Biochemical and Biophysical Research Communications 423 (2012) 247–252

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Forced expression of stabilized c-Fos in dendritic cells reduces cytokine production and immune responses *in vivo*

Ryoko Yoshida [a,b], Mayu Suzuki [a,b], Ryota Sakaguchi [a,b], Eiichi Hasegawa [a,b], Akihiro Kimura [a,b], Takashi Shichita [a,b], Takashi Sekiya [a,b], Hiroshi Shiraishi [c], Kouji Shimoda [d], Akihiko Yoshimura [a,b,*]

[a] Department of Microbiology and Immunology, Keio University School of Medicine, 35 Shinanomachi, Shinjyuku-ku, Tokyo 160-8582, Japan
[b] Japan Science and Technology Agency, CREST, Chiyoda-ku 102-0075, Japan
[c] Division of Medical Biochemistry, Department of Biomolecular Sciences, Saga Medical School, Saga, Japan
[d] Department of Laboratory Animal Center, Keio University School of Medicine, Tokyo, Japan

ARTICLE INFO

Article history:
Received 14 May 2012
Available online 24 May 2012

Keywords:
c-Fos
Cytokine
Dendritic cell
Immune response
EAE

ABSTRACT

Intracellular cyclic adenosine monophosphate (cAMP) suppresses innate immunity by inhibiting proinflammatory cytokine production by monocytic cells. We have shown that the transcription factor c-Fos is responsible for cAMP-mediated suppression of inflammatory cytokine production, and that c-Fos protein is stabilized by IKKβ-mediated phosphorylation. We found that S308 is one of the major phosphorylation sites, and that the S308D mutation prolongs c-Fos halflife. To investigate the role of stabilized c-Fos protein in dendritic cells (DCs) *in vivo*, we generated CD11c-promoter-deriven c-FosS308D transgenic mice. As expected, bone marrow-derived DCs (BMDCs) from these Tg mice produced smaller amounts of inflammatory cytokines, including TNF-α, IL-12, and IL-23, but higher levels of IL-10, in response to LPS, than those from wild-type (Wt) mice. When T cells were co-cultured with BMDCs from Tg mice, production of Th1 and Th17 cytokines was reduced, although T cell proliferation was not affected. Tg mice demonstrated more resistance to experimental autoimmune encephalomyelitis (EAE) than did Wt mice. These data suggest that c-Fos in DCs plays a suppressive role in certain innate and adaptive immune responses.

1. Introduction

The toll-like receptor (TLR) signaling pathway plays a central role in innate immunity and is linked to the activation of adaptive immunity. TLRs are activated by interaction with their cognate ligands, such as LPS (lipopolysaccharide), followed by the activation of two distinct adaptor molecules, MyD88 and TRIF. The MyD88 and TRIF signaling pathways activate the transcription factors NF-κB and IRF3, leading to the transcriptional activation of proinflammatory cytokines (TNF-α, IL-6, and IL-12) and interferons (IFNs) [1]. To maintain homeostasis, the TLR signal is strictly regulated by anti-inflammatory cytokines such as IL-10 and TGF-β [2,3] as well as various chemical and peptide mediators, including prostaglandin E2 (PGE2) [4], histamine [5], extracellular ATP [6], vasoactive intestinal peptide (VIP), and pituitary adenylate cyclase-activating peptide (PACAP) [7]. IL-10 has been shown to suppress proinflammatory cytokine production through the transcription factor STAT3 [8], whereas other mediators act by elevating intracellular cAMP [9].

We previously identified c-Fos as a strong candidate for the cAMP mediator [9]. It has been reported that deletion of the *c-fos* gene (encoding c-Fos) results in hyperinduction of proinflammatory cytokines in response to LPS [10], but the mechanism of c-Fos-mediated suppression of proinflammatory cytokine production had not been elucidated. We found that the amounts of c-Fos protein induced by LPS plus cAMP were much higher than those induced by LPS or cAMP alone. cAMP upregulates *c-fos* mRNA expression, whereas the kinase IKKβ activated by LPS, directly phosphorylates c-Fos, leading to c-Fos protein stabilization and accumulation. We also identified one of the critical c-Fos residues for IKKβ-mediated phosphorylation and stabilization [9]. Our data suggested that c-Fos should be viewed as a therapeutic target for endotoxin shock and inflammatory diseases, but the effect of c-Fos overexpression or stabilization *in vivo* remained to be clarified.

To investigate the immune-suppressive functions of c-Fos *in vivo*, we generated *c-fos* transgenic (Tg) mice in which the mutant *c-fos* gene is expressed in dendritic cells (DCs). Since c-Fos is very unstable without phosphorylation, we introduced an S308D substitution to stabilize the c-Fos protein. Although the expression levels of c-Fos were within normal physiological levels, proinflammatory

* Corresponding author at: Department of Microbiology and Immunology, Keio University School of Medicine, 35 Shinanomachi, Shinjyuku-ku, Tokyo 160-8582, Japan. Fax: +81 3 5360 1508.
E-mail address: yoshimura@a6.keio.jp (A. Yoshimura).

