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Epidermal-type fatty acid binding protein as a negative regulator of IL-12 production in dendritic cells ☆

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Abstract

Fatty acids and their metabolites have recently been shown to modulate various functions of dendritic cells (DCs) including their differentiation and cytokine production, although the mechanisms underlying their cellular functions are not fully understood. In view of our previous finding that epidermal-type fatty acid binding protein (E-FABP) was exclusively expressed in splenic DCs among FABP family, we examined the phenotype of E-FABP-null mutant mice in order to elucidate the functional significance of E-FABP expression in DCs. Although E-FABP-null mutant mice showed no apparent abnormalities in the population density and subset distribution of DCs as well as the microscopic morphology in the spleen, DCs isolated from E-FABP-null spleen showed enhanced production of IL-12p70, a key cytokine for innate immune responses, in response to appropriate stimuli as compared with wild-type. In real-time PCR, the expression level of IL-12p35 mRNA after LPS stimuli was much higher in mutant DCs when compared with wild-type, while no apparent change of IL-12p40 mRNA level was detected. Phosphorylated forms of p38 mitogen-activated protein kinase (p38MAPK) and IκB-α, molecules critical for IL-12 production, were detected at higher levels in E-FABP-null-mutant DCs after LPS stimuli when compared with wild-type counterparts. Collectively, it is suggested that E-FABP may be a novel negative regulator of IL-12 production in DCs, and this regulation may be exerted via its involvement in the p38MAPK-mediated transcription of IL-12p35.

Keywords: E-FABP; Dendritic cells; IL-12; Mouse; Gene-knockout mice; Fatty acids

☆ *Abbreviations:* AA, arachidonic acid; A-FABP, adipocyte-type fatty acid binding protein; APC, antigen-presenting cell; B-FABP, brain-type fatty acid binding protein; BMDC, bone marrow-derived dendritic cell; BSA, bovine serum albumin; CD, cluster of differentiation; cDNA, complementary DNA; COX, cyclooxygenase; DAPI, 4′,6-diamidino-2-phenylindole; DC, dendritic cell; DTH, delayed type hypersensitivity; EDTA, ethylenediaminetetraacetic acid; E-FABP, epidermal-type fatty acid binding protein; ELISA, enzyme-linked immunosorbent assay; EPA, eicosapentaenoic acid; FA, fatty acid; FABP, fatty acid binding protein; FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; FCS, fetal calf serum; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HBSS, Hank's balanced salt solution; H-FABP, heart-type fatty acid binding protein; HRP, horseradish peroxidase; IFN, interferon; Ig, immunoglobulin; IκB, inhibitor of NF-κB; IL, interleukin; JNK, c-Jun N-terminal kinase; L-FABP, liver-type fatty acid binding protein; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MHC, major histocompatibility complex; NF-κB, nuclear factor κB; NK cell, natural killer cell; PBS, phosphate-buffered saline; PCR, polymerase chain reaction; PE, phycoerythrin; PMSF, phenylmethylsulfonylfluoride; PUFA, polyunsaturated long chain fatty acid; PPAR, peroxisome proliferators-activated receptor; PVDF, polyvinylidene difluoride; RPMI 1640, Roswell Park Memorial Institute medium 1640; RT-PCR, reverse transcription PCR; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; Th1/2, T helper type 1/2; TNF, tumor necrosis factor; UDG, uracil DNA glycosylase.

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Dendritic cells (DCs), the most potent antigen-presenting cells (APCs), release multiple cytokines upon initial contact with pathogens and play a role in triggering subsequent adaptive immune responses against infective pathogens, serving as an essential link between innate and adaptive immunity [1,2]. DCs are the most potent producer of IL-12, one of key cytokines for the innate immune response [3]. IL-12 acts on resting natural killer (NK) and $CD8^+$ T cells and induces their cytotoxic activity as well as the activation of macrophages, which contributes to the elimination of cells infected with intracellular pathogens and that of malignant tumors [3]. Furthermore, IL-12 polarizes naïve T cells to Th1 effector cells, and simultaneously suppresses the differentiation of Th2 cells, which controls the humoral immunity with production of immunoglobulins, such as IgG1, IgE, and IgA [4]. In contrast to the beneficial aspects of IL-12 in the host defense, excessive IL-12 secretion can lead to immunopathology and host mortality, such as allograft rejection and auto-immune diseases [5,6]. Thus, IL-12 production in DCs should be strictly regulated positively or negatively, although the molecular mechanism underlying the negative regulation of IL-12 production in DCs is not fully understood yet.

