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Molecules and Cells

The Effector Functions of Mature T Lymphocytes Are Impaired in Transgenic Mice Expressing the SH2 Domain of TSAd/Lad

Youngbong Choi[1], Eunkyung Park[1], Eunseon Ahn, Inyoung Park, and Yungdae Yun*

TSAd/Lad is a T cell adaptor molecule involved in p56^{lck}-mediated T cell activation. To investigate the functions of TSAd in T cells, we generated transgenic (TG) mice expressing the SH2 domain of TSAd (TSAd-SH2) under the control of the p56^{lck} proximal promoter. In T cells from TSAd-SH2 TG mice, T cell receptor (TCR)-mediated early signaling events, such as Ca^{2+} flux and ERK activation, were normal; however, late activation events, such as IL-2 production and proliferation, were significantly reduced. Moreover, TCR-induced cell adhesion to extracellular matrix (ECM) proteins and migration through ECM proteins were defective in T cells from TSAd-SH2 TG mice. Furthermore, the contact hypersensitivity (CHS) reaction, an inflammatory response mainly mediated by T helper 1 (Th1) cells, was inhibited in TSAd-SH2 TG mice. Taken together, these results show that TSAd, particularly the SH2 domain of TSAd, is essential for the effector functions of T cells.

INTRODUCTION

The TCR engagement initiates multiple intracellular signaling pathways that lead to the alteration of gene expression required for cell proliferation and effecter functions (Lanzavecchia and Sallusto, 2001). The biochemical mechanisms that couple TCR stimulation with the intracellular signaling events have been investigated intensively. The signaling process is initiated by the activation of cytosolic protein tyrosine kinases (PTKs), such as p56^{lck} and ZAP-70 (Mustelin and Tasken, 2003). Subsequently, the activated PTKs phosphorylate transmembrane adaptor proteins, such as LAT, which, once phosphorylated, recruit other adaptor proteins, including Grb2, GADS, and SLP-76, which in turn amplify the TCR-mediated signaling pathways (Leo and Schraven, 2001).

We previously identified a murine adaptor protein named Lad (p56^{lck}-associated adaptor protein) as a binding partner of p56^{lck} (Choi et al., 1999). The human orthologue of TSAd was cloned, and named as a T cell-specific adaptor, TSAd (Spurkland et al., 1998). Murine TSAd/Lad was also found to be a Rlk/Txk- and Itk-binding adaptor protein (RIBP), based on its ability to interact with Tec family kinases (Rajagopal et al., 1999). Here, to avoid confusion, we will refer to this adaptor protein as TSAd. TSAd expression is restricted to T cells and is upregulated substantially after T cell activation (Choi et al., 1999). TSAd contains a SH2 domain, a proline-rich SH3-binding motif, and several phosphotyrosine sites. Upon T cell activation, TSAd is tyrosine phosphorylated, associates with p56^{lck}, and mediates signaling that leads to the enhancement of IL-2 promoter activity (Choi et al., 1999). Consistently, TSAd-deficient T cells are defective in TCR stimulation-dependent proliferation, and the production of interleukin (IL)-2 and interferon-γ (Marti et al., 2006; Rajagopal et al., 1999). These characteristics of TSAd suggest that TSAd plays an important role in TCR-dependent T cell activation. In addition to a role in T cell activation, we have recently shown that TASd mediates chemokine-dependent T cell migration by associating with p56^{lck} and ZAP-70 (Park et al., 2007).

In the present study, we generated transgenic mice expressing the TSAd SH2 domain under control of the mouse p56^{lck} proximal promoter to investigate the impact of TSAd-mediated signals on T cell functions. We found that TSAd was required for the effector functions of T cells, especially for the TCR stimulation-dependent adhesion and migration of T cells on ECM proteins, and inflammatory response.

