

Regulation of Dendritic Cell Function and T Cell Priming by the Fatty Acid-Binding Protein aP2¹

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The fatty acid-binding protein (FABP) family consists of a number of conserved cytoplasmic proteins with roles in intracellular lipid transport, storage, and metabolism. Examination of a comprehensive leukocyte gene expression database revealed strong expression of the adipocyte FABP aP2 in human monocyte-derived dendritic cells (DCs). We isolated bone marrow-derived DC from aP2-deficient mice, and showed that expression of DC cytokines including IL-12 and TNF was significantly impaired in these cells. Degradation of I κ B α was also impaired in aP2-deficient DCs, indicative of reduced signaling through the I κ B kinase-NF- κ B pathway. The cytokine defect was selective because there was no effect on Ag uptake or expression of MHC class II, CD40, CD80, or CD86. In an MLR, aP2-deficient DCs stimulated markedly lower T cell proliferation and cytokine production than did wild-type DCs. Moreover, aP2-deficient mice immunized with keyhole limpet hemocyanin/CFA showed reduced production of IFN- γ by restimulated draining lymph node cells, suggesting a similar defect in DC function in vivo. Similarly, infection of aP2-deficient mice with the natural mouse pathogen ectromelia virus resulted in substantially lower production of IFN- γ by CD8⁺ T cells. Thus, FABP aP2 plays an important role in DC function and T cell priming, and provides an additional link between metabolic processes and the regulation of immune responses. *The Journal of Immunology*, 2006, 177: 7794–7801.

Dendritic cells (DCs)³ are potent APCs, and play an essential role in the activation of naive T cells. Although the picture is growing increasingly complex, a simplified scheme of DC function involves Ag uptake in the periphery followed by migration to local lymph nodes where the DCs activate T cells through provision of high levels of Ag/MHC and costimulatory signals (1–3). During this process, DCs undergo maturation, a complex cellular transition that includes up-regulation of MHC and costimulatory molecules, increased production of cytokines, chemokines, and other bioactive mediators, and down-regulation of Ag uptake. The end result of DC maturation is conversion of the DCs from a cell type specialized for Ag uptake to one specialized for T cell activation (2, 3). Numerous stimuli can mediate DC maturation, the best characterized being TLR ligands, and signals

such as CD40L delivered by T cells and innate lymphocytes (4, 5). In addition to their essential role in T cell priming, DCs are also involved in innate immunity through production of cytokines and other factors, and the activation of NK and NKT cells (2).

Lipids have broad and complex roles in cellular physiology, acting as metabolic substrates, structural components, bioactive mediators, and signaling intermediaries. Due to their hydrophobic nature, the transport and storage of lipids presents special challenges to cells. This is usually dealt with through interaction with proteins such as members of the fatty acid-binding protein (FABP) family. This family comprises a number of homologous cytoplasmic proteins with affinity for fatty acids and with distinct patterns of tissue expression. Although the precise mechanism of action of FABPs is not well-understood, emerging data indicate roles in uptake, transport, storage, and metabolism of lipids (6–8).

Macrophages express three different FABP family members, FABP3, FABP4 (aP2), and FABP5 (mal1) (9–11). Recently, specific roles for aP2 have been identified in macrophages. In macrophages, the expression of aP2 is regulated by peroxisome proliferator-activated receptor- γ (PPAR γ) agonists and oxidized low-density lipoprotein, and it is present at high levels in foam cells within atherosclerotic lesions (10, 12). aP2 deficiency provides considerable protection in the apoE^{-/-} mouse model of atherosclerosis, and this effect is mediated largely at the level of the macrophage (10). Two aP2-regulated pathways have been identified in macrophages that are likely to be involved in atherosclerosis. First, aP2 negatively regulates PPAR γ function (13). This leads to defects in cholesterol accumulation and foam cell differentiation, by affecting both CD36-mediated uptake of oxidized low-density lipoprotein, and ABCA1-mediated cholesterol efflux. Second, aP2-deficient macrophages have diminished NF- κ B activity, leading to a reduction in the production of proinflammatory mediators such as TNF- α , IL-6, and MCP-1 (13). The PPAR γ and I κ B kinase (IKK)-NF- κ B pathways are both highly dependent on lipid signals, and aP2 is thought to act by regulating availability of ligands for these, and other, signaling pathways (13).

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³ Abbreviations used in this paper: DC, dendritic cell; FABP, fatty acid-binding protein; PPAR, peroxisome proliferator-activated receptor; IKK, I κ B kinase; rm, recombinant murine; BMDC, bone marrow-derived DC; EV, ectromelia virus; WT, wild type; imDC, immature DC; KLH, keyhole limpet hemocyanin.

We have recently developed comprehensive gene expression profiles of essentially all the major leukocyte subsets (14–17). Due to the emerging roles of FABPs in inflammation, we probed their expression across the data set. Human monocyte-derived DCs expressed the FABPs aP2, mal1, and FABP3. This pattern of FABP expression is the same as that found in macrophages (9, 10). Based on the ability of aP2 to regulate PPAR γ and NF- κ B function in macrophages (13), and the important role these transcription factors play in DCs (18–21), we hypothesized that aP2 might modulate DC responses. In the present study, we show that aP2 regulates DC cytokine production, and as a consequence, it impacts the specific ability of DCs to activate naive T cells *in vitro* and *in vivo*.