Polyunsaturated long chain fatty acids (PUFAs) arouse beneficial effects on a variety of inflammatory diseases, such as IgA nephropathy and rheumatoid arthritis [7,8]. Dietary supplementation of fish oils, enriched in n-3 series of PUFAs, is known to suppress the lymphocyte proliferation and the secretion of pro-inflammatory cytokines, such as IL-2 [9,10]. It has recently been revealed that various lipid metabolites and/or molecules which modulate the lipid signaling and metabolism are involved in the negative feed back mechanism of IL-12 production in DCs [11–14]. PUFAs, i.e., arachidonic acid (AA) and eicosapentaenoic acid (EPA), block the production of IL-12 and TNF-α in human DCs in response to lipopolysaccharide (LPS), and they could consequently contribute to the well known anti-inflammatory effects on various inflammatory diseases [15]. However, little information is so far available about the molecular mechanism of such PUFAs' effect on IL-12 production in DCs.

Fatty acid binding proteins (FABPs) constitute a multigene family of structurally homologous cytosolic proteins capable of binding PUFAs and various eicosanoids [16], and may work as vehicles of their ligands for given cellular functions such as cellular FA uptake and FA-mediated signal transduction. FABPs show distinct patterns of tissue expression and their individual physiological functions have so far been investigated using genetic manipulation studies in mice [17]. Among members of FABP family, epidermal-type FABP (E-FABP), also called mal1 or fabp5, has been shown to be expressed not only in keratinocytes [18,19] but also in adipocytes [20], macrophages in various tissues [21–23] and brain cells including neuron and glia [24,25], and its gene ablation in mice results in altered water barrier function in the skin [26] and increased insulin sensitivity in the adipocytes [27]. We have recently shown E-FABP to be localized specifically to DCs in the splenic white pulp in vivo, suggesting its possible involvement in the antigen-presenting function [28]. Considering that PUFAs, ligands of E-FABP, have inhibitory effects on IL-12 production in DCs [15,29], it is possible that E-FABP expressed in DCs may be involved in the process of IL-12 production via modulation of intracellular PUFA-metabolism and/or -mediated signal transduction. The present study was undertaken to clarify the functional significance of E-FABP in DCs based on a knockout approach by making use of E-FABP-null mice [26], and showed E-FABP to act as a negative regulator of IL-12 production in the DCs.

Materials and methods

Mice. Generation of E-FABP gene-knockout mice was described previously by the present authors [26]. The mice used in this study were backcrossed from a mixed 129SVJ × C57BL/6 background onto the C57BL/6 for at least 8 generations and were intercrossed to produce wild-type and E-FABP-null mutant mice. They were maintained under specific pathogen-free conditions. The genotype of the mice was confirmed before experiments by PCR analysis of tail chip digests [26]. All experimental protocols were reviewed by the Committee on the Ethics of Animal Experiments of Tohoku University, and were carried out in accordance with the guidelines for animal experiments issued by graduate school of medicine, Tohoku University.

DC preparation. Splenic DCs were separated as described previously [30] with minor modifications. Mice were sacrificed by cervical dislocation and their spleens were aseptically removed. Splenocytes were prepared by homogenizing collagenase-treated spleens in all experiments. The spleen was digested in Hank's balanced salt solution (HBSS, Sigma, St. Louis, MO, USA) containing 1 mg/ml collagenase type IV (Sigma). The collagenase-treated cells were suspended in a dense bovine serum albumin (BSA) solution ($P = 1.080$), overlaid with an equal volume of Roswell Park Memorial Institute medium 1640 (RPMI1640, Sigma), and centrifuged in a swing bucket rotor at 9500*g* for 15 min at 4 °C. Cells in the low-density APC fraction at an interface between the medium and BSA solution were collected and washed in RPMI 1640. DCs were positively purified with anti-mouse CD11c microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and VarioMACS (Miltenyi Biotec) directly from APC fraction. All procedures, except the collagenase-digestion step, were done on ice. Approximately >95% of collected cells were $CD11c^+$ $I\text{-}A^+$ as judged by FACS (Caliber, BD Bioscience, Mountain View, CA, USA) and were used as immature splenic DCs.

Immunocytochemistry. Immunocytochemistry was performed as described previously with slight modifications [28]. Briefly, isolated splenic DCs were attached onto glass slides using a centrifugal cell collector (TOMY SEIKO, Japan) or using Celltak (BD Sciences, Bedford, MA, USA). The cells on glass slide were incubated with a rabbit anti-rat antibody against E-FABP [28] at a concentration of 0.5 μg/ml for 12 h at 4 °C. After incubation with the primary antibody, the cells were incubated with an anti-rabbit antibody labeled by Alexa488 (Invitrogen, Carlsbad, CA, USA). After nuclear-counterstaining with 4′,6-diamidino-2-phenylindole (DAPI, Sigma), the cells were covered with Gel/Mount (Biomeda, Foster City, CA, USA) and observed by a confocal laser microscope (LSM5 Pascal; Carl Zeiss, Oberkochen, Germany).