MATERIALS AND METHODS

Generation of transgenic mice and analysis of transgene expression

For transgene construction, the fragment corresponding to the SH2 domain (aa 116-211) of murine TSAd was generated by PCR amplification. Subsequently, the PCR product was ligated into the *Bam*HI site of the p1017 expression vector carrying the proximal murine p56^{lck} promoter and the transcription termination sequence of the human growth hormone gene sequence (Chaffin et al., 1990). From the resulting p1070-TSAd-SH2-HA plasmid, a 5.6 kb *Not*I fragment was isolated and microinjected into FVB embryos for the generation of transgenic mice (Macrogen Co.). Three different founder mice were generated and maintained in FVB background for the experiments.

For the analysis of transgene expression by RT-PCR analy-

Department of Life Science, Ewha Womans University, Seoul 120-750, Korea, [1]These authors contributed equally to this work.
*Correspondence: YUNYUNG@ewha.ac.kr

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sis, mRNA was extracted from splenic T cells using the μMACS mRNA isolation method (MACS, Miltenyl Biotec), and was reverse transcribed using reverse transcriptases (Qiagen), according to the manufacturer's instructions. Twenty nanograms of mRNA and 0.6 μM primer were used in a 50 μl reaction for 45 cycles. The PCR primers (sense, 5′-CAG TCA GGA GCT TGA ATC CCA CGA TTG-3′; and antisense, 5′-GG AGT GGT TCG GGG AGT TGG GCC TTG-3′) amplified a 350 bp fragment.

For Western blot analysis of transgene expression, thymocytes, lymph nodes (LN), and splenocytes were isolated from wild type and TSAd-SH2 transgenic mice. Following harvest, 2×10^7 lymphocytes from the indicated organs were lysed in 1% Nonidet P-40 lysis buffer [50 mM Tris-HCl (pH 7.6), 120 mM NaCl, 1 μg/ml leupeptin, 1 μg/ml aprotinin, and 1 μg/ml pepstatin] followed by high speed centrifugation at 4°C for 30 min to remove debris. Subsequent Western blotting was performed using the enhanced chemiluminescence protocol (Amersham).

Flow cytometry

Flow cytometry was performed using a Becton Dickinson FACScalibur. Briefly, cells from the lymphoid organs of four-week-old mice were incubated with conjugated monoclonal antibodies in PBS for 15 min at room temperature, washed three times in PBS, and then fixed in 1% neutralized formaldehyde. The monoclonal antibodies used were fluorescein isothiocyanate (FITC)-conjugated anti-CD8 and phycoerythrin (PE)-conjugated anti-CD4 (BD Pharmingen).

Cell stimulation

To analyze the proximal TCR signaling events, LN T cells were washed three times with RPMI 1640 and then incubated on ice for 30 min with 500 μl of RPMI 1640 containing 10 μg/ml of anti-CD3ε antibody (145-2C11, BD Pharmingen). After being washed with RPMI 1640, the cells were stimulated by incubation with 10 μg/ml anti-mouse IgG for 10 min at 37°C, and were subsequently lysed in 1% Nonidet P-40 lysis buffer. For the analysis of late parameters of T cell activation, purified T cells were stimulated by culture in 96-well flat-bottomed microtiter plates (2×10^6 cells/ml) for 24 h in the presence of either 2 μg/ml soluble anti-CD3ε antibody plus 2 μg/ml soluble anti-CD28 antibody (37.51, BD Pharmingen) or 100 ng/ml PMA plus 200 ng/ml ionomycin.

Calcium measurement

Lymph node T cells were loaded with 5 μM acetoxymethyl ester of fura-2 (fura-2/AM, Molecular Probes) and 0.02% Pluronic F-127 in HBSS buffer (137 mM NaCl, 1 mM $NaHCO_3$, 0.34 mM Na_2HPO_4, 0.34 mM KCl, 5.36 mM KH_2PO_4, 5 mM HEPES, adjusted to pH 7.2) at 37°C for 30 min. Loaded cells were incubated with 5 μg/ml of hamster anti-CD3ε antibody (145-2C11, BD Pharmingen) on ice for 30 min. After being washed, cells were stimulated with 30 μg/ml of anti-hamster IgG (G94-56, BD Pharmingen). The cytosolic free calcium concentration was monitored using an RF-5301PC spectrofluorometer.

