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Intracellular cytoplasm-specific delivery of SH3 and SH2 domains of SLAP inhibits TcR-mediated signaling

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ABSTRACT

Signaling events triggered by T cell receptor (TcR) stimulation are important targets for the development of common therapeutics for various autoimmune diseases. SLAP is a negative regulator of TcR-mediated signaling cascade via targeting TcR zeta chain for degradation through recruiting the ubiquitin ligase c-Cbl. In this study, we generated a transducible form of SH3 and SH2 domains of SLAP (ctSLAPΔC) which can be specifically targeted to the cytoplasm of a cell. ctSLAPΔC inhibited tyrosine phosphorylation of signaling mediators such as ZAP-70 and LAT involved in T cell activation, and effectively suppressed transcriptional activity of NFAT and NFκB upon TcR stimulation. The transduced ctSLAPΔC in T cells blocked the secretion of T cell-specific cytokines such as IL-2, IFNγ, IL-17A, and IL-4 and induced the expression of CD69 and CD25 on effector T cells without influencing the cell viability. Inhibition of TcR-mediated signaling via SLAP blocked the differentiation of naïve T cells into Th1, Th2 or Treg cells with different sensitivity, suggesting that qualitative and quantitative intensity of TcR-mediated signaling in the context of polarizing cytokines environment may be a critical factor to determine the differentiation fate of naïve T cells. These results suggest that cytoplasm-specific transduction of the SH3 and SH2 domains of SLAP has a therapeutic potential of being an immunosuppressive reagent for the treatment of various autoimmune diseases.

1. Introduction

Stimulation of the T cell antigen receptor (TcR) is a central event in the adaptive immune response. TcR engagement leads to phosphorylation of tyrosine residues in the TcR-associated CD3 complex subunits by the Src family protein tyrosine kinase Lck [1,2]. Phosphorylation of CD3 zeta chains results in recruitment and activation of ZAP-70, and then ZAP-70 phosphorylates multiple adaptor and linker molecules, such as SLP-70 and LAT. LAT plays crucial roles for activating downstream signaling molecules, including the Ras pathway and MAP kinase activation [3,4]. These signaling events eventually lead to IL-2 production, T cell proliferation, and activation.

Src-like adaptor protein (SLAP) is a negative regulator of TcR signaling and targets TcR zeta chain for degradation through recruiting the ubiquitin ligase c-Cbl [5]. In thymocytes, SLAP is expressed at the highest level in the $CD4^{+}CD8^{+}$ double positive T cells, and SLAP-deficient T cells increase the level of TcR on their surface and enhance TcR-mediated signaling [6–8]. Similar to the structure of Lck, SLAP has a unique myristolated N-terminus followed by SH3 and SH2 domains, which are 55% and 50% homologous to the SH3 and SH2 domains of Lck, respectively. Instead of a kinase domain, SLAP contains a unique COOH terminus of 104 residues responsible for TcR zeta chain degradation [9].

Chronic and sustained T cell activation is implicated in the pathogenesis of several autoimmune-associated inflammatory diseases [10,11]. Therefore, modulation of T cell activation by suppressing TcR signaling with natural and functional specificity can be an effective treatment strategy for autoimmune diseases.

Although there are many intracellular signal regulators inside the T cells to inhibit T cell activation, the major limitation of the usage of these proteins directly for therapeutic purpose is the difficulty of protein delivery into the cells across the cellular membrane. Protein transduction domains (PTDs) such as Tat, MTS, pep1, Hph-1 and Antp have been reported to transduce the functional

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biomolecules including proteins, double stranded DNA, antisense oligonucleotides, and liposomes into cells [12–14]. However, the biomolecules delivered into the cells by most PTDs are eventually translocated into the nucleus of the cells within a short period of time so that the delivered signal regulators cannot exert their therapeutic functions effectively. Previous study reported that cytoplasmic transduction peptide (CTP: YGRRARRRRRR) has the capacity to deliver the biomolecules preferentially into the cytoplasm [15].

In this study, we generated the cell-permeable form of SH2 and SH3 domains of SLAP (SLAPΔC) by fusing SLAPΔC with CTP, which can be effectively and specifically delivered into the cytoplasm of the cells. ctSLAPΔC can inhibit TcR-proximal and -distal intracellular signaling events as well as their functional outcomes. Differentiation of naïve T cells into each T cell subset was suppressed by ctSLAPΔC, but with different sensitivity. With these results, our study demonstrates that ctSLAPΔC can be a potential therapeutics for the treatment of autoimmune diseases.