Application of PUFAs or COX inhibitors to isolated splenic DCs. Isolated splenic DCs were seeded at 5×10^5 cells per well onto 96-well plates. Fatty acids including palmitic acid (C16:0), arachidonic acid (C20:4, n-6), and docosahexaenoic acid (C22:6, n-3), and COX inhibitors including NS-398 (a COX-2-specific inhibitor) and SC-560 (a COX-1-specific inhibitor) were purchased from Cayman Chemical (Ann Aobar, MI, USA). For PUFA treatment, the RPMI medium and a given fatty acid were mixed for 30 min to allow binding of the fatty acid with fatty acid-free albumin (Sigma) before addition to DCs at indicated concentrations. For treatment

of COX inhibitor, DCs were incubated with NS-398 or SC-560 at an appropriate concentration [31]. For DC activation, 1 μg/ml LPS, 10 μg/ml anti-CD40, and 10 ng/ml IL-4 were added to the culture medium and the cells were incubated at 37 °C with 5% CO_2. Cell supernatants were collected after 24 h of culture and used for cytokine measurement.

FACS analysis. Phenotypic analysis of DCs was performed with FITC-conjugated anti-CD11c and -CD3 or PE-conjugated anti-MHC class II, -B220 and -CD8α Abs (BD Biosciences Pharmingen, San Diego, CA, USA). DCs were suspended in FACS medium (0.2% BSA and 0.1% NaN_3 in PBS) at a concentration of 5×10^5 cells/ml followed by incubation for 15 min at 4 °C with properly diluted mAbs with FITC or PE. After staining, the cells were washed twice with FACS medium and brought to FACS analysis. Flow cytometry was done on a FACS with CellQuest software (Becton–Dickinson, San Jose, CA, USA).

RT-PCR. Total RNAs were isolated from wild-type and E-FABP-null splenic DCs at 6 h after LPS/anti-CD40/IL-4 stimulus using the guanidine isothiocyanate-based TRIzol solution (Gibco-BRL, Burlington, ON, Canada) according to the manufacturer's manual. Isolated RNAs were incubated with 1 U of DNase I (Invitrogen, Carlsbad, CA, USA) for 15 min at room temperature. The DNase I was inactivated by addition of 1 μl of 25 mM EDTA solution to the reaction mixture and heated for 10 min at 65 °C. The detail of semi-quantitative RT-PCR was described elsewhere [32]. For real-time RT-PCR, relative quantification of IL-12p35 and IL-12p40 mRNAs was done on an ABI PRISM 7700 Sequence Detection System (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) using a SuperScript III Platinum Two-Step qRT-PCR Kit with SYBR Green (Invitrogen). The reactions were carried out in a 96-well plate in a 25-μl reaction volume containing 12.5 μl of Platinum SYBR Green qPCR SuperMix-UDG (Invitrogen), 0.2 μM concentration of each forward and reverse primer, and cDNA obtained from the first-strand synthesis reaction. The thermal profile for all SYBR Green PCRs was 50 °C for 2 min and 95 °C for 2 min, followed by 50 cycles of 95 °C for 15 s and 60 °C for 30 s. The quantity of each transcript was calculated by the standard curve method [33] using ABI PRISM 7700 Sequence Detection System software version 1.7. GAPDH mRNA was used for each experimental sample as an endogenous control to account for differences in the amount and quality of total RNAs added to each reaction. Each sample was triplicated.

The nucleotide sequences of the primers used in this study were as follows:

GAPDH: 5′-GGTGCTGAGTATGTCGTGGAGTCTA
5′-CGGAGATGATGACCCGTTTG
IL-12p35: 5′-GTCCAGCATGTGTCAATCAC
5′-GCCAAACTGAGGTGGTTTAG
IL-12p40: 5′-GAGCACTCCCCATTCCTACT
5′-CCCTCCTCTGTCTCCTTCAT