IL-2 measurement

The amount of IL-2 from culture supernatants was measured by enzyme-linked immunosorbant assay (ELISA). Nunc-Immuno plates (Nalgen Nunc) were coated with anti-IL-2 antibody overnight at 4°C, washed in PBS, and blocked in 10% FBS/PBS for 1 h at room temperature. Supernatants diluted in 10% FBS/PBS were incubated on the coated plates overnight at 4°C. On the next day, plates were washed and incubated with anti-IL-2 detecting antibody and streptavidin-horseradish peroxidase (BD Pharmingen). Plates were developed using TMB One-Step Substrate (Dako). All antibodies and IL-2 standards were purchased from BD Pharmingen.

Proliferation assay

The 2×10^5 cells in 200 μl of HEPES-buffered RPMI 1640 media were added to each well of flat-bottomed 96-well plates. Cells were activated by incubation with 2 μg/ml anti-CD3 plus anti-CD28 Ab, or 50 ng/ml PMA plus 100 ng/ml ionomycin. After 48 h, cells were pulsed for 8 h with 1 μCi/well of [*methyl*-^{3}H] thymidine (25 Ci/mmol; Amersham). Subsequently, the cells were harvested on a filter and analyzed by scintillation counting.

Adhesion assay

Purified LN T cells were stimulated by culture in 96-well plates (2×10^6 cells/ml) for 4 h in the presence or absence of 2 μg/ml each of soluble anti-CD3ε antibody and anti-CD28 antibody. Subsequently, 4×10^5 stimulated T cells in 2.5% BSA/PBS were transferred onto 96-well plates coated with 10 μg/ml of purified BSA (Amresco), fibronectin, or laminin (Sigma), and incubated for 1 h at 37°C. After washing with 2.5% BSA in PBS, non-adherent cells were removed by centrifugation at 48 × *g* for 5 min. After three washes with PBS, the attached cells were fixed, and then stained for 30 min in 40 μl of a solution containing 1% formaldehyde, 0.5% crystal violet, and 20% methanol. The relative number of cells in each well was evaluated by measuring the absorbance at 595 nm in a microplate reader (Bio-Rad) after solubilization in 40 μl of 2% Triton X-100. All determinations were carried out in triplicate, and the data represent the means ± SD for a minimum of three independent experiments.

Migration assay

Cell migration assays were performed using 6.5-mm-diameter Transwell™ inserts with polycarbonate filters (3 μm pore size) (Costar). The filters were coated overnight with anti-CD3 Ab. After being washed, the filters were coated with either 5 μg/ml of fibronectin or 5 μg/ml of laminin for 2 h at room temperature. Then, 5×10^5 cells in 100 μl of RPMI 1,640 containing 0.6% BSA (BSA medium) were added to the upper chamber of the Transwell™, inserted into wells filled with 600 μl of the same medium. After incubation at 37°C for the appropriate duration of time, the filters were removed and the number of migrated cells was counted in four separate fields of each well. The experiment was performed three times in triplicate.

Contact hypersensitivity (CHS) response

CHS reactions were induced with 2,4-dinitrofluorobenzene (DNFB) (Sigma). The TSAd-SH2 TG or wild type mice were sensitized by the application of 20 μl of 0.5% DNFB in acetone/olive oil (4:1) to shaved abdominal skin, and were resensitized on day 2. On day 6, mice were challenged with an application of 10 μl of 0.2% DNFB in acetone/olive oil (4:1) on the dorsal surface of the left ear. The right ear, which was used as the control, received only acetone/olive (4:1) oil. Ear thickness was measured using an engineer's micrometer (Mistutoyo) 24 h after the treatment. To analyze proliferation of antigen-specific T cells, LN T cells were harvested on day 7 after DNFB sensitization of mice, and 1×10^5 cells were stimulated in triplicate by culture for 48 h in the presence or absence of 50 μg/ml 2,4-dinitrobenezenesulfonic acid (DNBS), the soluble form of DNFB, or 10 μg/ml concanavalin A (ConA), and then pulsed for 18 h with 0.5 μCi of [*methyl*-^{3}H] thymidine, as described above.