2. Materials and methods

2.1. Generation and purification of CTP fusion protein

The SH3 and SH2 domains of SLAP (1 -193AA) combined with cytoplasmic transduction peptide (CTP) were cloned into pET-28a(+) plasmid and transformed into *Escherichia coli* BL21 Codon plus (DE3) RIPL strain (Invitrogen) for proteins expression. The protein expression was induced for 4 h at 37 °C with 1 mM of isopropyl-β-D-thiogalactopyranoside (IPTG; DuchefaBiochemie), After harvesting, cells were resuspended and sonicated in SP binding buffer (50 mM NaH_2PO_4, 300 mM NaCl, pH 7.0). Soluble fraction of lysates was obtained by centrifugation (13,000 rpm for 10 min at 4 °C), and then mixed with SP sepharose fast flow (GE healthcare) for 1 h at 4 °C. The mixture was loaded to chromatography columns (Bio-rad) and washed enough to remove the endotoxins and LPS with SP wash buffer (50 mM NaH_2PO_4, 200 mM NaCl, pH 7.0), and the target proteins were eluted by SP elution buffer (50 mM NaH_2PO_4, 2 M NaCl, pH 8.0). Subsequently, the eluded proteins were mixed with Ni-NTA Agarose (Qiagen) in Ni-NTA binding buffer (10 mM imidazole, 50 mM NaH_2PO_4, 200 mM NaCl, pH 8.0) for 2 h at 4 °C. The proteins were loaded on HisTrap chromatography columns (Bio-rad), and non-specifically bound proteins were washed out with wash buffer (30 mM imidazole, 50 mM NaH_2PO_4, 200 mM NaCl, pH 8.0), and the recombinant proteins of interest were eluted by elution buffer (500 mM imidazole, 50 mM NaH_2PO_4, 200 mM NaCl, pH 8.0). The eluted proteins were desalted by PD-10 Sephadox G-25 supplemented with 10% glycerol PBS and the aliquots were to be stored at −80 °C.

2.2. In vitro intracellular transduction kinetics of ctSLAPΔC

Jurkat T cells were treated with proteins in concentration- or time-dependent manner, washed with PBS, and lysed with RIPA buffer (Sigma Aldrich). Protein extracts were separated by SDS-PAGE, and then the separated proteins were transferred to poly vinylidinedifloride (PVDF) transfer membrane (Millipore Billerica). The membrane was blocked in 5% Serum Bovine Albumin (BSA; Amresco), and then incubated with anti-FLAG antibody (1: 3000 dilution) and anti-β actin antibody (1: 5000 dilution) in TTBS for 3 h. After probing with anti-mouse IgG or anti-rabbit IgG antibody diluted by 1: 10,000, the target protein in the membrane was detected by WEST-ZOL (iNtRON Biotechnology).

2.3. Immunocytochemistry

HeLa cells seeded on Lab-Tek II chamber slides (Nunc) were treated with 2 μM of ctSLAPΔC for 1 h. The cells were washed twice with PBS, then fixed with 4% formaldehyde, permeabilized with 0.2% Triton X-100 (Sigma Aldrich), and blocked with blocking solution (0.5% BSA in PBS base). The transduced ctSLAPΔC was stained with anti-FLAG M2-FITC conjugated antibody (Sigma Aldrich), and the nucleus was stained with 4′, 6-diamino-2-phenylindole (DAPI) solution. The cells were mildly washed with distilled water and visualized by confocal micro scope (Carl Zeiss).

2.4. Cytotoxicity assay

C57BL/6mouse splenocytes in 96-well plate were incubated with varying concentration (0.01–5 μM) for 16 h. Then,CCK-8 solution was added to each well of plate, and then the samples were incubated for 4 h. The cell viability was determined by measuring the absorbance at 450 nm using a microplate reader (Bio-rad).

2.5. Phosphorylation of intracellular signaling proteins

Jurkat T cells were treated with 2 μM of ctSLAPΔC or 2 μM of SLAPΔC for 1 h followed by addition of anti-CD3 and anti-CD28 for 30 min at 4 °C, and then activated for 10 min at 37 °C. The cells were lysed with cell lysis buffer and the lysates were immuno blotted with anti-Phospho-ZAP70 (Tyr493) mAb (1: 1000; Cell Signaling), anti-Phospho-LAT (Tyr171) mAb (1: 1000; Cell Signaling), anti-Phospho-SAPK/JNK (Thr183/Tyr185) mAb (1: 2000; Cell Signaling), anti-Phospho-p38 MAP kinase (Tyr182) mAb (1:2000; Cell Signaling) and anti-β-actin mAb (1: 3000; Cell Signaling), and the target protein was detected by WEST-ZOL (iNtRON Biotechnology).

2.6. Luciferase assay

Jurkat T cells were transiently transfected with 10 μg of NFAT– or NFκB-luciferase reporter gene by electroporation (270 V/950 μF). After 24 h incubation, cells were harvested and treated with ctSLAPΔC, SLAPΔC or CsA in 12-well plate (5×10^5/well) for one additional hour. Then, the cells were stimulated with plate bound anti-CD3 (0.25 μg/ml) and anti-CD28 (0.5 μg/ml) monoclonal antibody for 24 h. Assays for luciferase activity were performed according to the manufacturer's instructions (Promega).

2.7. ELISA

After C57BL/6mouse splenocytes were stimulated with plate bound anti-CD3 (0.25 μg/ml) and anti-CD28 (0.5 μg/ml) monoclonal antibody for 24 h or 72 h, the cytokine level of IL-2, IFNγ, IL-17A,or IL-4 in the supernatant was measured by ELISA according to the manufacturer's directions (IL-2: R&D), (IFNγ, IL-17A, IL-4: eBioscience).

