation sites of STAP-2 are also shown. PH, Pleckstrin homology; SH2, Src homology 2; Pro-rich, Proline rich region.

(STAT3) (Fig. 1).

Western blotting analyses by Mitchell *et al.* showed that STAP-2 protein was detectable in various human tissues tested.¹² In contrast, STAP-1 mRNA has been shown to have hematopoietic-specific expression.^{14,15} It is also shown by Minoguchi *et al.* that STAP-2 mRNA was inducible in some tissues or cell lines. The STAP-2 mRNA was markedly elevated at 3 to 6 h in mouse liver after intraperitoneal injection of lipopolysaccharide (LPS). In addition, STAP-2 mRNA expression was also strongly induced in M1 cells, a murine myeloid leukemia cell line treated with leukemia inhibitory factor, LIF.¹³ In contrast, STAP-1 mRNA expression in M1 cells was diminished after treatment with LIF.¹⁴ Figure 2 compares the expression levels of STAP-1 and STAP-2 mRNAs in mouse, and demonstrates that STAP-1 mRNA is highly expressed in immune tissues, including the spleen and thymus, whereas STAP-2 mRNA is ubiquitously expressed, not solely in immune tissues.

Ectopically expressed STAP-2 localizes throughout the cytoplasm and nucleus in many different types of cell lines, including the human epithelial carcinoma cell line, A431,¹³ African green monkey kidney cell line, Cos-7,¹⁶ human T-cell leukemia cell line, Jurkat¹⁷ and human cervix carcinoma cell line, HeLa.¹⁸ In the case of A431 cells, epidermal growth factor (EGF) induces rapid (5–10 min) translocation of green fluorescent protein (GFP)-fused STAP-2 wild type (WT) protein to the plasma membrane, while mutant STAP-2 that lacks

Fig. 2. Expression Profile of STAP-1 and STAP-2 mRNA in Mouse Tissues

Total RNA samples isolated from these tissues were subjected to quantitative real-time PCR analysis using STAP-1 and STAP-2 primers. Data represent the levels of these mRNA normalized to that of the GAPDH internal control.

the PH domain fails to translocate to the plasma membrane in response to EGF.¹³⁾ Hence, the PH domain of STAP-2 is necessary for plasma membrane recruitment in response to EGF.

3. STAP-2 REGULATES JANUS KINASE (JAK)/STAT SIGNALING PATHWAYS

The Jak/STAT pathway is one of the major pathways in cytokine signaling.^{19–22)} Binding of a cytokine to its cognate receptor results in receptor dimerization and the activation of Jak tyrosine kinases. The activated Jak subsequently phosphorylates specific tyrosine residues on the cytokine receptor and serves as a docking site for STATs. Recruited STATs are tyrosine phosphorylated by activated Jak, then dimerize *via* SH2 domain phosphotyrosine interactions, and subsequently leave the receptor and translocate into the nucleus where they activate target gene transcription. Seven STAT proteins have been identified: STAT1, STAT2, STAT3, STAT4, STAT5A, STAT5B, and STAT6. STAT2, STAT4 and STAT6 are activated by a relatively small number of cytokines and play a distinct role in the development of T-cells and in interferon (IFN)- γ signaling, whereas STAT1, STAT3 and STAT5 are activated by a variety of ligands in several different tissues and are also involved in cell-cycle, apoptosis and tumorigenesis.²³⁾

STAP-2 contains a YXXQ motif, which is located in its C-terminal region, is conserved among various mammals, and has been expected as a potential STAT3 binding site. Indeed, STAP-2 interacted with STAT3 through its YXXQ motif, whereas the STAP-2 Y322F mutant, in which the tyrosine residue at 322 is substituted with phenylalanine, failed to bind.¹³⁾ It should be noted that STAP-2 expression was elevated in response to interleukin (IL)-6 in primary hepatocytes. On the other hand, STAT3 activation, as well as the acute phase responses that are regulated by the IL-6/STAT3 pathway, was impaired in the liver of STAP-2 deficient mice.¹³⁾ Furthermore, transient over-expression of STAP-3 in cultured cells results

in the STAT3 activation depending on its YXXQ motif.¹³⁾ It has also been shown that STAP-2 tyrosine-250 (Tyr250), a major tyrosine phosphorylation site by v-Src, Jak2 and LIF, is involved in the STAT3-enhancing activity of STAP-2, since the substitution of tyrosine residue at 250 with phenylalanine failed to enhance STAT3 transcriptional activity.^{13,24)} These observations demonstrate that tyrosine phosphorylation of STAP-2 is important for enhancing STAT3 transcriptional activation. In conclusion, these data indicate that STAP-2 is an adaptive protein in IL-6- and LIF-mediated STAT3 signaling.