Determination of cytokine production by ELISA. Commercially available ELISA Kits (BD OptEIA Set; BD Biosciences Pharmingen) were used for measuring the concentration of IL-12p70, TNF-α, IFN-γ, and IL-10 in supernatant fraction samples of the cell culture according to the manufacture's manual. In brief, 96-well ELISA plates (Nunc, Wiesbaden, Germany) were coated with a capture antibody overnight. The next day, the plates were treated with a blocking buffer (10% FCS/PBS) to block non-specific binding. Various dilutions of samples and the standards were incubated in the plates. After 2 h, the plates were washed five times with a wash buffer (PBS with 0.05% Tween 20) followed by addition of working detector (detection antibody + Avidin-horseradish peroxidase (HRP) reagent). Following a 1 h equilibration period, the plates were washed seven times with wash buffer and substrate solution was added. After color development for 30 min, the reaction was stopped with 2 N H_2SO_4. The absorbance was measured on an ELISA plate reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm within 30 min of stopping the reaction. All samples were assayed in duplicate.

Immunoblotting. Cells were lysed in a lysis buffer solution that consisted of 12.5 mM Tris–HCl (pH 6.8), 4% SDS, 20% glycerin, 0.01% bromophenol blue, 1 mM PMSF, 1 mM Na_3VO_4, and protease inhibitor cocktail tablet (Roche, Basel, Switzerland). Lysates were cleared by centrifugation at 15,000 rpm for 5 min and boiled in a sample buffer solution. Cell lysates were further fractionated on 10% SDS–PAGE and transferred to PVDF membranes (Osmonic Inc., Livemore, CA, USA). After blocking with 5% normal goat serum, the membrane was incubated with an appropriate primary antibody. Primary antibodies used in the present study were anti-rat E-FABP antibody [26], anti-human p38 (Cell Signaling Technology, Beverly, MA, USA), anti-human phosphorylated p38 (Cell Signaling Technology), anti-human phosphorylated IκB-α (Calbiochem, San Diego, CA, USA), and anti-tubulin α (Neomarkers, Fremont, CA, USA). After incubation with the primary antibody, the membrane was subsequently incubated with an anti-rabbit secondary antibody conjugated with HRP. The immuno-positive bands were visualized with the enhanced chemiluminescence immunoblotting detection system (Amersham Bioscience, Buckinghamshire, UK), and the images were digitally captured by ChemiDoc XRS (Bio-Rad Laboratories, Hercules, CA, USA). The density of immuno-positive bands for p-IκB-α, p-p38MAPK, p38MAPK, and tubulin was measured by Quantity One software (Bio-Rad Laboratories) and the density ratio (p-IκB-α/tubulin, p-p38MAPK/tubulin, and p38MAPK/tubulin) was obtained for comparison.

Allogenic mixed lymphocyte reaction. The mixed lymphocyte reaction was performed as described previously [34]. Briefly, $CD4^+$ T cells were isolated from splenocytes of BALB/c mice using anti-mouse CD4 microbeads (Miltenyi Biotec) and MACS (Miltenyi Biotec). The purity of the isolated cells was >98% as determined by CD4 expression. Splenic DCs (1×10^5) prepared from wild-type and E-FABP-null splenocytes of C57BL/6 background were cultured with purified $CD4^+$ T cells (2×10^5) in a 96-well flat-bottomed plate for 2 days. The titers of IFN-γ in the supernatants were then measured by ELISA Kit.

Statistical analysis. Results are expressed as means ± standard errors of the means (SEM). The statistical difference was determined by two-sided Student's t test. The difference with $P < 0.05$ was considered as significant.

Results

In immuno-light microscopy, the population density of E-FABP-positive cells was approximately 30% in the splenic DCs isolated from wild-type mice (Fig. 1A), while it increased up to 80% after LPS stimulation (Fig. 1B), while no positive cells were detected for adipocyte-type (A-), brain-type (B-), heart-type (H-) FABP or liver-type (L-) FABP (data not shown). The immunopositivities for E-FABP were localized in both cytoplasm and nucleus of DCs before and after LPS stimulation (Fig. 1A and B). In immunoblot analysis, a single immuno-positive band for E-FABP was detected before and after LPS stimulation on DCs, and the intensity of immuno-positive band was increased by 15-fold at 24 h after LPS stimulation (Fig. 1C). In RT-PCR, the gene expression of E-FABP was further confirmed in DCs isolated from wild-type, while no bands for A-, B-, H-, or L-FABP were detected in the cells (Fig. 1D).