2.8. Analysis of T cell activation

Naïve $CD4^+$ T cells were purified from C57BL/6mouse spleen by using magnetic-activated cell sorting to >95% purity (MiltenyiBiotec). Naïve $CD4^+$ T cells were incubated with SLAPΔC, tEGFP, CsA or ctSLAPΔC and were activated by plate bound anti-CD3 (0.25 μg/ml) and anti-CD28 (0.5 μg/ml) monoclonal antibody for 12 h or 48 h. The cells were stained with FITC-conjugated anti-mouse CD69 (BD pharmingen) or PE-conjugated anti-mouse CD25 antibody (BD pharmingen), and then were analyzed by FACSCalibur (BD Biosciences).

2.9. T helper subtype cells differentiation

For T cell subset differentiation, purified $CD4^+$ T cells were activated with plate bound anti-CD3 (0.25 μg/ml) and anti-CD28 (0.5 μg/ml) monoclonal antibody in 96 flat-bottom plate for 5 days with various combination of antibodies and cytokines: for Th1 differentiation, 25 ng/ml of IL-12 (Pepro Tech) and 2 μg/ml of anti-IL-4mAb (11B11, eBioscience); for Th2 differentiation, 200 ng/ml of IL-4 (Pepro Tech) and 2 μg/ml of anti-IFNγmAb (XMG1.2, eBioscience); for inducible Treg cells generation, 5 ng/ml of TGFβ (R&D) and 100 U/ml IL-2.

2.10. Intracellular staining and flow cytometry analysis

For intracellular cytokine staining, differentiated T cells (Th1, Th2) were stimulated with cell stimulation cocktail (plus protein transporter inhibitor; eBioscience) for 4 h. At the end of the stimulation, the cells were harvested and washed twice in PBS, stained with APC-conjugated anti mouse CD4 (eBioscience), and subsequently fixed and permeabilized using Fixation/permeabilization buffer (eBioscience). For intracellular cytokines, the cells were stained with fluorescence-conjugated antibodies for IFNγ or IL-4 (eBioscience) diluted in permeabilization buffer (eBioscience) for 30 min. The stained cells were examined by FACSCalibur (BD Bioscience), and the results were analyzed using FlowJo software (Tree Star). Staining of the transcription factor was carried out with Foxp3 staining buffer set (eBioscience) according to the manufacturer's instructions. Anti-Foxp3 (FJK-16s) were purchased from eBioscience.

2.11. Statistical analysis

Data from at least five sets of samples were used for statistical analysis. Statistical significance was calculated by Student's t test. A p value less than 0.05 was considered significant.

3. Results

3.1. Generation of cell-permeable form of SH3-SH2 domain of SLAP specifically targeting the intracellular cytoplasm

The SH3 and SH2 domains and unique COOH terminus of 104 residues of SLAP have been known to play an important role for the inhibition of downstream signaling pathway of the TcR complex and TcR degradation, respectively. To modulate TcR-mediated signaling events without TcR down-regulation, we took the novel strategy to deliver SLAPΔC protein directly to the cytoplasm using CTP. The cell-permeable form of SLAPΔC specifically targeting the intracellular cytoplasm was generated in which SLAPΔC was fused with CTP, facilitating the efficient cytoplasmic delivery of CTP-fused protein [15]. We also generated the SLAPΔC protein without CTP as a control (Fig. 1A). The fusion proteins were made in the *E. coli* expression system, purified under native condition, and its biochemical identity was verified by SDS-PAGE (Fig. 1B).

Next, the transduction kinetics of ctSLAPΔC was examined in Jurkat T cells or HeLa cells. ctSLAPΔC was effectively delivered into the cytoplasm of the cells in a concentration-dependent manner while SLAP subdomain without CTP (SLAPΔC) was not delivered (Fig. 1C). Time course analysis of ctSLAPΔC delivery showed that the