RESULTS

Generation of transgenic mice expressing the SH2 domain of TSAd

The SH2 domain was previously established as being a dominant negative form of TSAd by the finding that overexpression of the SH2 domain blocked TCR-mediated IL-2 gene expression (Choi et al., 1999). In addition, the TSAd mutant in the phosphotyrosine binding pocket of the TSAd SH2 domain lacks the ability to enhance IL-2 gene expression (Marti et al., 2001). Together, these findings indicate that the SH2 domain is essential for the function of TSAd.

To examine the role of the TSAd SH2 domain, and, at the same time, to identify the functions of TSAd in T cells, we generated transgenic mice (TG) expressing the TSAd SH2 domain under the control of the lck proximal promoter, which restricts the expression of the transgene to T lineage cells (Fig. 1A) (Chaffin et al., 1990; Shimizu et al., 2001). Founder TGs were screened by PCR and Southern analysis and TG lines were maintained by inbreeding. The expression of the transgene was confirmed by RT-PCR (Fig. 1B) and Western blotting (Fig. 1C). To analyze T cell development, thymocytes and splenocytes were isolated from wild type or TSAd-SH2 TG mice, and stained with FITC-conjugated anti-CD8 and PE-conjugated anti-CD4 Ab. The percentages of $CD4^-CD8^-$, $CD4^+CD8^+$, $CD4^+CD8^-$, and $CD4^-CD8^+$ T cell populations of the TSAd-SH2 TG mice were approximately equal to those of the wild type (Fig. 1D). These results show that the TSAd SH2 domain is not involved in T cell development, and are consistent with the previously reported phenotypes of TSAd knockout mice (Rajagopal et al., 1999).

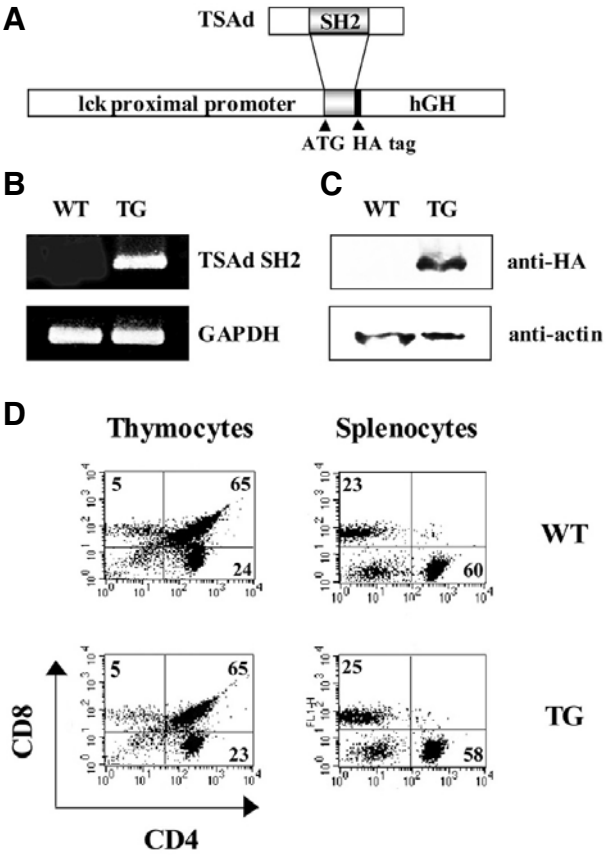

Fig. 1. Generation of transgenic mice expressing the SH2 domain of TSAd. (A) Schematic representation of the TSAd-SH2 transgene. The TSAd-SH2 domain was inserted between the proximal murine lck promoter and the transcription termination sequence of the human growth hormone (hGH) gene in the p1070 expression vector. HA, hemagglutinin tag; ATG, start codon. (B) RT-PCR analysis of transgene expression in splenocytes. Amplification of the housekeeping gene for GAPDH indicates equal mRNA recovery and RT-PCR efficiency. (C) Immunoblot analysis of transgene expression. Lysates were prepared from splenocytes of wild type and TSAd-SH2 TG mice, and subjected to immunoblotting with the anti-HA antibody. (D) Flow cytometric analysis of thymocytes and peripheral T cell populations in TSAd-SH2 TG mice. Thymocyte and splenocytes were isolated from six-week-old mice, and stained with FITC-conjugated anti-CD8 mAb and PE-conjugated anti-CD4 mAb.