0006-291X/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbrc.2012.05.097

cytokine production of bone marrow-derived DCs (BMDCs) from Tg mice was lower than that from wild type (Wt) mice. Compared with Wt mice, these Tg mice were more resistant to induction of experimental autoimmune encephalomyelitis (EAE). These data confirmed that c-Fos in DCs plays a suppressive role in immune responses.

2. Materials and methods

2.1. Generation of c-FosS308D Tg mice

The 5.5-kb CD11c promoter was kindly provided by Dr. Littman [11]. The cDNA encoding for human c-Fos was ligated into the EcoRI site of the CD11c-promoter vector. The orientation of the cDNA was controlled by restriction digestion and DNA sequence analysis. The linearized transgenic construct devoid of vector sequence was injected into fertilized oocytes and transgenic offspring were initially identified by tail genomic PCR. We back-crossed founder line 3, referred to in this study as CD11c *c-fos*-Tg mice, toward C57BL/6J for three generations. All experiments using these mice were approved by the Animal Ethics Committee of the Keio University School of Medicine, Tokyo, Japan, and performed according to their guidelines.

2.2. Cells, antibodies and reagents

Bone marrow-derived dendritic cells (BMDCs) were prepared as described previously [12]. BMDCs were stained with APC-conjugated anti-CD11c, FITC-conjugated anti-CD40 and PE-conjugated anti-I-A, or FITC-conjugated anti-CD86 and PE-conjugated anti-CD80. LPS (Escherichia coli serotype 055:B5) was purchased from Sigma. Anti-c-Fos (sc-52) antibody was from Santa Cruz Biotechnology, and anti-Actin antibody (A2066) was from Sigma.

2.3. ELISA and RT-PCR

For the assessment of cytokine production, supernatants were collected 24 h after LPS (10 ng/ml) with or without 100 μM dibutylic cAMP [5] or other TLR ligand stimulation of the BMDCs. The amounts of TNF-α, IL-12 p70, IL-6, and IL-10 were measured with OptEIA ELISA sets (BD Bioscience) [3]. Cells for total RNA analysis were prepared using the Sepasol RNA I Super G (Nacalai Tesque) and RT-PCR was carried out using the High Capacity cDNA reverse transcription kit (Applied Biosystems). Real-time PCR was carried out with EvaGreen Supermix (Bio-Rad). The data were normalized to G3PDH reference. The primers were described previously [13].

2.4. Western blot analysis

Western blot analysis was performed as described elsewhere [9]. In brief, BMDCs (1×10^6) were treated with LPS at the indicated times and then collected and lysed with a lysis buffer. Proteins were separated by 10% SDS–PAGE, transferred onto nitrocellulose membranes, and incubated with antibodies.

2.5. Experimental autoimmune encephalomyelitis (EAE)

Myelin oligodendrocyte glycoprotein (MOG) peptide 35–55 (MEVGWYRSPFSRVVHLYRNGK) was used to induce EAE in mice [14]. Paralysis was evaluated according to the following scale: 0, no disease; 1, tail limpness; 2, hind limb weakness; 3, hind limb paralysis; 4, fore limb weakness; 5, quadriplegia; and 6, death. Relative weight, meaning the ratio of that day's weight to the weight measured on day 0 was also measured daily. After 11 days, splenocytes (5×10^5 cells/well) from these mice were cultured with MOG peptide at the indicated concentrations in a 96-well plate and amounts of IL-17 in the culture supernatants were measured with ELISA [15]. FACS analyses of cytokines in T cells were performed as described [16].

2.6. Preparation of $CD4^+$ T cells and mixed lymphocyte reaction

Spleen and lymph node (LN) cells of C57BL/6 mice were incubated with anti-CD4-coated magnetic beads (Miltenyi Biotec), purified, and positively selected [4]. The purity of the $CD4^+$ T cells was >90%, as determined by flow cytometry. The $CD4^+$ T cells were co-cultured with LPS-stimulated BMDCs in the presence of 1 μg/ml anti-CD3 Ab for 3 days. Then, after a 60 h culture, cell proliferation was assessed by [^{3}H]-thymidine incorporation assay. Cytokine concentration in the supernatants was measured by ELISA [17].