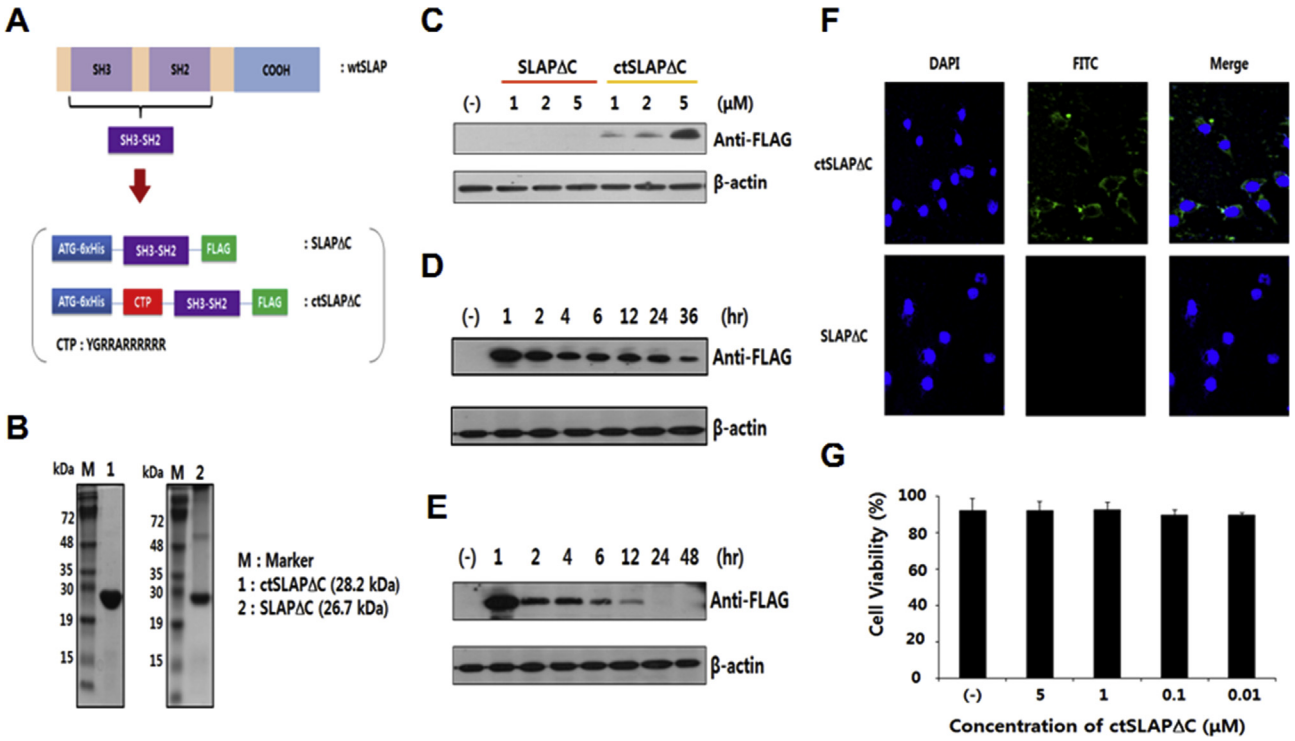

Fig. 1. Generation of cytoplasm-transducible protein of SLAPΔC (ctSLAPΔC). (A) Construct of SH3 and SH2 domains of SLAP with or without CTP. (B) The recombinant proteins were purified under native condition, and its identity was confirmed by SDS-PAGE. (C, D) Concentration- and time-dependent delivery of ctSLAPΔC into the cells (E) Intracellular stability of transduced ctSLAPΔC (2 μM) was examined by western blot with anti-FLAG mAb. (F) Cytoplasm-specific localization of ctSLAPΔC was analyzed by intracellular staining with FITC conjugated-FLAG mAb at 1 h of post-transduction followed by analysis using confocal microscopy. (G) Mouse splenocytes were treated with various concentrations (0.01–5 μM) of ctSLAPΔC, and cell viability was measured by CCK-8 assay. Error bars represent S.D..

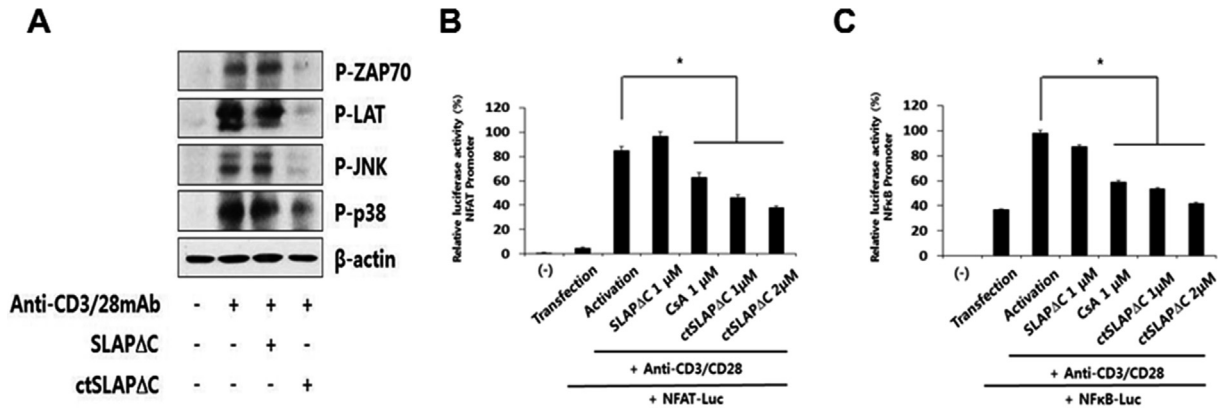

Fig. 2. ctSLAPΔC inhibited TcR-mediated activation signaling events. (A) After ctSLAPΔC (2 μM) or SLAPΔC (2 μM) was incubated with Jurkat T cells for 1 h, cells were activated with anti-CD3 and anti-CD28 Abs for 10 min at 37 °C, and then cell lysates were immuno blotted with anti-Phospho-ZAP70, -LAT, -JNK or -p38 Abs, and anti-β actin Ab was used as a lysate control. (B, C) Jurkat T cells transfected with NFAT- or NFkB- luciferase construct were incubated with ctSLAPΔC (1, 2 μM), SLAPΔC (1 μM) or CsA (1 μM) for 1 h, and then stimulated with plate bound anti-CD3/CD28 mAbs for 24 h. Luciferase activity was measured by luminometer. Error bars represent S.D. *P < 0.05 was considered to be significant.