Previously, STAP-1 has been shown to associate with STAT5 through its SH2 domain.¹⁴⁾ Interestingly, STAP-2 binds not only to STAT3 but also to STAT5A and STAT5B through its PH and SH2-like domains. Furthermore, the association of STAP-2 and STAT5 was attenuated by the treatment of cells with IL-2.¹⁶⁾ From these observations, STAP-2 is thought to interact with a non-phosphorylated form of STAT5 and to dissociate from a tyrosine-phosphorylated form of STAT5 that is activated in response to cytokine stimulation. Indeed, the level of STAT5 tyrosine phosphorylation and STAT5-dependent transcription in response to erythropoietin or IL-3 was shown to be decreased in cells that overexpress STAP-2. In addition, STAP-2 suppresses the proliferation of the mouse pro-B cell line, Ba/F3,²⁵⁾ which is an IL-3/STAT5-dependent cell line. It has been shown that there is a profound deficiency of peripheral T-cells in STAT5A/5B double knockout mice, indicating that STAT5 proteins play an essential role in IL-2 signaling.²⁶⁾ Interestingly, STAP-2-deficient thymocytes show enhanced cell growth in response to IL-2.¹⁶⁾ These data suggest that STAP-2 might negatively regulate STAT5-mediated cytokine signaling.

Taken together, in the Jak/STAT pathway, STAP-2 plays a role in regulating the transcriptional activity of STATs in response to a variety of cytokine stimulations. As its unique characteristics, STAP-2 shows pleiotropic function in cytokine signaling. The STAT3-mediated cytokine signaling through

Fig. 3. STAP-2 Regulates Jak/STAT Signaling Pathways

Schematic showing the proposed function of STAP-2 in the Jak/STAT signaling pathways. STAP-2 interacts with STAT3 through the YXXQ motif in the C-terminal region and positively regulates STAT3 transcriptional activation. STAP-2 interacts with STAT5 through its PH and SH2-like domain and negatively regulates STAT5-mediated signaling.

IL-6 and LIF is enhanced by STAP-2. On the other hand, STAP-2 down-regulates the STAT5-mediated cytokine signaling pathways triggered by IL-2, IL-3 or erythropoietin (Fig. 3).

4. STAP-2 IN MACROPHAGE

4.1 STAP-2 Regulates Toll-Like Receptor (TLR) Signaling Pathways²⁷⁾ TLRs are type-I transmembrane proteins containing leucine-rich repeat motifs in the extracellular region and a Toll/interleukin-1 receptor (TIR) domain in the intracellular region, and they play an essential role in innate immunity in organisms ranging from insects to mammals.^{28–31)} Microorganisms express highly conserved sequences known as pathogen-associated molecular patterns (PAMPs) which are recognized by innate immune cells such as macrophages and dendritic cells through a series of TLRs. Cell surface TLRs, including TLR1, TLR2, TLR4, TLR5, TLR6 and TLR11, recognize mainly microbial membrane components such as LPS, diacyl or triacyl lipopeptides and zymosan. On the other hand, microbial nucleic acids such as double-stranded RNA and bacterial CpG DNA, are detected by TLR3-, TLR7-, TLR8- and TLR9-expressing intracellular vesicles, in endoplasmic reticulum (ER), endosomes, lysosomes and endolysosomes.^{32–35)} PAMPs recognition by TLRs leads to the recruitment of various TIR domain-containing adaptors containing MyD88, TIR-associated protein (TIRAP), Toll-receptor-associated activator of interferon (TRIF) and Toll-receptor-associated molecule (TRAM), then activate subsequent signaling such as nuclear factor (NF)- κ B and mitogen-activated protein kinases (MAPK), resulting in the production of inflammatory cytokines and the expression of co-stimulatory molecules. The se-

creted inflammatory cytokines, then, not only stimulate macrophages and natural killer cells that directly kill pathogens but also enhance the clonal B and T lymphocyte responses.