First, we examined the modulatory effects of exogenously applied PUFAs and endogenous eicosanoids, all of which are possible ligands of E-FABP, on IL-12 production after LPS stimulation of isolated splenic DCs. In accord with previous findings about the effect of PUFAs on the production of IL-12p70 in DCs derived from mouse

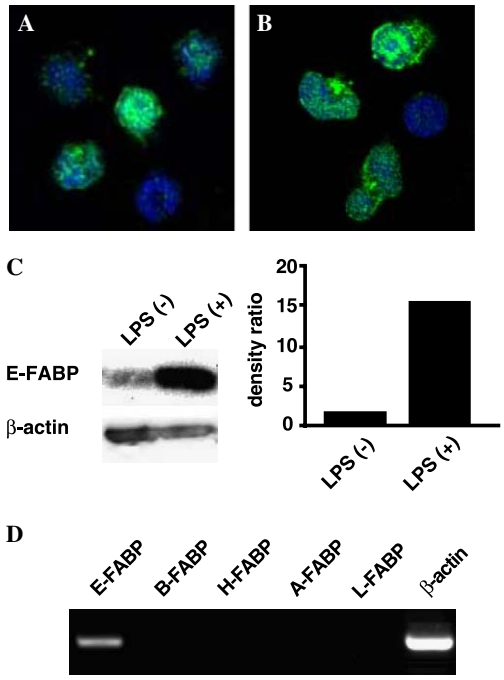

Fig. 1. Expression of E-FABP in the dendritic cells. (A,B) Immunocytochemical localization of E-FABP in the wild-type splenic DCs before (A) and after LPS stimulation (B). Note the increase in the population of E-FABP-positive DCs after stimulation. (C) Immunoblot analysis of E-FABP in cell lysates of wild-type splenic DCs before and after LPS stimulation (left). Note the 15-fold induction of E-FABP expression in the DCs from E-FABP-null mutant mice (right). Data are shown by the density ratio (E-FABP/β-actin). (D) RT-PCR analysis of E-FABP in the isolated splenic DCs. Note an exclusive expression of gene for E-FABP among FABP family in splenic DCs. Data are representative of two independent experiments.

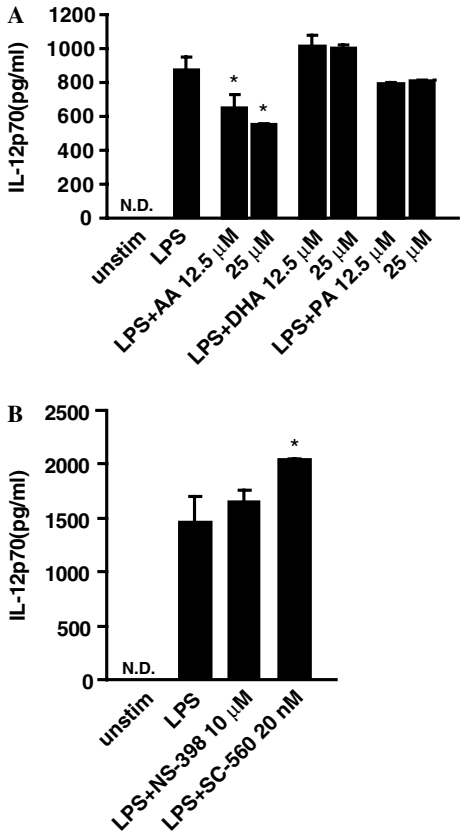

Fig. 2. Effect of PUFAs and COX inhibitors on the production of IL-12p70 in DCs. (A) Arachidonic acid inhibited the production of IL-12p70 in a dose-dependent manner in DCs from wild-type mouse, while palmitic acid and docosahexaenoic acid have no effect on the production of IL-12p70. (B) A COX-1-specific inhibitor, SC-560, enhanced the production of IL-12p70 from DCs. Data are shown as means ± SEM and representative of two independent experiments. $^{*}P < 0.05$, significantly different from the mean value of the corresponding control. N.D., not detected.

bone marrow [29] and from human mononuclear cells [15], arachidonic acid (AA) suppressed the production of IL-12p70 in a dose-dependent manner in splenic DCs from wild-type mice, while palmitic acid (PA) and docosahexaenoic acid (DHA) had no effect on their production of IL-12p70 (Fig. 2A). Furthermore, application of SC-560, an inhibitor for COX-1, to DCs was revealed to enhance the IL-12 production, while NS-398, an inhibitor for COX-2, had no significant effects (Fig. 2B). These findings strongly suggest that E-FABP may play a role as a cellular shuttle of AA and its metabolites, negatively modulating the IL-12 production in DCs.