Analysis of TCR-dependent activation of peripheral T cells

We next analyzed the early signaling events occurring immediately after TCR stimulation. Lymph node T cells of wild type mice and transgenic mice expressing TSAd-SH2 were stimulated with anti-CD3ε and analyzed for TCR-mediated calcium flux and the activity of Erk1/Erk2. Both calcium influx and ERK phosphorylation were only marginally affected by the transgene expression (Figs. 2A and 2B), showing that the TSAd SH2 domain may not interfere with early TCR signaling events.

Next, we analyzed late activation events, occurring within two to three days of TCR stimulation. Lymph node T cells from the TSAd-SH2 TG and wild type mice were stimulated with anti-CD3 plus anti-CD28 Ab or PMA plus ionomycin for 48 h, and the amount of IL-2 secreted in the culture media was measured. As shown in Fig. 2C, CD3 plus CD28 crosslinking-dependent IL-2 production by T cells from TSAd-SH2 TG mice was significantly decreased compared to that of wild type mice, while PMA plus ionomycin-mediated IL-2 production was not affected by expression of the transgene. In addition, CD3 + CD28 crosslinking-dependent proliferation of LN T cells from TSAd-SH2 TG mice was also significantly reduced compared to that of wild type mice (Fig. 2D). These data show that TSAd, particularly the SH2 domain of TSAd, is important in late activation events, such as IL-2 production and proliferation.

TCR stimulation-dependent adhesion to ECM is defective in T cells from TSAd-SH2 TG mice

Upon TCR stimulation, T cells can adhere to the extracellular matrix (ECM), and the adhesion is mediated mainly by the integrin αβ heterodimers (Burbach et al., 2007). Therefore, we tested whether TSAd could regulate T cell adhesion to fibronectin (FN) or laminin (LM), which bind to α4β 1 (VLA4) or α6β 1 (VLA6) integrins, respectively. As shown in Fig. 3, T cells from the TSAd-SH2 TG mice showed reduced adhesion to FN (Fig. 3A) or LM (Fig. 3B) in response to CD3-stimulation compared to those from wild type mice. In contrast, when treated with PMA, the extent of adhesion was found to be comparable in T cells from TSAd-SH2 TG mice and in those from wild type mice. These results show that the defect in TCR-induced adhesion in T cells of the TSAd-SH2 TG mice is due to a blockade in inside-out signaling between the TCR and integrin, and is not due to an intrinsic defect of the integrin. Taken together, these data indicate that TSAd is required in TCR-induced adhesion to ECM, and that the SH2 domain is essential in this process.

TCR stimulation-dependent migration through ECM is defective in T cells from TSAd-SH2 TG mice

T cell trafficking is a critical element of cell-mediated immune responses (Pribila and Shimizu, 2003). The engagement of TCR enhances the binding of integrins to their ligands and