As mentioned above, up-regulation of STAP-2 mRNA was observed in mouse liver after intraperitoneal injection of LPS, and in LIF-treated M1 cells that underwent differentiation into macrophages.¹³⁾ The expression patterns of STAP-2 mRNA in macrophages suggest the involvement of STAP-2 in LPS signaling. Indeed, compared to WT mice, STAP-2 knock out (KO) mice showed a significantly reduced response in terms of LPS-induced TNF- α production in the serum.²⁷⁾ In addition, macrophages isolated from STAP-2 KO mice showed lowered production of TNF- α and IL-6 in response to LPS, compared with those from WT mice.²⁷⁾

Phosphorylation and degradation of inhibitor (I) κ B α leads to NF- κ B transcriptional activation, subsequently producing inflammatory cytokines in the LPS/TLR4 signaling pathway. In contrast to WT mice-derived macrophages, LPS-induced phosphorylation of I κ B α was diminished in macrophages from STAP-2-deficient mice.²⁷⁾ However, activation of p38 MAP kinase (MAPK) and JNK in response to LPS was similarly observed in both STAP-2-deficient macrophages and in WT ones.²⁷⁾ Furthermore, a STAP-2-overexpressing macrophage cell line Raw264.7, Raw/STAP-2, produced significantly higher amounts of TNF- α and IL-6 after LPS stimulation than Raw/pcDNA3, which represents parental Raw264.7 transfected with pcDNA3 vector alone.²⁷⁾ It was also observed that LPS-stimulated Raw/STAP-2 cells showed enhanced NF- κ B activation.²⁷⁾

Co-immunoprecipitation experiments showed that STAP-2 interacted with MyD88, IKK α and IKK β through its SH2-like domain, but not with IRAK1 and TRAF6.²⁷⁾ Interestingly, the

Fig. 4. STAP-2 Regulates Macrophage Functions

STAP-2 enhances the LPS/TLR4 signaling pathway. STAP-2 interacts with MyD88 and IKK- α/β , leading to the enhancement of NF- κ B activity and the production of inflammatory cytokines. STAP-2 may constitute an alternative pathway from LPS/TLR4 to NF- κ B activation instead of the TRAF6-IRAK1 pathway. STAP-2 regulates the M-CSF/c-Fms signaling pathway. STAP-2 interacts with c-Fms, leading to the suppression of ERK and Akt phosphorylation. The suppression of M-CSF/c-Fms signaling by STAP-2 results in the regulation of macrophage migration.

association between MyD88 and IKK β was only observed in the presence of STAP-2.²⁷⁾ This data suggests that STAP-2 is able to interact with both MyD88 and IKK, and plays a role in forming a molecular complex that leads to NF- κ B activation (Fig. 4).

Bacterial or virus CpG motifs are recognized by TLR9 and induce inflammatory cytokine secretion through MyD88-dependent NF- κ B activation, whereas polyinosinic-polycytidylic acid (poly(I:C))/TLR3 signaling uses TRIF instead of MyD88 to produce inflammatory cytokines.²⁸⁾ It should be noted that the production of TNF- α and IL-6 through the CpG/TLR9 pathway was impaired in STAP-2-deficient macrophages, while that through the poly(I:C)/TLR3 pathway remained intact.²⁷⁾ Thus, STAP-2 augments the signaling triggered by LPS/TLR4 and CpG/TLR9 mediated through MyD88, thereby stimulating cytokines production by macrophages, while it does not participate in the MyD88-independent signaling pathway.

Taken together, STAP-2 positively regulates TLR signaling by interacting with MyD88 and IKKs to activate NF- κ B and induce inflammatory cytokines in macrophage cells. Furthermore, the data suggest the possibility that STAP-2 can be considered a novel candidate for anti-inflammatory drug development to regulate the expression of NF- κ B-dependent genes.

4.2 STAP-2 Regulates Macrophage-Colony Stimulating Factor (M-CSF) Receptor Signaling Pathways STAP-2 plays a role in macrophages, not only in regulating TLR signaling but also in M-CSF (macrophage-colony stimulating factor)-mediated signaling.^{36,37)} M-CSF, known as CSF-1, is the principal molecule responsible for the development, differentiation, proliferation, survival and motility of macrophage lineages.^{38–40)} M-CSF binds to its receptor c-Fms which is encoded by the *c-fms* proto-oncogene,⁴¹⁾ and subsequently induces the dimerization of c-Fms, leading to the autophosphorylation of a number of tyrosine residues throughout the cytoplasmic domain of the receptor. Tyrosine-phosphorylation of c-Fms induces the recruitment of intracellular signaling molecules, resulting in activation of both the PI3K/Akt and MAPK/ERK signaling cascades.^{42–44)} M-CSF/PI3K signaling regulates macrophages' cytoskeletal rearrangement and motility.^{44,45)}