Thus, we studied the phenotype of DCs deficient for E-FABP, especially focusing on the production of cytokines. When first examined whether E-FABP deficiency in splenic DCs may impact the DC population and/or subset using FACS, no differences in the population density of splenic DCs with high expression of CD11c and MHC class II were discerned between wild-type and E-FABP-null mutant mice (Fig. 3A). Furthermore, no significant differences of the population density of $CD8\alpha^{+}$ to $CD8\alpha^{-}$ DC subsets were found between E-FABP-null mutant and wild-type counterpart (Fig. 3B). These features indicate that the development of splenic DCs in E-FABP-null mutant mice appears to be intact. In addition, no histological abnormalities were apparent in the spleen from E-FABP-null mutant mice as compared with wild-type mice (data not shown).

Next, we examined whether E-FABP deficiency affected the cytokine production in splenic DCs. In ELISA, the amount of IL-12p70 in culture media containing DCs under appropriate stimuli [35,36] was markedly enhanced in E-FABP-null mutants compared with wild-type counterparts: approx. 1.5-fold increase by LPS/anti-CD40/IFN-γ, LPS/anti-CD40/IL-4, and LPS/anti-CD40/IFN-γ/IL-4 stimuli; 2.5-fold increase by LPS/IL-4 stimulus (Fig. 4A). In contrast to IL-12, no significant differences were detected in amounts of IL-10 or TNF-α between the two groups (Fig. 4B). In quantitative RT-PCR, the expression of mRNA for IL-12p35 was elevated at 6 h after LPS stimulation in E-FABP-null DCs as compared with wild-type counterparts, while no significant difference was detected at the level of mRNA for IL-12p40 between the two groups (Fig. 4C).

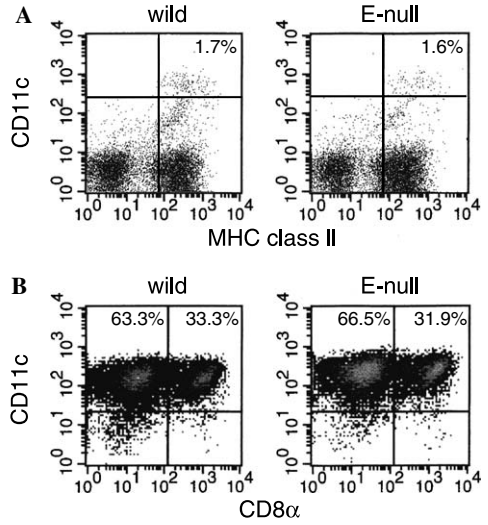

Fig. 3. Population density of DC subsets expressing CD11c, MHC class II, and CD8α examined by FACS in wild-type and E-FABP-null mutant mice. (A) No significant differences in population density of DCs with high expression of CD11c and major histocompatibility complex (MHC) class II in the spleen between wild-type (left) and E-FABP-null mutant mice (right). (B) No significant differences in population density of $CD8\alpha^+$ and $CD8\alpha^-$ DC subsets in isolated splenic DCs showed no significant differences between wild-type (left) and E-FABP-null mutant mice (right). Data are representative of two independent experiments.

Then we examined the levels of phosphorylated forms of p38MAPK (p-p38) and IκB-α (p-IκB-α), which are known to be critical signaling molecules for IL-12 production after LPS stimulation. The levels of both p-p38 and p-IκB-α were significantly increased in E-FABP-null DCs as compared with wild-type counterparts at 30 min after LPS stimulation (Fig. 5A and B).

Finally, we examined whether enhanced IL-12 production of E-FABP-null DCs would result in the enhanced antigen presentation to T-cells in the present allogenic mixed lymphocyte reaction (MLR). In MLR in which $CD4^+$ T cells purified from the spleen of wild BALB/c mice were co-cultured with splenic DCs from E-FABP-null mutant mice or wild-type counterpart, the amount of IFN-γ was significantly higher in the medium for splenic DCs from E-FABP-null mutants than wild-type mice (Fig. 6).