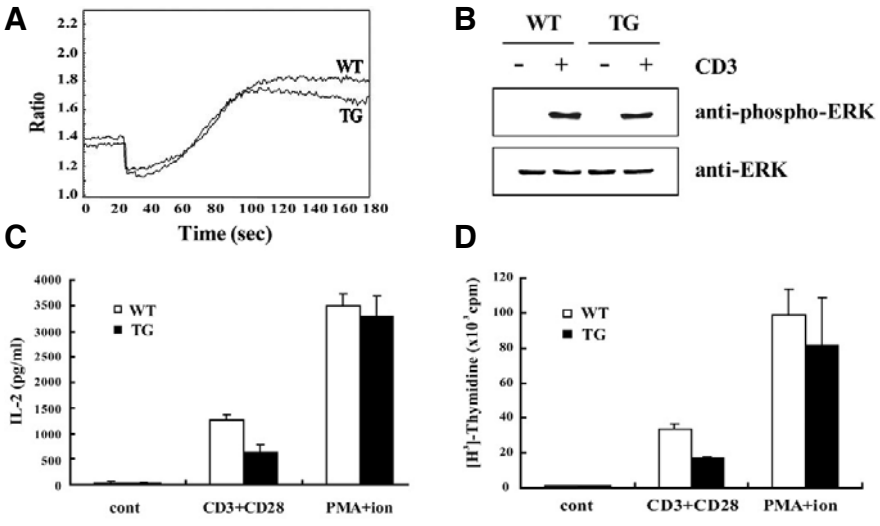

Fig. 2. Analysis of early and late T cell activation events in TG mice. (A) Calcium mobilization. Purified T cells from LN were loaded with Fura-2 and stimulated with anti-CD3ε mAb. Ca^{2+} flux was measured with a spectrofluorometer. The intracellular calcium flux was presented as the ratio of emitted fluorescence intensities of Fura-2 which was exited at 340 nm and 380 nm, respectively (EM 340 nm/EM 380 nm). (B) Activation of ERK. LN T cells were stimulated with anti-CD3ε mAb for 10 min, and lysates were analyzed by western blotting using anti-phospho-ERK mAb. (C) IL-2 production. LN T cells from wild type and TSAd-SH2 TG mice were stimulated with soluble anti-CD3ε (2 μg/ml) plus anti-CD28 mAb (2 μg/ml). Supernatants were collected at 48 h, and the concentration of IL-2 was determined by ELISA. (D) Proliferation of purified LN T cells. Cells were stimulated with anti-CD3ε plus anti-CD28, or with PMA plus ionomycin. Proliferation was determined by measuring [^{3}H]-thymidine incorporation after 48 h. All the experiment were carried out three times in triplicate, and the data represent the means ± STD.

triggers the locomotion of T cells within tissues (Burbach et al., 2007). The finding that TSAd mediates inside-out signaling (Fig. 3) led us to test whether TSAd mediates migration through ECM proteins. T cells from wild type and TSAd-SH2 TG mice were transferred to the upper chambers of transwells containing filters coated either with anti-CD3 mAb + FN or with FN alone, and were allowed to migrate through the filters for 6 h. In this assay, the wild type T cells showed a markedly increased migration through the anti-CD3 Ab + FN-coated filter compared to the FN only-coated filter. However, only a slight increase was observed for the TSAd-SH2 TG T cells (Fig. 4A). Similar results were observed in anti-CD3 Ab + LM-induced cell migration (Fig. 4B). These findings indicate that TSAd acts as a mediator of TCR signaling, and thereby enhances migration of T cells through ECM.

The Contact hypersensitivity response (CHS) is defective in TSAd-SH2 TG mice

The adhesion to ECM is important for the migration of effector T cells to inflammatory sites (Ben-Horin and Bank, 2004; Weninger et al., 2001). Therefore, we investigated whether CHS, an inflammatory response mediated by T cells, is affected in TSAd-SH2 TG mice. The TSAd-SH2 TG and wild type mice were sensitized with surface applications of DNFB on their shaved abdomens on day 1 and day 2. On day 6, mice were challenged with surface applications of DNFB on their ears, and, 24 h after challenge, ear thickness was measured using a micrometer. As shown in Fig. 5A, compared to wild type mice, the TSAd-SH2 TG mice showed a significantly reduced ear-swelling response (approximately 50% that of the wild type). In addition, the proliferation of DNBS- or ConA-primed T cells was significantly reduced in the T cells from TSAd -SH2 TG mice compared to that from wild type mice (Fig. 5B). Combined with the observed migration defect (Fig. 4), these results suggest that the defect in the CHS response can be attributed to the defect in TCR-stimulation-dependent cell migration, as well as cell proliferation. Thus these results suggest that TSAd plays important roles in the inflammatory immune response, presumably by regulating T cell proliferation and migration to inflammatory sites.