Originally, STAP-2 was cloned by yeast two-hybrid screening using c-fms as bait.¹³⁾ Co-immunoprecipitation assays using mammalian cells revealed that STAP-2 interacts with c-Fms through its PH domain independently of M-CSF stimulation.³⁶⁾ M-CSF stimulation does not affect the interaction between c-Fms and STAP-2, although M-CSF stimulation induces tyrosine-phosphorylation of STAP-2 *via* c-Fms. M-CSF/c-Fms-mediated tyrosine-phosphorylation of STAP-2 is observed mainly at Tyr-250 and weakly at Tyr-310. Intriguingly, tyrosine-phosphorylation of c-Fms after the stimulation with M-CSF was suppressed in Raw/STAP-2 cells.³⁶⁾ Furthermore, phosphorylation of Akt and ERK in response to M-CSF was also impaired in Raw/STAP-2 cells.³⁶⁾ In contrast, bone marrow-derived macrophages (BMMs) isolated from STAP-2 KO mice showed enhanced phosphorylation of Akt and ERK in response to M-CSF, compared to BMMs from WT mice.³⁷⁾ Thus, STAP-2 modulates M-CSF-mediated PI3K/Akt and Ras/ERK signaling pathways in macrophages. More importantly, STAP-2 regulates macrophage cell migration. When Raw/STAP-2 cells were subjected to *trans*-well assay, fewer

numbers of cells migrated toward M-CSF, compared to Raw/pCDNA3 cells.³⁶⁾ In contrast, the wound-healing assay using STAP-2-deficient BMMs demonstrated elevated cell motility in response to M-CSF.³⁷⁾

These findings suggest that STAP-2 controls not only TLR-mediated cytokine production, but also M-CSF-induced cell migration in macrophage cells (Fig. 4). It is likely that STAP-2 maintains the homeostasis of macrophage-mediated functions by regulating cytokine production and cell motility during infection or inflammatory diseases.

5. STAP-2 IN T-CELLS

5.1 STAP-2 Modulates T-Cell Motility In the immune system, lymphocyte trafficking and homing to specific micro-environments are very important to maintaining homeostasis and controlling the immune response.^{46–49)} Lymphocyte motility is mainly controlled by selectin- and integrin-mediated cell adhesion to the extracellular matrix (ECM) and chemokine-mediated cell migration to specific sites. Integrins are known to regulate rolling and firm adhesion of leukocytes.⁴⁷⁾ STAP-2 plays a role in regulating T-cell adhesion by modulating integrin-mediated signaling.¹⁷⁾ It is not surprising that STAP-2 regulates the motility of T-cells, as it does in M-CSF-stimulated macrophages, because STAP-2 is expressed in T-cells, in which it participates in IL-2/STAT5-mediated cell growth.¹⁶⁾ Splenocytes and T-cells from STAP-2-deficient mice showed enhanced cell adhesion to fibronectin (FN), a component of ECM, after treatment with phorbol myristate acetate (PMA).¹⁶⁾ In addition, Jurkat cells that stably over-express STAP-2 (Jurkat/STAP-2) demonstrated reduced cell adhesion to FN after integrin activation with a β 1-integrin specific antibody. In the case of STAP-2 deficient T-cells, the expression level of focal adhesion kinase (FAK) proteins was elevated.¹⁶⁾ On the other hand, overexpression of STAP-2 induces a dramatic decrease in the protein content of FAK in Jurkat T-cells.¹⁶⁾ FAK is a ubiquitously expressed non-receptor protein tyrosine kinase, which has emerged as a crucial molecule for integrating signals from integrins and receptor tyrosine kinases in processes such as cell survival, proliferation and motility.^{50,51)} It is noteworthy that STAP-2 were shown to associate with FAK through its SH2-like domain, and the treatment of cells with proteasome inhibitors resulted in the accumulation of polyubiquitinated forms of FAK in Jurkat/STAP-2 cells.¹⁷⁾ Furthermore, STAP-2 can also interact with the E3-ubiquitin ligase, Cbl, and recruits it to FAK.¹⁷⁾ In addition, a decrease in Cbl caused increased levels of FAK proteins in Jurkat/STAP-2 cells.¹⁷⁾ Interestingly, it is also demonstrated that STAP-2 protein expression levels are also regulated by this proteasomal degradation.¹⁸⁾ These lines of evidence indicate that STAP-2 is a potential novel regulator of integrin/FAK-mediated T-cell adhesion (Fig. 5).