3. Results

3.1. CD11c c-Fos transgene expression

To investigate the role of c-Fos in DCs, we generated Tg mice that express Wt or S308D mutant human c-Fos protein via a transgene under the control of the mouse CD11c promoter (Fig. 1A). This promoter has been used to express various genes in myeloid cells, mostly DCs [18]. We obtained four Wt *c-fos*-Tg lines, however, none of the BMDCs from these mice showed strong accumulation of c-Fos protein after LPS treatment. No difference in cytokine production was observed in BMDCs from Wt *c-fos*-Tg mice (data not shown). Therefore, we did not analyze Wt *c-fos*-Tg lines in further

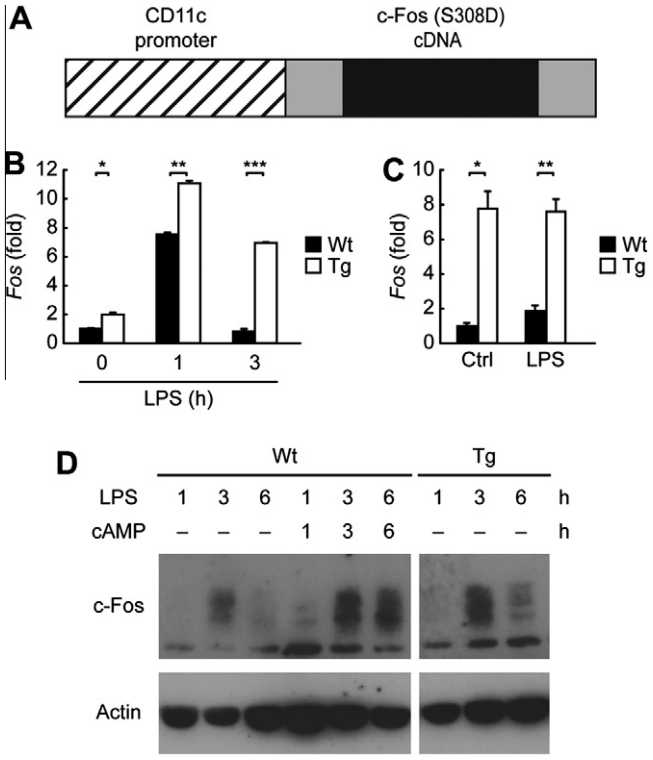

Fig. 1. Generation of c-Fos transgenic mice. (A) ß c-Fos cDNA containing the S308D mutation (black bar) was inserted into the mouse CD11c promoter-containing plasmid (striped bar). The rabbit *β-globin* gene fragment providing an intron and polyadenylation signal is indicated by gray bars. (B and C) BMDCs (B) and $CD11c^+$ splenic DCs (C) were simulated with LPS (10 ng/ml) for the indicated time and for 3 h, respectively. The c-Fos mRNA was determined by quantitative RT-PCR. (D) BMDCs were stimulated with LPS and cAMP for the indicated time. The c-Fos protein was analyzed by immunoblotting. $^*P < 0.05$ $^{**}P < 0.01$, $^{***}P < 0.001$.