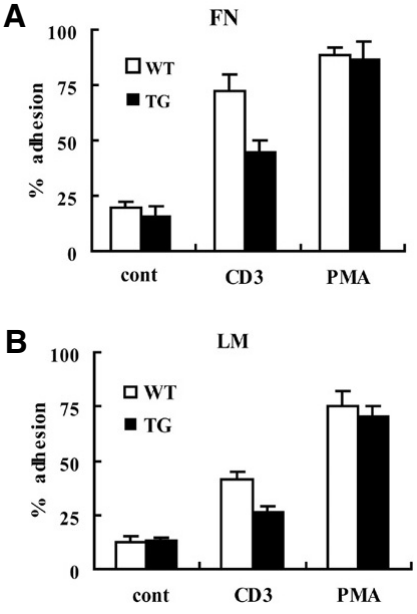

Fig. 3. TCR-induced adhesion to fibronectin (FN) or laminin (LM) is defective in T cells from TSAd-SH2 TG mice. (A-B) 4×10^5 LN T cells were treated in triplicate with anti-CD3ε Ab (2 μg/ml) or PMA (100 ng/ml) + ionomycin (200 ng/ml), and allowed to adhere to FN (A) or LM (B). Results are presented as the percentage of adhered cells. The experiments were repeated three times with reproducible results, and STDs are indicated as error bars.

DISCUSSION

In this study, we presented data showing that TSAd, particularly the TSAd SH2 domain, plays important roles in the effector functions of T cells. T cells from TSAd-SH2 TG mice showed no significant defects in proximal TCR signaling events, such as calcium flux and MAP kinase activation (Figs. 2A and 2B). This finding can be explained by the previous observations that

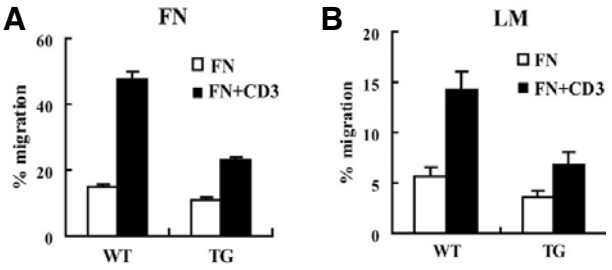

Fig. 4. TCR-induced migration through integrin is impaired in T cells from TSAd-SH2 TG mice. LN T cells were incubated for 6 h in the upper chamber of transwells containing porous filters coated with FN (5 μg/ml) alone or FN+anti-CD3 mAb (2 μg/ml) (A), or coated with LM (5 μg/ml) alone or LM+anti-CD3 mAb (2 μg/ml) (B). The results are expressed as the percentage of fully migrated cells on the lower side of the filters. The cell numbers were counted in five different microscope fields. Bars represent the mean ± STD of triplicate samples. Data are representative of three independent experiments.

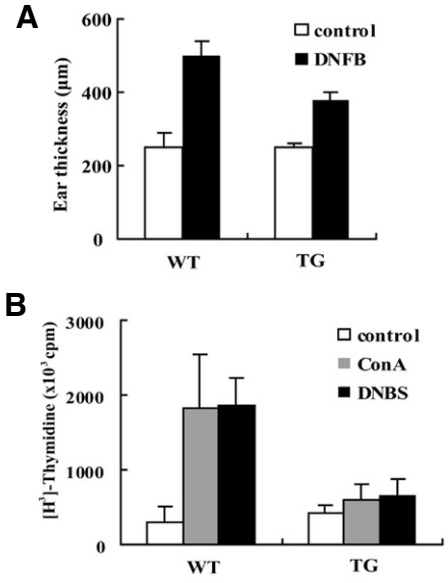

Fig. 5. The inflammatory response is impaired in TSAd-SH2 TG mice. (A) In TSAd-SH2 TG mice, the CHS response is significantly reduced. TSAd-SH2 TG mice were sensitized with 0.5% DNFB, and the CHS reaction was analyzed by measuring the extent of ear swelling. (B) LN T cells were isolated on day 7 after DNFB sensitization, stimulated with concanavalin A (ConA) or DNBS for 72 h, and then pulsed for 18 h with [^{3}H]-thymidine. Results are representative of three independent experiments and are presented as the mean of triplicate values ± STD.