transduced ctSLAPΔC reached its maximal concentration in the cytoplasmat 1 h after transduction, and then started to decrease as time passed (Fig. 1D). Once ctSLAPΔC was transduced into the cells, it was detectable in the cells until 12 h after transduction at which point it slowly degraded (Fig 1E).

The transduced ctSLAPΔC was specifically present in the cytoplasm when intracellular localization of ctSLAPΔC in HeLa cells was visualized by confocal microscopy (Fig 1F). Importantly, ctSLAPΔC treatment did not induce any cellular cytotoxicity (Fig. 1G). These results suggest that ctSLAPΔC is efficiently and specifically delivered into the cytoplasm of the cells without any adverse effects.

3.2. ctSLAPΔC inhibits the T cell activation at the level of TCR proximal signaling events

To prove whether the transduced ctSLAPΔC can inhibit tyrosine phosphorylation of various intracellular signaling proteins proximal to TcR complex, Jurkat T cells were treated with ctSLAPΔC or SLAPΔC, activated by anti-CD3 and anti-CD28 antibody for 10 min. Then the tyrosine phosphorylation of ZAP-70, LAT, JNK and p38 were analyzed by phospho tyrosine immunoblot (Fig. 2A). Tyrosine phosphorylation induction of these signal mediators was

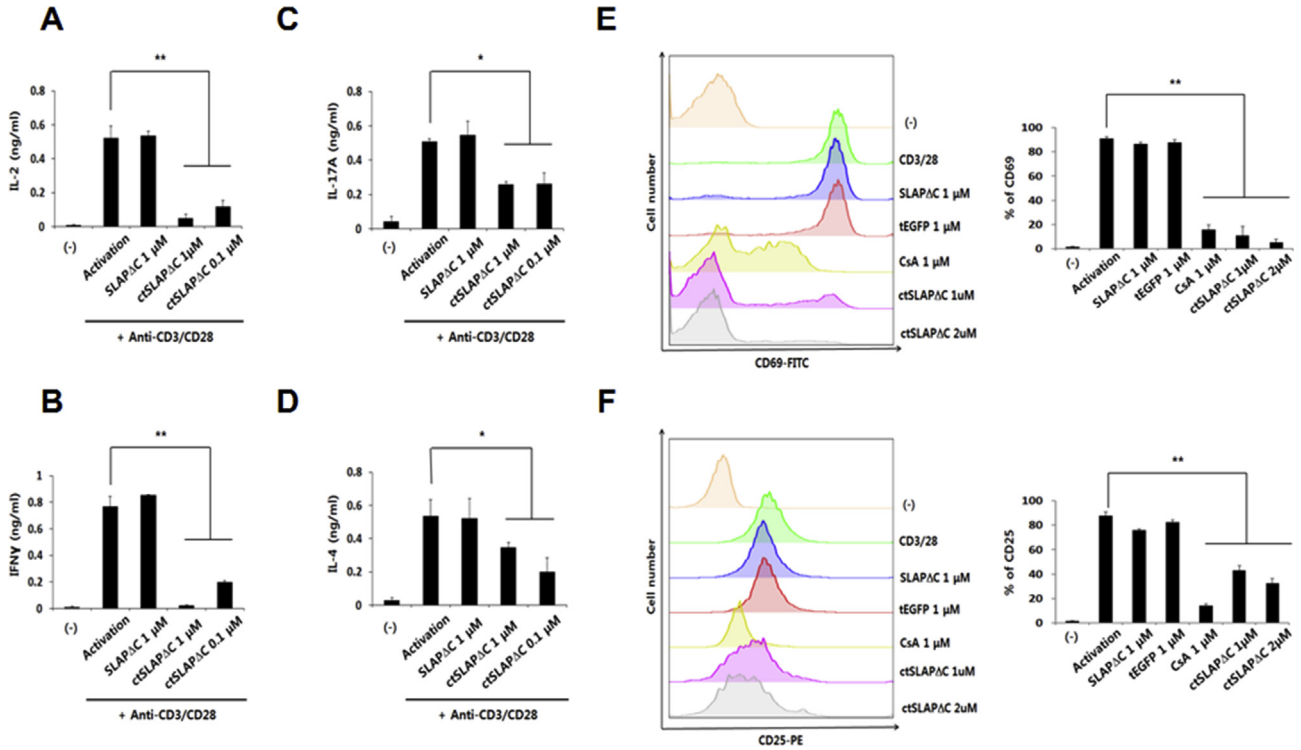

Fig. 3. Inhibition of TcR-induced activation markers by ctSLAPΔC. Mouse splenocytes were incubated with SLAPΔC (1 μM) or ctSLAPΔC (1 and 0.1 μM) for 1 h followed by TcR stimulation with plate bound anti-CD3/28 mAbs for 24 h (for IL-2) or 72 h (for IFN, IL-17A or IL-4). The level of IL-2 (A), IFNγ (B), IL-17A (C) or IL-4 (D) in the culture media was measured by ELISA. (E,F) Mouse naïve CD4$^+$ T cells (CD4$^+$CD25$^-$CD62L^{high}) were purified by MACS, and incubated with SLAPΔC (1 μM),tEGFP(1 μM), CsA(1 μM) or ctSLAPΔC (1 and 2 μM) for 1 h. Then, the cells were activated with plate bound anti-CD3/28 mAbs for 12 h (for CD69), or for 48 h (for CD25), and stained with FITC-conjugated anti-mouse CD69 Ab (E) or PE-conjugated anti-mouse CD25 Ab (F).Error bars represent S.D. *P < 0.05, **P < 0.01 was considered to be significant.

substantially inhibited by 2 μM of ctSLAPΔC while SLAPΔC without CTP did not affect their tyrosine phosphorylation.