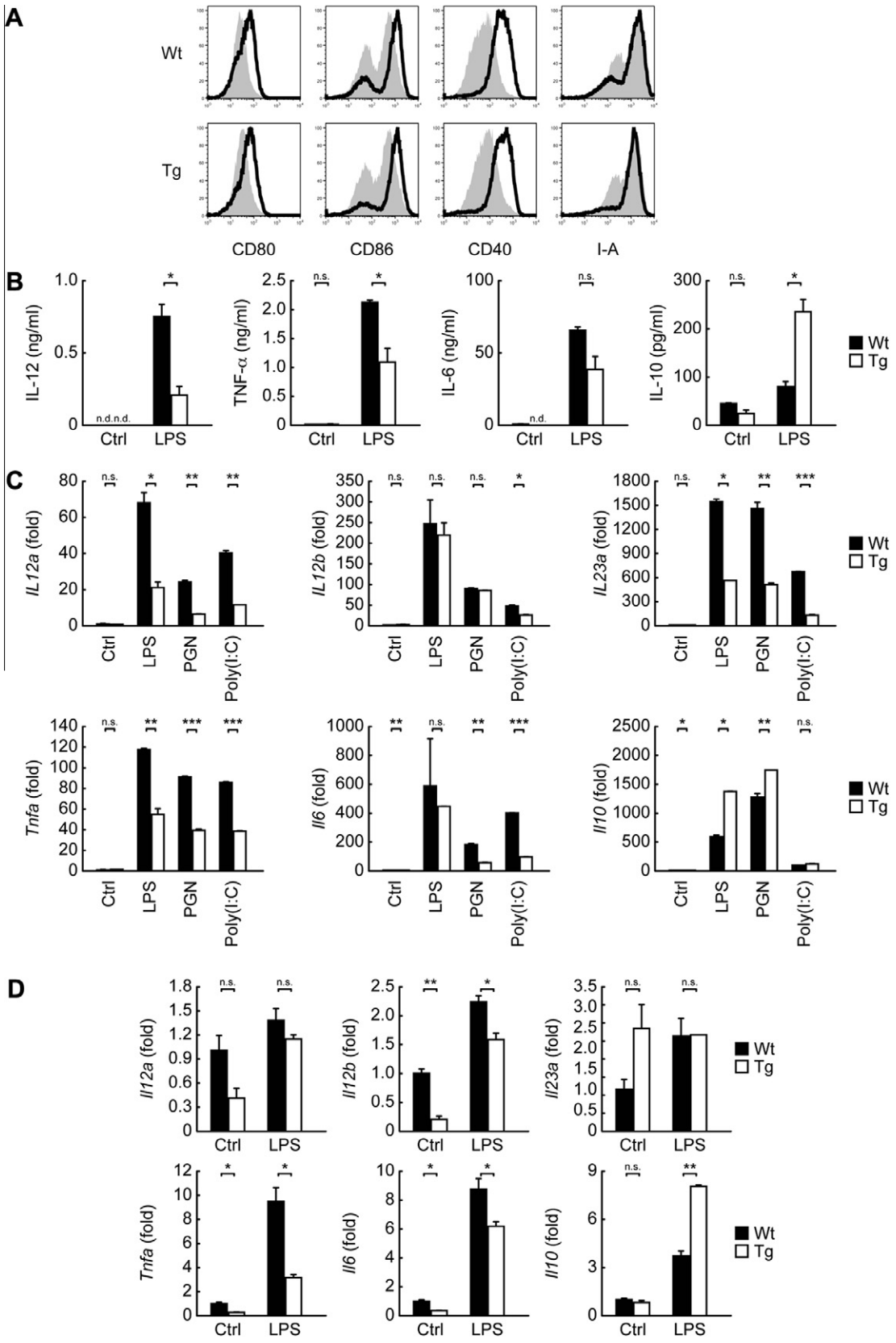

Fig. 2. Effect of c-Fos expression on BMDC activation. (A) Flow cytometric analysis of CD80, CD86, CD40, and MHC class II expression of BMDCs from Tg mice and Wt littermates. BMDCs were cultured in the presence or absence of LPS for 18 h, then stained and analyzed for expression of cell surface markers. (B) BMDCs were stimulated with LPS (10 ng/ml) for 24 h. The concentration of indicated cytokines in culture supernatants was determined by ELISA. (C) BMDCs were stimulated with LPS (10 ng/ml), PGN (10 μg/ml) or Poly(I:C) (10 μg/ml) for 3 h. Cytokine mRNA induction was measured by quantitative RT-PCR and normalized to G3PDH expression for each sample. (D) $CD11c^+$ splenic DCs were stimulated with LPS for 3 h. Cytokine mRNA induction was measured by quantitative RT-PCR and normalized to G3PDH expression for each sample. $^*P < 0.05$ $^{**}P < 0.01$, $^{***}P < 0.001$; n.s., not significant; n.d., not detected.

experiments. We then examined S308D *c-fos*-Tg lines. After microinjection of the plasmids to oocytes, three Tg mice were obtained. Two of them expressed high levels of *c-fos*, showed osteopetrosis, and were sterile. One Tg mouse that expressed relatively low levels of *c-fos* mRNA (Fig. 1B and C) had offspring, so we analyzed this line further. After 1 h LPS stimulation, we could not detect c-Fos protein even in Tg BMDCs by Western blotting (Fig. 1D). As shown in Fig. 1D, the protein expression levels in the BMDCs treated with LPS for 3 h from this Tg line were increased and higher than those in Wt BMDCs were treated with LPS for 3 h. However, this c-Fos levels in Tg BMDCs were comparable to those in Wt BMDCs treated with LPS plus cAMP. Thus, the protein levels of c-Fos in these Tg mice seemed to be within physiological levels. c-Fos protein levels in Tg BMDCs were increased after LPS stimulation, suggesting that LPS activates CD11c promoter.

3.2. Effect of c-Fos on DC maturation

First, we investigated the DC population of Tg mice. The number and population of $CD11b^+CD11c^+$ DCs as well as $CD11c^+B220^+$ pDCs in the spleen were similar between Wt and Tg mice (data not shown). Wt and Tg mice showed similar responses to *in vivo* administration of LPS, probably because macrophages were normal in Tg mice. Thus, we examined DC maturation *in vitro*. LPS induces DC maturation associated with the enhanced expression of class II MHC (I-A), CD40, and costimulators (CD80 and CD86). Upregulation of these surface molecules of BMDCs from Tg mice was comparable to that of the BMDCs from control mice (Fig. 2A). Next, we evaluated the TLR-mediated proinflammatory cytokines. Fully matured DCs produce various cytokines, such as TNF-α, IL-12, IL-6, and IL-10. The production of TNF-α and IL-12 in response to LPS was markedly reduced in BMDCs from Tg mice (Fig. 2B). These cytokine patterns were confirmed by mRNA levels (Fig. 2C) and IL-23p19 (*Il23a*) levels were also reduced (Fig. 2C). In contrast, secretion of IL-10 was enhanced in DCs from Tg mice compared with that of control DCs (Fig. 2B–D). Furthermore, BMDCs from Tg mice suppressed the production of inflammatory cytokines by other TLR ligands such as peptideglycan (PGN) or Poly(I:C) (Fig. 2C). Impairment of TLR-dependent cytokine production was also detected in DCs from Tg mice. These last results confirmed that c-Fos suppresses LPS-induced proinflammatory cytokine production.