TSAd expression is low in naïve T cells, and TSAd is induced by TCR stimulation (Choi et al., 1999). In contrast, late T cell activation events, such as IL-2 secretion and proliferation, were significantly affected in T cells from the TG mice (Figs. 2C and 2D). Moreover, TCR stimulation-dependent adhesion to ECM and migration through ECM, which are required for the inflammatory response, were significantly affected in T cells from the TG mice (Figs. 3 and 4). Based on these findings, we propose that TSAd, especially the TSAd SH2 domain, plays important roles in effector phase immune responses.

In this study, we showed that T cells from TSAd-SH2 TG mice were defective in CD3-induced adhesion to ECM proteins, fibronectin, and laminin. During T cell activation, TCR ligation initiates inside-out signaling, which enhances integrin avidity toward ECM (Burbach et al., 2007). For inside-out signaling, the SLP76-ADAP complex was previously shown to connect TCR stimulation with the activation of the small GTPase Rap1, which is required for integrin clustering (Kliche et al., 2006). To understand the role of TSAd in inside-out signaling, it will be interesting to test whether TSAd interacts with any of the proteins in the SLP-76-ADAP-Rap1 axis.

In TSAd TG mice, the overexpressed SH2 domain is presumed to block the binding of the endogenous binding partner(s) to the TSAd SH2 domain. Until now, endogenous binding partners of the SH2 domain have not been identified, although several tyrosine phosphorylated proteins were suggested to bind to the TSAd SH2 domain. Marti and King have isolated p57 and p95-100 kDa proteins as TSAd-SH2 binding partners, and subsequently found p95-100 as a Valosin-containing protein (VCP) (Marti and King, 2005; Marti et al., 2001). VCP is a member of the AAA (ATPases associated with a variety of cellular activities) family, involved in various signaling pathways such as ubiquitin signaling pathways, growth factor-mediated survival signaling pathways, and possibly caspase-dependent signaling pathways during apoptosis (Dai and Li, 2001; Jang et al., 2008; Vandermoere et al., 2006). The ortholog of VCP in yeast is the cell cycle control protein CDC48 (Madeo et al., 1998). Upon TCR stimulation, VCP is rapidly tyrosine phosphorylated and interacts with SH2 domain of TSAd. Therefore, it is possible that the reduced proliferation of T cells of TSAd-SH2 TG mice is caused by the disruption of the interaction between endogenous TSAd and VCP. However, at this stage, there is no evidence that the TSAd-VCP interaction is involved in the observed TCR-mediated integrin activation and migration of T cells. Further experiments for the identification of the binding partners of TSAd-SH2 domain will be helpful to understand the molecular mechanism and the biological function of TSAd.

It is interesting to note that p56^{lck} and Tec family kinases do not bind to the TSAd SH2 domain (Marti et al., 2001); p56^{lck} was shown to bind to the C-terminal phosphotyrosine of TSAd through its SH2 domain (Granum et al., 2008; Marti et al., 2006). Therefore, it is presumed that TSAd regulates effector functions, such as TCR-mediated integrin activation, by binding to as-yet unidentified binding partner(s) other than p56^{lck}.

T cells from TSAd-deficient mice were shown to be defective in the production of IL-2 and interferon-γ (IFN-γ), but not of IL-4 (Rajagopal et al., 1999), suggesting that the lack of TSAd more profoundly affects the ability of T cells to produce Th1-type cytokines than Th2-type cytokines. This previous observation is consistent with our current finding that the CHS response is downregulated in SH2 TG mice, because the CHS response is known to be mediated by Th1/T cytotoxic 1 (Tc1) effector cells, and to be downregulated by Th2/T regulatory (Treg) CD4$^+$ T cells (Cavani et al., 2001; Grabbe and Schwarz, 1998; Xu et al., 1996).

In summary, we have established TSAd as an essential signaling molecule for the effector functions of T cells, such as TCR stimulation-dependent adhesion/migration and inflammation.

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