Next, the influence of ctSLAPΔC on the transcriptional activities of Nuclear Factor Activated T-cells (NFAT) and Nuclear Factor kappa-light-chain-enhancer of activated B cells (NFκB), both of which play a key role in the expression of activation-induced cytokines in T cells, were investigated. We transiently transfected Jurkat T cells with NFAT- or NFκB- promoter luciferase reporter gene in which the expression of luciferase gene is induced by T cell stimulation. ctSLAPΔC or SLAPΔC was transduced into the transfected Jurkat T cells, followed by the activation with anti-CD3 and anti-CD28 antibodies, and then the luciferase activity was measured. Treatment of ctSLAPΔC significantly reduced the transcriptional activity of NFAT or NFκB in a concentration-dependent manner, and the level of inhibition of the transcriptional activity by 1 μM of ctSLAPΔC was equivalent to that by 1 μM of Cyclosporin A (CsA). However, the luciferase activity was not affected by SLAPΔC (Fig. 2B and C). With these results, we suggest that ctSLAPΔC strongly inhibits the signaling events for T cell activation at TcR proximal and distal levels.

3.3. Inhibition of TcR-induced activation markers and T cell subset differentiation by ctSLAPΔC

To investigate whether ctSLAPΔC suppresses secretion of T cell activation-associated cytokines, splenocytes were isolated from C57BL/6 mice, incubated with ctSLAPΔC, and activated with plate bound anti-CD3 and anti-CD28 antibodies for 24 h. Then the level of T cell proliferation cytokine (IL-2)or inflammatory cytokines such as IFNγ, IL-17A and IL-4 in culture media was analyzed. As shown in Fig. 3A–D, ctSLAPΔC significantly reduced production of these cytokines from the splenocytes in concentration dependent manner, while SLAPΔC lacking CTP did not show any inhibitory effects. Additionally, we examined whether ctSLAPΔC can block the induced expression of activation markers on a T cell surface. Naïve $CD4^{+}CD25^{-}CD62L^{high}$ T cells were purified from mouse spleens using a magnetic-activated cell sorting (MACS), then incubated with ctSLAPΔC, SLAPΔC, CsA, or tEGFP (transducible EGFP), followed by TcR stimulation with anti-CD3 and anti-CD28 antibodies. The induced expression of CD69 and CD25 on the surface of activated effector T cell was analyzed by flow cytometry. Induction of CD69 and CD25 expression was markedly inhibited by ctSLAPΔC in dose-dependent manner, but non-transducible SLAPΔC or tEGFP did not affect its induction (Fig. 3E and F). Inhibitory potential of ctSLAPΔC was almost equivalent to that of CsA.

Stimulation through TcR complex in the proper cytokine milieu is important for T cell subset differentiation. To test whether the complete or partial blockage of TcR signaling via SLAP can distinctively influence the differentiation of naïve T cells into each T cell, differentiation of naïve $CD4^{+}CD25^{-}CD62L^{high}$ T cells into Th1, Th2 or Treg cells was induced under the specific polarizing