We then used Western blot analyses to examine the effect of c-Fos on TLR signaling pathways. ERK, IkBα, p38, and JNK were phosphorylated by stimulation with LPS, but comparisons between BMDCs from Tg mice and Wt mice revealed little alteration (data not shown). These results suggest that the suppressive effects of c-Fos are not due to the inhibition of LPS-mediated intracellular signal transduction pathways.

3.3. Low production of inflammatory cytokines from T cells stimulated with DCs from c-fos-Tg mice

Next, we examined T cells expanded by transgenic DCs *in vitro*. Naïve $CD4^+$ T cells were co-cultured with BMDCs from Tg mice or control mice for three days in the presence of anti-CD3. T cell proliferation was unchanged (Fig. 3A). However, the expression level of Th17-type cytokine IL-17 and RORγt, a master transcription factor of Th17, was much lower in the co-cultured T cells than those of control mice (Fig. 3B). Furthermore, the level of Th1-type cytokine IFN-γ was also lower in DCs from Tg mice. These results indicate that DCs from Tg mice suppressed Th17-type and Th1-type response *in vitro*, compared with those of control mice. These results also suggest that BMDCs from Tg mice are less immunogenic *in vitro* than BMDCs from Wt mice.

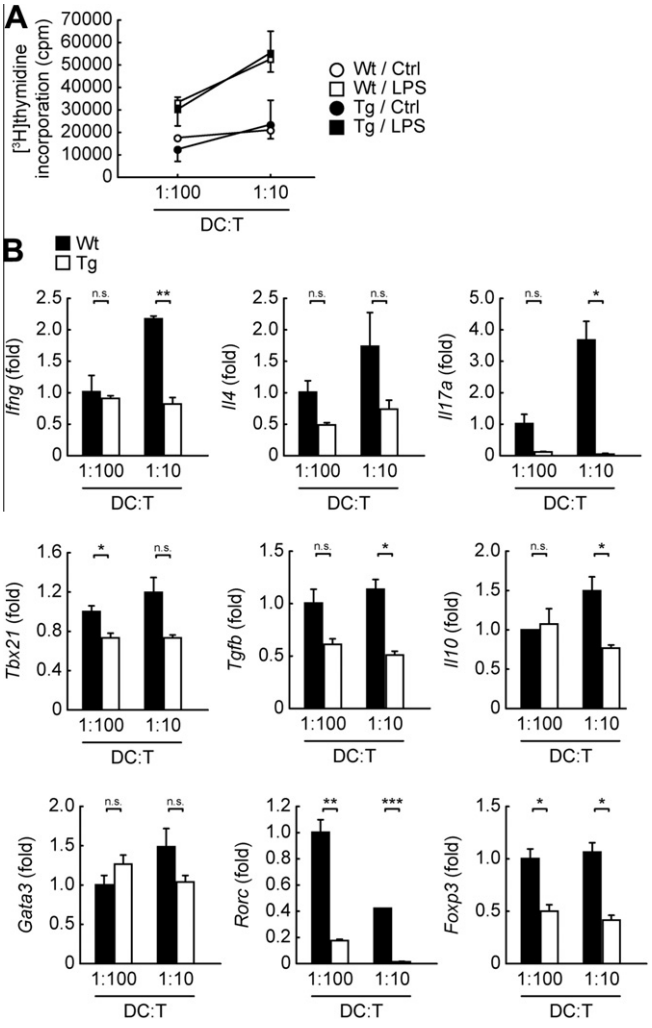

Fig. 3. Low production of cytokines in T cells stimulated with BMDCs from c-Fos transgenic mice. (A) BMDCs from Wt or Tg mice were stimulated with LPS for 24 h, and $CD4^+$ T cells from Wt mice were then co-cultured with BMDCs in the presence of anti-CD3e antibody for 3 days. Proliferation of T cells was determined by [^{3}H]-thymidine incorporation. (B) Cytokines and transcription factor levels in re-stimulated T cells were measured by quantitative RT-PCR and normalized to G3PDH. $^*P < 0.05$ $^{**}P < 0.01$, $^{***}P < 0.001$; n.s., not significant.

3.4. Reduced adaptive immunity in c-fos-Tg mice

We investigated the *in vivo* immunosuppressive effect on *c-fos*-Tg mice of MOG-induced EAE in Th17-mediated disease. Control mice exhibited characteristic signs of EAE starting on day 9, whereas Tg mice developed less severe EAE symptoms compared with those in control mice (Fig. 4A).