STAP-2 regulates not only integrin-mediated T-cell adhesion but also chemokine-induced T-cell migration.⁵²⁾ Chemokines are chemoattractive cytokines which bind to seven-transmembrane G protein-coupled receptors, and are involved in chemotaxis, transendothelial migration of leukocytes during immune and inflammatory responses, non-inflammatory functions during T and B cell development, lymphocyte trafficking, and cell compartmentalization in lymphoid tissues.^{46,53,54)} Stromal cell-derived factor-1 α (SDF-1 α ; CXCL12) is a member

Fig. 5. STAP-2 Modulates T-Cell Functions

STAP-2 is a crucial modulator of T-cell motility. T-Cells from STAP-2 deficient mice show enhancement of integrin-mediated cell adhesion to fibronectin. STAP-2 interacts with both FAK and Cbl in T-cells and plays a role in the regulation of proteasome-mediated degradation of FAK by recruiting Cbl. STAP-2 also interacts with Vav1 in the downstream of SDF-1 α mediated signaling. STAP-2 regulates SDF-1 α -induced Vav1/Rac1 activation, controlling chemotaxis toward to SDF-1 α in T-cells.

of the CXC subfamily of chemokines and uses CXCR4 as the receptor.^{55–58} CXCR4 is expressed on a number of cell types such as T cells, hematopoietic stem cells and progenitor cells, and mediates the signaling that leads to the development and migration of these cells.^{55–58}

SDF-1 α -dependent migration of T-cells isolated from STAP-2 KO was lower than that of T-cells from WT mice.⁵² In contrast, the chemotactic response of Jurkat/STAP-2 cells is elevated in response to SDF-1 α compared with Jurkat/pcDNA3 cells.⁵² Thus, STAP-2 can up-regulate the directional migration of T cells toward SDF-1 α . ERK is not involved in STAP-2-dependent up-regulating signaling during T-cell migration, since the levels of ERK phosphorylation in Jurkat/pcDNA3 and Jurkat/STAP-2 cells were comparable after the stimulation with SDF-1 α .⁵² It is known that SDF-1 α -induced activation of Rho GTPases, including RhoA, Rac1 and Cdc42, controls T-cell migration.^{59,60} Indeed, SDF-1 α causes robust activations of Rac1 and Cdc42 in Jurkat/STAP-2 cells, but not in Jurkat/pcDNA3 cells.⁵² Furthermore, the colocalization and association of STAP-2 with Rac1 was observed after stimulation with SDF-1 α .⁵² Vav1, a guanine-nucleotide exchange factor (GEF), plays a critical role in T-cell development and activation.^{61,62} In the SDF-1 α signaling pathway, Vav1 acts as a GEF for Rac1, and inhibition of Vav1/Rac1 impairs SDF-1 α -promoted T-cell migration.⁶³ STAP-2 was shown to associate with Vav1 in the absence of SDF-1 α stimulation in T-cells,⁵² indicating that STAP-2 constitutively interacts with Vav1. When endogenous Vav1 protein expression was lowered, binding between STAP-2 and Rac1 was abolished, thereby resulting in decreased Rac1 activation.⁵² Furthermore, phosphorylated levels of Vav1 after SDF-1 α stimulation are elevated in Jurkat/STAP-2 cells, but lowered in STAP-2-deficient T-cells.⁵² Accumulating data suggest that STAP-2 enhances the phosphorylation of Vav1 after SDF-1 α stimulation and subsequently recruits Rac1 to Vav1, resulting in augmented Rac1 GTPase activation and facilitation of T-cell migration (Fig. 5).