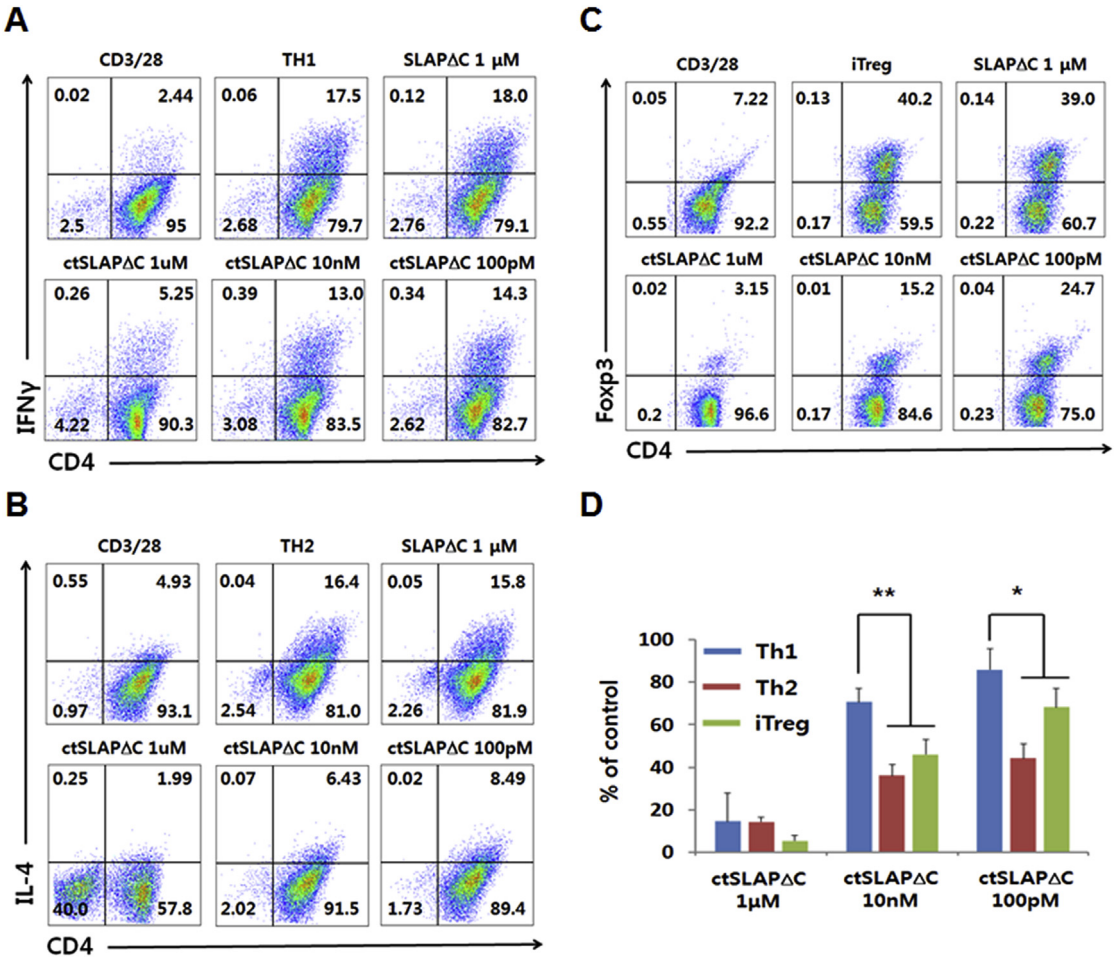

Fig. 4. Differentiation capacity of naïve T cells into each T cell subset is differentially sensitive to inhibition of TcR stimulation. The mouse naïve $CD4^{+}$ T cells ($CD4^{+}CD25^{-}CD62L^{high}$) were transduced with SLAPΔC (1 μM),tEGFP(1 μM), CsA(1 μM) or ctSLAPΔC (1 μM, 10 nM and 100 pM) for 1 h, and activated with plate bound anti-CD3/28 mAbs for 5 days, (A)in the presence of recombinant IL-12 and anti-IL-4 antibody, (B)in the presence of recombinant IL-4 and anti-IFNγ antibody, (C) in the presence of recombinant IL-2 and TGFβ. Data are representative of five independent experiments with similar results. (D) Quantification of data in A–C, presents the percentage of suppression of T cell subset differentiation. Error bars represent S.D. $^{*}P < 0.05$, $^{**}P < 0.01$ were considered to be significant.

condition for each T cell subset in the presence of ctSLAPΔC. As shown in Fig. 4A–D, iTreg and Th2 differentiation was more sensitive to inhibition of TcR signaling via SLAP than Th1 cell differentiation. Therefore, in addition to different context of cytokine signaling, qualitative and quantitative intensity of TcR-mediated signaling determines the differentiation fate of naïve T cells.

4. Discussion

The autoimmune diseases were associated with excessive T cell activation and pathogenic T cell response. T cell activation is initiated by the interaction of the TcR with self or foreign antigen loaded on MHC-II molecules. Therefore, the high level of specific inhibition of TcR signaling without using virus-mediated delivery is important to treat various autoimmune diseases.

Several molecular and cellular methods have been used to inhibit TcR-mediated signaling, such as using small molecules to inhibit the functions of signal mediators, utilizing antagonistic mAb specific to surface molecules, or delivering the genes encoding inhibitory protein. However, lack of specificity associated with small molecules, complete turn-off of all signaling pathways downstream of the surface molecule by mAb, or unwanted immune and genetic outcome by viral vector may cause significant side effects or cellular toxicity.