We then examined cytokine secretion by splenocytes from mice with EAE. Splenocytes were isolated from mouse spleens on day 11 and re-stimulated with MOG peptide. Re-stimulated splenocytes from Tg mice showed reduction in IL-17 and IFN-γ production compared with those from control mice (Fig. 4B). In addition, IL-17 and IFN-γ expression and the number of $CD4^+$ T cells infiltrated in the spinal cord and brain on day 15 was examined by intracellular cytokine staining and flow-cytometry (Fig. 4C and D). The expression of IL-17 and IFN-γ and the number of infiltrated $CD4^+$ T cells were markedly reduced in cells from Tg mice compared with that of control cells (Fig. 4C). These data indicate that excessive c-Fos expression leads to suppressed IL-17 and IFN-γ production, which leads to improvement in symptoms of Th17-mediated autoimmune disease.

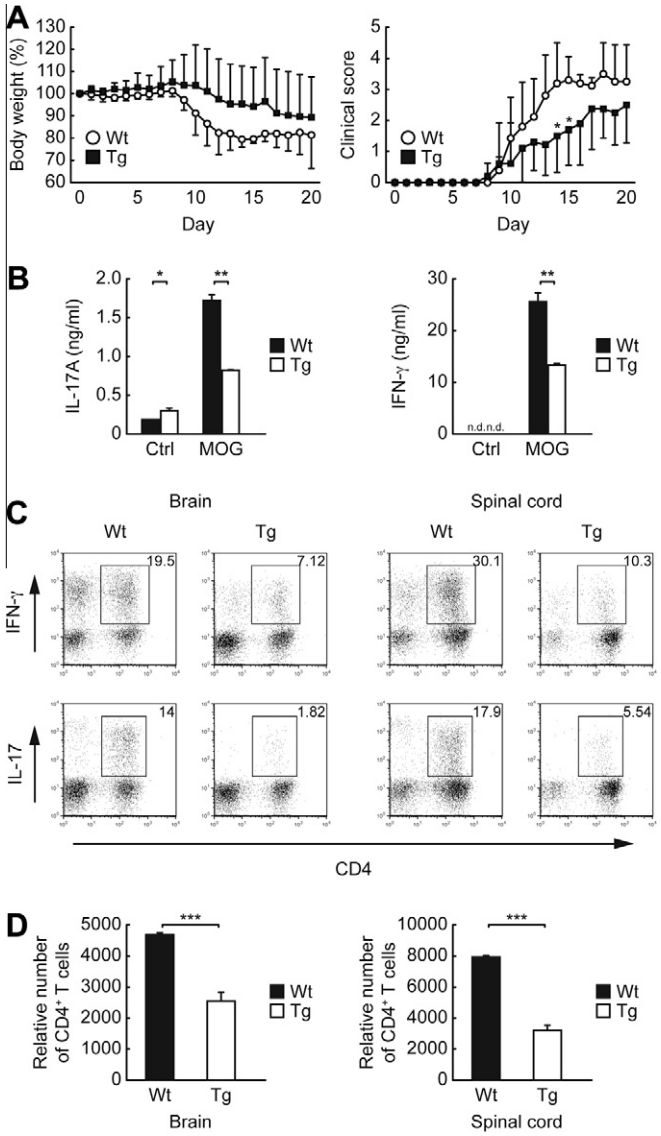

Fig. 4. The level of c-Fos expression affected adaptive immunity. (A) Effect of c-Fos expression on autoimmune responses in a MOG-induced EAE as a Th17-dependent disease model. Tg mice and their littermates were immunized with MOG peptide, and their clinical scores and relative body weights were monitored daily. (B) On day 11 after immunization, the splenocytes from these mice were re-stimulated with MOG peptide for 4 days, and the IL-17 and IFN-γ concentrations in the cultured media were determined by ELISA. (C) MNCs (mononuclear cells) were isolated from brain and spinal cord of MOG-immunized mice on day 15, and intracellular IFN-γ and IL-17 expression in T cells was examined by flow cytometry. (D) The number of $CD4^+$ T cells from brain and spinal cord of MOG-immunized mice on day 15. $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; n.s., not detectable.

4. Discussion

Prior research has shown that intracellular cAMP, when elevated by stimulation by prostaglandin E2 (PGE2), vasoactive intestinal peptide (VIP), pituitary adenylate cyclase-activating peptide (PACAP), histamine, or extracellular ATP, has an immunosuppressive effect on various cell types, including macrophages, DCs and T cells [4,9,19]. However, the mechanism for cAMP-mediated immune suppression is still controversial. We proposed that c-Fos protein accumulated in the nucleus by phosphorylation suppresses NF-κB transcriptional activity. Other groups reported that ICER induced by CREB [20] or p105 phosphorylated by PKA [21] is responsible for suppression of the TLR-mediated cytokine production. Since IL-10 is also upregulated by cAMP, IL-10 is also one of the key factors involved in cAMP-mediated immune suppression. Here, we showed that the forced expression of stabilized c-Fos in dendritic cells at physiological levels was sufficient for suppression of pro-inflammatory cytokines production as well as induction of IL-10, and immunosuppression of T cells. Furthermore, c-Fos expression in DCs can potentially suppress T-cell-mediated EAE, an autoimmune disease model. Thus, our data support our hypothesis that c-Fos is an important factor that mediates the immunosuppressive effect of cAMP. We wish to emphasize that c-Fos stability is important for this immune modulation, because no apparent changes in DCs was observed in Wt *c-fos*-Tg mice.