In addition, studies on STAP-2 during T-cell migration have shown that STAP-2 controls T-cell accumulation in tissues undergoing inflammation.⁵²

5.2 STAP-2 Regulates Apoptosis Signaling in T-Cells⁶⁴
During the course of an immune response, T-cells clonally expand after encountering the antigen presented by antigen presenting cells (APC), after which activated peripheral T-cells are removed in the termination phase. The death of these cells is important in preventing autoimmunity and maintaining T-cell homeostasis. However, a certain number of T-cells survive, forming a pool of memory T cells, which are specialized cells that respond rapidly to subsequent exposure to the same antigen.^{65–67} Elimination of activated T-cells during the termination phase of immune responses is mediated through two mechanisms: activation-induced cell death (AICD) and activated T-cell autonomous death (ACAD).⁶⁸ AICD is mainly triggered by death receptor signaling causing programmed cell death, or apoptosis. In contrast to AICD, ACAD is also referred to as death by neglect, death by cytokine withdrawal or passive cell death.

AICD is mediated through interactions between the death receptor Fas, known as CD95, and its ligand, FasL, expressed on activated T-cells. Fas oligomerization leads to the formation of the Fas-death-inducing signaling complex (Fas-DISC) that initiates apoptosis. The Fas-DISC consists of oligomerized, probably trimerized, Fas, the adaptor molecule FADD, procaspase-8, procaspase-10 and c-FLIP. Autoproteolytic activation of pro-caspase-8 is induced after oligomerization at the receptor complex, followed by the release of active caspase-8 heterotetramer into cytosol for apoptotic signal propagation.^{69–71}

The T-cell development in STAP-2-deficient mice, assessed by the expression of CD4 and CD8, was comparable to that in WT mice.¹⁶ In addition, expression levels of CD3 on thymocytes and splenocytes were also indistinguishable between WT and STAP-2-deficient mice.¹⁶ AICD is known

to be induced in WT mice by the injection of anti-CD3 antibody, which can directly stimulate TCR, resulting in the reduced T-cell-to-B-cell ratio and increased terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL)-positive CD4-positive T-cells in the spleen.⁷²⁾ However, neither a reduction of the T/B cell ratio nor an increase in TUNEL-positive cells was observed in the spleen of STAP-2-deficient mice subjected to anti-CD3 antibody injection.⁶⁴⁾ Furthermore, while WT mice injected with a superantigen, SEB, displayed an elimination of $V\beta 8^+$ (superantigen responsive) T-cells in a Fas-dependent manner,⁷³⁾ no elimination of $V\beta 8^+CD4^+$ -responsive cells was observed in SEB-injected STAP-2 deficient mice.⁶⁴⁾ In neither WT mice nor STAP-2 deficient mice was elimination of the $V\beta 6^+CD4^+$ T-cell population (unresponsive to SEB) observed.⁶⁴⁾ These *in vivo* data suggest STAP-2 is involved in T-cell AICD.

The involvement of STAP-2 in AICD was confirmed by studies *in vitro*. Jurkat/STAP-2 cells showed significantly enhanced cell death after treatment with phytohemagglutinin (PHA), an inducer of T-cell AICD, compared to Jurkat/pcDNA3.⁶⁴⁾ In addition to the death receptor stimulation, *i.e.*, Fas activation by anti-Fas antibody, TNF- α and TRAIL-induced cell death was also enhanced in Jurkat/STAP-2 cells.⁶⁴⁾ In contrast, in T-cells from STAP-2-deficient mice, FasL-induced apoptosis was impaired.⁶⁴⁾ Furthermore, the activation of caspase-3 and -8 was significantly enhanced in Jurkat/STAP-2 cells treated with anti-Fas antibody, whereas activation of those was impaired in FasL-stimulated STAP-2 deficient T-cells.⁶⁴⁾ Moreover, a caspase-8 specific inhibitor suppressed Fas-induced cell death in Jurkat/STAP-2 cells,⁶⁴⁾ suggesting that the enhanced activation of caspase-8 is critical to increasing the susceptibility to Fas-mediated cell death.

In the FasL/Fas signaling pathway, STAP-2 was shown to interact with Fas and caspase-8, but not with FADD.⁶⁴⁾ Interestingly, Fas-DISC complex formation after the treatment with anti-Fas antibody was dramatically augmented in STAP-2-over-expressing Jurkat cells.⁶⁴⁾ In contrast, T-cells from STAP-2-deficient mice that show suppressed activation of caspase-8 exhibited a reduced level of Fas-DISC complex formation after Fas ligation.⁶⁴⁾ Thus, STAP-2 is likely to enhance the Fas-DISC formation and subsequent caspase-8 activation.