In this study, to modulate TcR-mediated intracellular activation events while avoiding the problems listed above, we used the novel strategies: (1) cytoplasm-specific intracellular delivery of negative regulator protein (SLAP) involved in TcR signaling using CTP, (2) usage of only SH3 and SH2 domains of SLAP to inhibit T cell activation without down-regulation of TcR level. Cytoplasmic transducible SLAPΔC (ctSLAPΔC) effectively suppressed the signaling events proximal to TcR complex, resulting in inhibition of transcriptional activity of NFkB and NFAT, secretion of cytokines, and surface expression of markers induced by TcR stimulation without any cellular cytotoxicity. Interestingly, the partial inhibition of TcR-signaling via SLAP in naïve T cells using ctSLAPΔC enabled us to find that differentiation potential of naïve T cells into Th2 or iTreg cells is more sensitive to inhibition of T cell activation than that to Th1 cells. Therefore, qualitative and quantitative intensity of TcR-mediated signaling in the context of polarizing cytokines environment may be a critical factor to determine the differentiation fate of naïve T cells.

From these results, we conclude that cytoplasm-specific delivery of SH3 and SH2 domains of SLAP protein using CTP may be a novel therapeutic strategy for the treatment of various autoimmune diseases.

Conflict of interest

None.

Acknowledgments

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References

[1] J.P. Deans, S.B. Kanner, R.M. Torres, J.A. Ledbetter, Interaction of CD4-LCK with the T-cell receptor CD3 complex induces early signaling events in the absence of CD45 tyrosine phosphatase, Eur. J. Immunol. 22 (1992) 661–668.
[2] K. Sugie, M.S. Jeon, H.M. Grey, Activation of naive CD4 T cells by anti-CD3 reveals an important role for fyn in lck-mediated signaling, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 14859–14864.
[3] W.E. Paul, R.A. Seder, Lymphocyte-responses and cytokines, Cell 76 (1994) 241–251.
[4] M. Raab, H. Kang, A. da Silva, X.C. Zhu, C.E. Rudd, FYN-T-FYB-SLP-76 interactions define a T-cell receptor zeta/CD3-mediated tyrosine phosphorylation pathway that up-regulates interleukin 2 transcription in T-cells, J. Biol. Chem. 274 (1999) 21170–21179.
[5] M.D. Myers, T. Sosinowski, L.L. Dragone, C. White, H. Band, H. Gu, A. Weiss, Src-like adaptor protein regulates TCR expression on thymocytes by linking the ubiquitin ligase c-Cbl to the TCR complex, Nat. Immunol. 7 (2006) 57–66.
[6] M.D. Myers, L.L. Dragone, A. Weiss, Src-like adaptor protein down-regulates T cell receptor (TCR)-CD3 expression by targeting TCR zeta for degradation, J. Cell Biol. 170 (2005) 285–294.
[7] T. Sosinowski, A. Pandey, V.M. Dixit, A. Weiss, Src-like adaptor protein (SLAP) is a negative regulator of T cell receptor signaling, J. Exp. Med. 191 (2000) 463–473.
[8] T. Sosinowski, N. Killeen, A. Weiss, The Src-like adaptor protein downregulates the T cell receptor on CD4(+)CD8(+) thymocytes and regulates positive selection, Immunity 15 (2001) 457–466.
[9] A. Pandey, H.J. Duan, V.M. Dixit, Characterization of a novel SRC-like adapter protein that associates with the ECK receptor tyrosine kinase, J. Biol. Chem. 270 (1995) 19201–19204.
[10] R.E. Voll, E. Jimi, R.J. Phillips, D.F. Barber, M. Rincon, A.C. Hayday, R.A. Flavell, S. Ghosh, NF-kappa B activation by the pre-T cell receptor serves as a selective survival signal in T lymphocyte development, Immunity 13 (2000) 677–689.
[11] A. Lanzavecchia, G. Iezzi, A. Viola, From TCR engagement to T cell activation: a kinetic view of T cell behavior, Cell 96 (1999) 1–4.
[12] M.C. Morris, J. Depollier, J. Mery, F. Heitz, G. Divita, A peptide carrier for the delivery of biologically active proteins into mammalian cells, Nat. Biotechnol. 19 (2001) 1173–1176.
[13] H. Nagahara, A.M. Vocero-Akbani, E.L. Snyder, A. Ho, D.G. Latham, N.A. Lissy, M. Becker-Hapak, S.A. Ezhevsky, S.F. Dowdy, Transduction of full-length TAT fusion proteins into mammalian cells: TAT-p27(Kip1) induces cell migration, Nat. Med. 4 (1998) 1449–1452.
[14] H. Noguchi, M. Matsushita, T. Okitsu, A. Moriwaki, K. Tomizawa, S.Y. Kang, S.T. Li, N. Kobayashi, S. Matsumoto, K. Tanaka, N. Tanaka, H. Matsui, A new cell-permeable peptide allows successful allogeneic islet transplantation in mice, Nat. Med. 10 (2004) 305–309.
[15] D. Kim, C. Jeon, J.H. Kim, M.S. Kim, C.H. Yoon, I.S. Choi, S.H. Kim, Y.S. Bae, Cytoplasmic transduction peptide (CTP): new approach for the delivery of biomolecules into cytoplasm in vitro and in vivo, Exp. Cell Res. 312 (2006) 1277–1288.