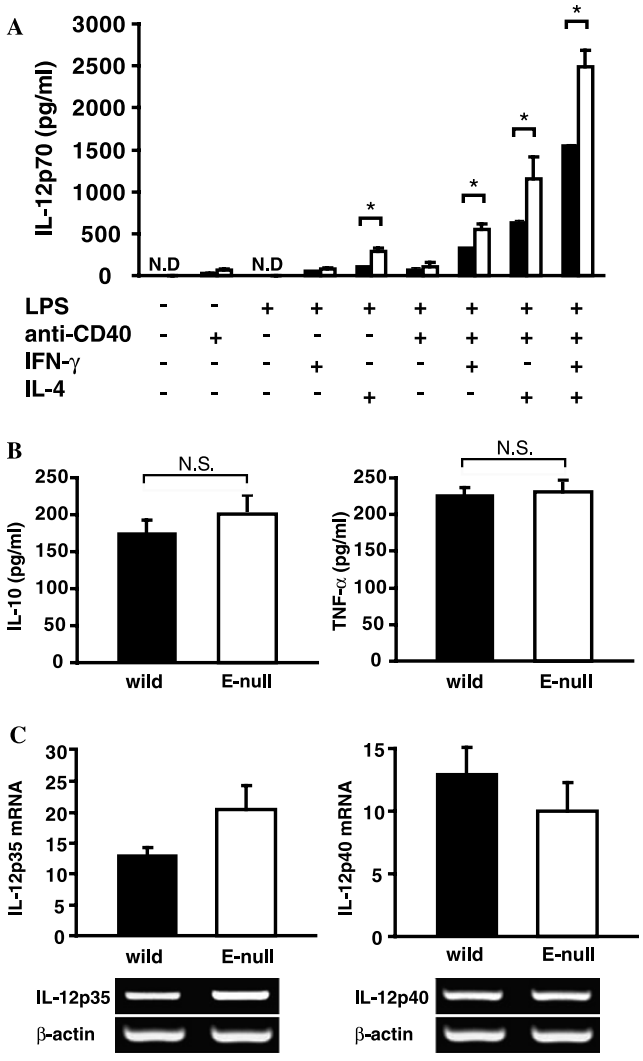

Fig. 4. Comparison of IL-12 production after LPS stimulation between wild-type and E-FABP-null mutant splenic DCs examined by ELISA (A,B) and RT-PCR (C). (A) A marked increase in amount of IL-12p70 in media containing DCs 24 h after appropriate stimuli in E-FABP-null mutants (open column) as compared with wild-type counterparts (filled column). (B) No significant differences in amounts of IL-10 (left) or TNF-α (right) in media containing DCs at 24 h after LPS/anti-CD40/IL-4 stimuli between wild-type (filled column) and E-FABP-null mice (open column). (C) A significant increase in the expression of mRNA for IL-12p35 (left graph) at 6 h after LPS/anti-CD40/IL-4 stimuli in E-FABP-null DCs (open column) compared with wild-type counterparts (filled column), in contrast to the absence of significant difference in levels of mRNA for IL-12p40 (right graph). Data are shown as means ± SEM and representative of two independent experiments $^*P < 0.05$, significantly different from the mean value of the corresponding control. N.D., not detected.

Discussion

In the present study, splenic DCs, in which E-FABP is exclusively expressed among FABP family in accord with our previous in vivo finding [28], are shown to markedly increase the production of IL-12p70 after LPS stimulation in E-FABP-null mutant, indicating the inhibitory effect of E-FABP on the production of IL-12 in splenic DCs.

Several molecules have so far been shown to regulate IL-12 production negatively during the immune responses: among them, IL-10, a major cytokine produced in B cells as well as DCs themselves, has been shown to negatively regulate the production of IL-12 in DCs [37]. In this study, no significant differences in the number of B-cells in the spleen or IL-10 production in splenic DCs are discerned between E-FABP-null mutant and wild-type mice. It is thus reasonable to conclude that E-FABP deficiency in splenic DCs contributes cell-autonomously to the increase of their IL-12 production. In addition, mechanisms of DC exhaustion [38] and DC paralysis [39], which have recently been shown to occur for alteration of cytokine production in

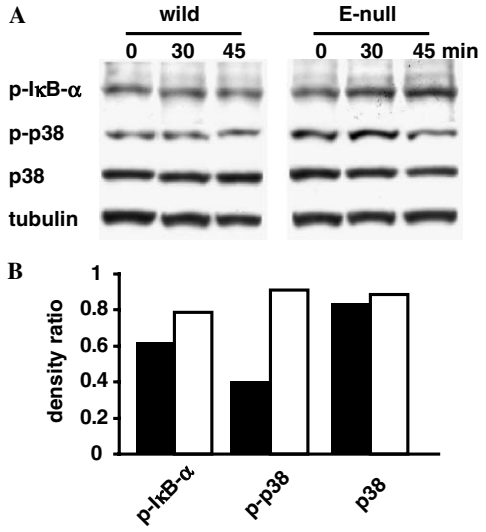

Fig. 5. Changes in expression of phosphorylated form of IκB-α and that of p38MAPK after LPS stimulation in wild-type and E-FABP-null DCs in immunoblot analysis. (A) Phosphorylated forms of both p38MAPK (p-p38) and of IκB-α (p-IκB-α) are significantly increased in amount E-FABP-null DCs as compared with wild-type counterparts at 30 min after LPS stimulation, while no significant changes are observed in the total p38 expression. (B) The increases in expression of p-IκB-α and p-p38 in E-FABP-null DCs are clearly demonstrated in the density ratio of immunoblot bands corrected by tubulin. Density ratio (p-p38/tublin): wild-type (filled column) vs E-FABP-null (open column) = 0.38 vs 0.92; p-IκB-α/tubulin: wild-type vs E-FABP null = 0.61 vs 0.78. Data are representative of two independent experiments.