A consensus caspase-8 cleavage site was reported to be (I/L/V)EXD.⁷⁴⁾ STAP-2 has a VEAD sequence (residues 257–260) that may be a potential caspase-8 cleavage site. It is noteworthy that cleaved STAP-2 fragment was detected after Fas stimulation in Jurkat/STAP-2 and HUT78 cells.⁶⁴⁾ Furthermore, a STAP-2 mutant (STAP-2 DA), in which aspartic acid residue was substituted with alanine, did not generate any Fas-mediated cleavage fragments, although it interacted with caspase-8, but not with Fas.⁶⁴⁾ Thus, Fas-DISC formation is abolished in Jurkat/STAP-2 DA, and proteolytic cleavage of STAP-2 at Asp260 by caspase-8 is required for the enhancement of Fas-induced apoptosis and caspase-8 enzymatic activation. In summary, these data indicate that STAP-2 is a novel participant in the regulation of T-cell AICD by controlling Fas-mediated caspase-8 activation (Fig. 6).

6. STAP-2 MODULATES ALLERGIC RESPONSE

STAP-2 regulates immune and inflammatory responses through interactions with a variety of signaling or transcrip-

Fig. 6. STAP-2 Regulates Fas-Induced Cell Death Signaling

T-Cells from STAP-2 deficient mice show resistance to Fas-mediated apoptosis. STAP-2 interacts with both Fas and caspase-8 but not FADD in T-cells. STAP-2 increases Fas-DISC complex formation and results in the activation of caspase-8 and subsequent induction of apoptosis.

tional molecules.⁷⁵⁾ It is also reported that the overexpression of STAP-2 in a rat basophilic leukemia cell line, RBL-2H3, resulted in a dramatic suppression of tyrosine phosphorylation of PLC- γ , calcium mobilization, and degranulation, all of which were triggered by immunoglobulin E (IgE)/Fc ϵ RI, a high-affinity receptor for IgE.⁷⁶⁾ These facts suggest that STAP-2 may have the potential to negatively regulate Fc ϵ RI-mediated signals. IgE/Fc ϵ RI signaling plays a central role in allergic diseases.^{77–80)} In mast cells and basophils, the aggregation of Fc ϵ RI eventually leads to the release of granule components such as histamine and cytokines. Although further experiments using STAP-2-deficient mast cells or basophils are required to demonstrate physiological STAP-2 functions in IgE/Fc ϵ RI-mediated pathogenesis of allergic diseases, STAP-2 has a potential to regulate allergic signaling and is a possible target for allergic disorder drug development.

CONCLUSION

STAP-2 expression is detected in numerous tissues and cell lines such as lymphocytes, macrophages, hepatocytes and breast tumor cell lines, and its patterns of inducible expression imply that it is a multifunctional protein. Specifically, STAP-2 regulates not only immune systems, but also controls other signal transduction pathways involved in leukemia and breast tumor cells.^{81–85)} Indeed, the constitutive expression of STAP-2 mRNA is observed in macrophages and T-cells, where STAP-2 regulates cellular functions. Furthermore, the induction of STAP-2 mRNA was detected in various types of cells treated with corresponding stimulatory factors; *e.g.*, hepatocytes treated with IL-6,¹³⁾ M1 cells subjected to LIF stimulation,¹³⁾ and Epstein–Barr virus (EBV)-positive human B cells in parallel with latent membrane protein 1 (LMP1) expression.²³⁾

In these stimulatory signaling pathways, STAP-2 plays a role as an important modulator. Importantly, STAP-2 functions are regulated by tyrosine-phosphorylation, proteasome-dependent degradation and caspase-8-dependent cleavage.

Recently, it has been reported that STAP-1 expression is induced in pro-inflammatory activated microglia and mac-

rophages. Additionally, cell migration and M-CSF-induced chemotaxis is inhibited in STAP-1-expressing microglia cells. Furthermore, STAP-1 increases microglial phagocytosis, NO (nitric oxide) secretion and neurotoxicity.⁸⁶⁾ These findings are interesting because STAP-2 regulates M-CSF-induced macrophage migration similar to STAP-1's actions in microglia. In fact, STAP-2 mRNA is expressed in the brain, especially the cerebellum (data not shown). This may point to a role of STAP-2 in the brain. Further experiments are necessary to understand the function of STAP family proteins in the central and peripheral nervous systems.

Accumulating data suggest that a novel adaptor protein, STAP-2, is an important modulator not only for the innate immune system, as well as and the adaptive one, but also for tumorigenic signaling.

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