Since immature DCs treated with PGE2 or other cAMP-elevating agents have been shown to induce IL-10-producing Tr1-like regulatory T cells [22], we expected an expansion of Tr1-like cells in mixed lymphocyte reaction (MLR) experiments using *c-fos*-Tg BMDCs. However, all regulatory gene expression, including TGF-β, Foxp3, and IL-10, was reduced (Fig. 3B). It would be interesting to determine whether or not the expansion of T cells by *c-fos*-Tg BMDCs is regulatory. In any case, cytokine production indicates that these T cells are less inflammatory, which is consistent with the EAE data of *c-fos*-Tg mice. Further study is necessary to define the nature of the T cells induced by *c-fos*-Tg DCs, both *in vitro* and *in vivo*.

c-Fos is a component of AP-1, which is usually thought to be involved in immune activation. For example, AP-1 in T cells is known to enhance IL-2 transcription, in cooperation with NF-AT [23]. By microarray analysis, we noticed that IL-1β mRNA levels were higher in *c-fos*-Tg BMDCs than in Wt BMDCs (data not shown). Thus, in certain situations, c-Fos may promote immune responses by inducing some cytokines such as IL-1β and IL-2. EP2 and EP4, PGE2 receptors that upregulate cAMP, have also been shown to upregulate Th17 responses *in vivo* [24]. We observed that *c-fos*-Tg mice were resistant to EAE, a disease in which Th17 induction is important. This may be explained by the notion that c-Fos upregulation stems from the effects of cAMP or PGE2. Thus, c-Fos can modulate a particular immune disorder but may also exhibit different effects in other diseases.

Acknowledgments

The authors are grateful to M. Asakawa, K. Fukuse, and N. Shiino for their technical assistance. This work was supported by special Grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, the Program for Promotion of Fundamental Studies in Health Science of the National Institute of Biomedical Innovation (NIBIO), the SENSHIN Research Foundation, the Mochida Memorial Foundation, and the Takeda Science Foundation.

References

[1] K. Takeda, S. Akira, TLR signaling pathways, Semin. Immunol. 16 (2004) 3–9.
[2] K. Takeda, B.E. Clausen, T. Kaisho, T. Tsujimura, N. Terada, I. Forster, S. Akira, Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils, Immunity 10 (1999) 39–49.
[3] Y. Sugiyama, K. Kakoi, A. Kimura, I. Takada, I. Kashiwagi, Y. Wakabayashi, R. Morita, M. Nomura, A. Yoshimura, Smad2 and Smad3 are redundantly essential for the suppression of iNOS synthesis in macrophages by regulating IRF3 and STAT1 pathways, Int. Immunol. 24 (2012) 253–265.
[4] H. Shiraishi, H. Yoshida, K. Saeki, Y. Miura, S. Watanabe, T. Ishizaki, M. Hashimoto, G. Takaesu, T. Kobayashi, A. Yoshimura, Prostaglandin E2 is a major soluble factor produced by stromal cells for preventing inflammatory cytokine production from dendritic cells, Int. Immunol. 20 (2008) 1219–1229.
[5] M. Jutel, T. Watanabe, M. Akdis, K. Blaser, C.A. Akdis, Immune regulation by histamine, Curr. Opin. immunol. 14 (2002) 735–740.
[6] A. la Sala, D. Ferrari, S. Corinti, A. Cavani, F. Di Virgilio, G. Girolomoni, Extracellular ATP induces a distorted maturation of dendritic cells and inhibits their capacity to initiate Th1 responses, J. Immunol. 166 (2001) 1611–1617.