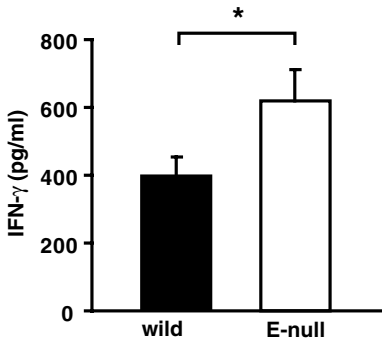

Fig. 6. Comparison of IFN-γ production between wild-type and E-FABP-null DCs in the mixed lymphocyte reaction. The amount of IFN-γ is significantly higher in the medium for E-FABP-null DCs (open column) than wild-type counterpart (filled column). Data are shown as means ± SEM and representative of two independent experiments $^{*}P < 0.05$, significantly different from the mean value of the corresponding control.

DCs, are not likely to underlie the present phenomena, because the present study showed that the kinetic properties of IL-12 production were not affected in E-FABP-null DCs as compared with wild-type counterparts (data not shown).

With regard to the molecular mechanisms for E-FABP to regulate the production of IL-12 in DCs, a recent study of A-FABP by Makowski et al. [16] on macrophages should be noted. Their study has shown that A-FABP, also called ap2, is intimately involved in the modulation of cytokine production in macrophages: the ablation of A-FABP in macrophages results in decreased expression of IL-1α, IL-1β, IL-6, and IL-12p40 mRNAs. Although the physiological ligand of A-FABP in macrophages and the molecular mechanism underlying the modulation in production of the cytokines remain to be elucidated, the authors have proposed a hypothesis that A-FABP may exert the modulatory action on the cytokine production through controlling the availability of its ligands, fatty acids, and/or their metabolites, which were shown to act as positive or negative regulators of inflammatory responses in immune cells [40–42]. Indeed, we show in the present study that application of AA or COX-1 inhibitor to DCs under LPS stimulation alters the IL-12 production. In analogy to the hypothetical exertion of A-FABP, it is possible that E-FABP may also work as a regulator of IL-12 production in DCs through fatty acids or their metabolites.

There have been growing bodies of evidence for PUFAs to suppress the IL-12 production in bone marrow-derived DCs (BMDCs) of mice and mononuclear cell-derived DCs of human [15], and for PUFA feeding to inhibit selectively murine Th1 response [29]. Considering the preferable binding of E-FABP with PUFAs including AA [43] and a possible role of E-FABP as a cellular shuttle of these PUFAs to various cell organelles including the nucleus and plasma membranes, impaired intracellular transports of PUFAs and/or their active derivatives into nuclear receptors may occur in the present E-FABP-null splenic DCs. With regard to the possible nuclear receptors, it should be noted that E-FABP has been shown to enhance the transactivation of peroxisome proliferator-activated receptors (PPARs) by their ligands [44], and to bind with some of the identified PPAR ligands [45]. In support of this consideration, a recent study has shown that the application of PPARγ ligand inhibits the production of IL-12 in splenic DCs and controls the differentiation of Th1 cells in response to LPS [12,46]. According to the authors, such a negative regulation of IL-12 transcription by PPARs is supposed to be exerted not through their direct transcriptional control of IL-12, but through the ability of PPARs to antagonize the NF-κB signaling pathway by physical interaction of PPAR-ligand complex with NF-κBp65 [47], a key transcription factor for IL-12 transcription [48]. Furthermore, the activation of PPARs is capable of suppressing the activation of both c-Jun N-terminal kinase (JNK) and p38MAPK [49]. In the same token, the present study shows that AA, which is a ligand of PPARs, inhibits the production of IL-12p70 in splenic DCs, and that the phosphorylation of p38MAPK and IκB-α is enhanced in E-FABP-null DCs compared with wild-type counterparts.

In conclusion, E-FABP is a negative modulator of IL-12 production in splenic DCs and E-FABP-null mutant mice would be a useful model for studying function of DCs. Further elucidation of the molecular mechanism underlying the negative regulation of IL-12 transcription by E-FABP may provide a cue for understanding anti-inflammatory action of PUFAs.

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