[7] D. Pozo, VIP- and PACAP-mediated immunomodulation as prospective therapeutic tools, Trends Mol. Med. 9 (2003) 211–217.
[8] H. Yasukawa, M. Ohishi, H. Mori, M. Murakami, T. Chinen, D. Aki, T. Hanada, K. Takeda, S. Akira, M. Hoshijima, T. Hirano, K.R. Chien, A. Yoshimura, IL-6 induces an anti-inflammatory response in the absence of SOCS3 in macrophages, Nat. Immunol. 4 (2003) 551–556.
[9] K. Koga, G. Takaesu, R. Yoshida, M. Nakaya, T. Kobayashi, I. Kinjyo, A. Yoshimura, Cyclic adenosine monophosphate suppresses the transcription of proinflammatory cytokines via the phosphorylated c-Fos protein, Immunity 30 (2009) 372–383.
[10] N. Ray, M. Kuwahara, Y. Takada, K. Maruyama, T. Kawaguchi, H. Tsubone, H. Ishikawa, K. Matsuo, C-Fos suppresses systemic inflammatory response to endotoxin, Int. Immunol. 18 (2006) 671–677.
[11] S. Jung, D. Unutmaz, P. Wong, G. Sano, K. De los Santos, T. Sparwasser, S. Wu, S. Vuthoori, K. Ko, F. Zavala, E.G. Pamer, D.R. Littman, R.A. Lang, In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens, Immunity. 17 (2002) 211–220.
[12] Y. Matsumura, T. Kobayashi, K. Ichiyama, R. Yoshida, M. Hashimoto, T. Takimoto, K. Tanaka, T. Chinen, T. Shichita, T. Wyss-Coray, K. Sato, A. Yoshimura, Selective expansion of foxp3-positive regulatory T cells and immunosuppression by suppressors of cytokine signaling 3-deficient dendritic cells, J. Immunol. 179 (2007) 2170–2179.
[13] T. Chinen, K. Komai, G. Muto, R. Morita, N. Inoue, H. Yoshida, T. Sekiya, R. Yoshida, K. Nakamura, R. Takayanagi, A. Yoshimura, Prostaglandin E2 and SOCS1 have a role in intestinal immune tolerance, Nat. Commun. 2 (2011) 190.
[14] K. Ichiyama, T. Sekiya, N. Inoue, T. Tamiya, I. Kashiwagi, A. Kimura, R. Morita, G. Muto, T. Shichita, R. Takahashi, A. Yoshimura, Transcription factor Smad-independent T helper 17 cell induction by transforming-growth factor-beta is mediated by suppression of eomesodermin, Immunity 34 (2011) 741–754.
[15] H. Yoshida, A. Kimura, T. Fukaya, T. Sekiya, R. Morita, T. Shichita, H. Inoue, A. Yoshimura, Low dose CP-690,550 (tofacitinib), a pan-JAK inhibitor, accelerates the onset of experimental autoimmune encephalomyelitis by potentiating Th17 differentiation, Biochem. Biophys. Res. Commun. 418 (2012) 234–240.
[16] T. Sekiya, I. Kashiwagi, N. Inoue, R. Morita, S. Hori, H. Waldmann, A.Y. Rudensky, H. Ichinose, D. Metzger, P. Chambon, A. Yoshimura, The nuclear orphan receptor Nr4a2 induces Foxp3 and regulates differentiation of CD4+ T cells, Nat. Commun. 2 (2011) 269.
[17] R. Takahashi, S. Nishimoto, G. Muto, T. Sekiya, T. Tamiya, A. Kimura, R. Morita, M. Asakawa, T. Chinen, A. Yoshimura, SOCS1 is essential for regulatory T cell functions by preventing loss of Foxp3 expression as well as IFN-gamma and IL-17A production, J. Exp. Med. 208 (2011) 2055–2067.
[18] D.A. Hume, Applications of myeloid-specific promoters in transgenic mice support in vivo imaging and functional genomics but do not support the concept of distinct macrophage and dendritic cell lineages or roles in immunity, J. Leu. Biol. 89 (2011) 525–538.
[19] R. Mosenden, K. Tasken, Cyclic AMP-mediated immune regulation–overview of mechanisms of action in T cells, Cell. Signal. 23 (2011) 1009–1016.
[20] M.D. Harzenetter, A.R. Novotny, P. Gais, C.A. Molina, F. Altmayr, B. Holzmann, Negative regulation of TLR responses by the neuropeptide CGRP is mediated by the transcriptional repressor ICER, J. Immunol. 179 (2007) 607–615.
[21] E.A. Wall, J.R. Zavzavadjian, M.S. Chang, B. Randhawa, X. Zhu, R.C. Hsueh, J. Liu, A. Driver, X.R. Bao, P.C. Sternweis, M.I. Simon, I.D. Fraser, Suppression of LPS-induced TNF-alpha production in macrophages by cAMP is mediated by PKA-AKAP95-p105, Sci. Signal. 2 (2009) ra28.
[22] T. Bopp, C. Becker, M. Klein, S. Klein-Hessling, A. Palmetshofer, E. Serfling, V. Heib, M. Becker, J. Kubach, S. Schmitt, S. Stoll, H. Schild, M.S. Staege, M. Stassen, H. Jonuleit, E. Schmitt, Cyclic adenosine monophosphate is a key component of regulatory T cell-mediated suppression, J. Exp. Med. 204 (2007) 1303–1310.
[23] J. Jain, Z. Miner, A. Rao, Analysis of the preexisting and nuclear forms of nuclear factor of activated T cells, J. Immunol. 151 (1993) 837–848.
[24] C. Yao, D. Sakata, Y. Esaki, Y. Li, T. Matsuoka, K. Kuroiwa, Y. Sugimoto, S. Narumiya, Prostaglandin E2-EP4 signaling promotes immune inflammation through Th1 cell differentiation and Th17 cell expansion, Nat. Med. 15 (2009) 633–640.