Materials and Methods

Reagents and mice

LPS (*Escherichia coli* serotype 0111:B4) and FITC-conjugated dextran (FITC-dextran) were purchased from Sigma-Aldrich. FITC-conjugated OVA (FITC-OVA) was purchased from Molecular Probes. Recombinant mouse (rm) GM-CSF and rmIL-4 were obtained from PeproTech. All Abs were purchased from BD Pharmingen. Cell culture medium was RPMI 1640 (Invitrogen Life Technologies) supplemented with 10% (v/v) heat-inactivated FBS (HyClone), 100 U/ml penicillin (Invitrogen Life Technologies), 100 μ g/ml streptomycin (Invitrogen Life Technologies), 2 mM L-glutamine (Invitrogen Life Technologies), and 50 μ M 2-ME (Invitrogen Life Technologies). C57BL/6 and aP2-deficient mice (22) were bred under specific pathogen-free conditions at the Biological Testing Facility, Garvan Institute for Medical Research. For all experiments, mice were used at 8–12 wk of age.

Generation of bone marrow-derived DCs (BMDCs)

DCs were generated from mouse bone marrow cells as previously described (23), with minor modifications. Bone marrow cells were disaggregated by vigorous pipetting, and the cells were filtered through a 70- μ m nylon cell strainer (BD Biosciences) to remove debris. RBCs were lysed by incubating with RBC lysis buffer containing ammonium chloride (0.8% w/v) in distilled water for 2 min at room temperature. Cells were washed twice with culture medium and the remaining cells were depleted of erythroid precursors, T and B lymphocytes, NK cells, and granulocytes using MACS. Briefly, cells were incubated for 45 min with gentle mixing in a mixture of hybridoma supernatants containing mAbs against CD4 (clone GK1.5, rat IgG2b), CD8 (clone 53-6.7, rat IgG2b), Gr-1 (clone RB6-8C5, rat IgG2b), MHC class II (clone M5/115, rat IgG2b), and B220 (clone RA3-3A1, rat IgG2b) mAbs. After washing in ice-cold PBS containing 2 mM EDTA and 0.5% (v/v) FBS (MACS buffer), cells were incubated with magnetic bead-conjugated goat anti-rat IgG mAb (Miltenyi Biotec) and negative selection was performed through a paramagnetic column (LS column; Miltenyi Biotec), according to the manufacturer's instructions. During immunodepletion, all incubation and centrifugation steps were performed at 4°C.

Immunodepleted bone marrow cells were resuspended at 2×10^5 cells/ml in prewarmed culture medium containing 20 ng/ml rmGM-CSF and 20 ng/ml rmIL-4. Cells were plated on 100-mm bacteriological dishes (BD Biosciences) in 10 ml of culture medium per dish, and incubated at 37°C in 5% CO₂ humidified air (day 0). On day 3, a further 10 ml of culture medium containing 20 ng/ml rmGM-CSF and 20 ng/ml rmIL-4 were added to the culture. On days 5 and 7, half of the culture supernatant was removed and replaced with fresh medium. After 8 days of culture, nonadherent and loosely adherent cells, resembling mainly immature DCs (imDCs), were collected by gently pipetting off the culture supernatant. At this stage, cultures contained ~80% DC as assessed by CD11c staining.

Stimulation of BMDCs

For maturation of imDCs, nonadherent cells at day 8 of culture were resuspended at 1×10^6 cells/ml in fresh culture medium containing 10 ng/ml rmGM-CSF and 20 ng/ml rmIL-4, and were plated on a 100-mm tissue culture dish (BD Biosciences). DC maturation was induced by stimulating with 1 μ g/ml LPS for 24 h. Following stimulation, the nonadherent and loosely adherent cells were harvested, and DC maturation and purity was determined using flow cytometry. After maturation, DC purity was repeatedly determined to be ~85% in these cultures. Levels of IL-12 p40, IL-12 p70, and TNF in DC culture supernatants were measured using commercially available ELISA kits (BD Pharmingen).

Immunostaining and flow cytometric analysis

DC maturation and purity of cultured BMDCs was assessed using flow cytometry. Surface expression of DC markers was analyzed using anti-CD11c (clone HL3), -MHC class II (clone 2G9), -CD40 (clone 3/23), -CD80 (16-10A1), and -CD86 (clone GL1) mAbs. All incubation and centrifugation steps were performed at 4°C. Cells were first incubated with purified anti-CD16/CD32 (clone 2.4G2) mAb for 15 min on ice, followed by mAb staining for 30 min in the dark. Cells were washed twice, resuspended in 200 μ l of FACS buffer, and analyzed on a FACSCalibur flow cytometer (BD Pharmingen). For each sample, a total of 20,000 cells was counted and data analysis was performed using CellQuest Pro software (BD Pharmingen).

Western blot analysis of aP2 and I κ B

Whole cell protein extracts were isolated from white adipose tissue, thioglycolate-elicited macrophages, and resting wild-type (WT) and aP2^{-/-} DCs in lysis buffer containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 1% Triton X-100, 10% glycerol, 1.5 mM MgCl₂, 1 mM EGTA, 10 mM pyrophosphate, 100 mM NaF, and protease inhibitors (Sigma-Aldrich). Equal amounts (10 μ g) of protein were subjected to SDS-PAGE and Western blot was performed using a primary mAb against aP2 generated in our laboratory (24) and appropriate HRP-conjugated secondary Ab (Amersham Biosciences).

For analysis of the IKK-NF- κ B pathway, BMDCs were cultured as described and were stimulated with 1 μ g/ml LPS for the indicated times before harvesting cells and extracting cytoplasmic protein fractions (Nuclear Extract kit; Active Motif). SDS-PAGE was performed using 15 μ g of cytoplasmic extract from each sample. Western blots were performed using a rabbit anti-mouse I κ B α primary Ab (clone C-21; Santa Cruz Biotechnology) and appropriate HRP-conjugated secondary Ab (Amersham Biosciences). Bands were detected using ECL and quantified using densitometry.

Allelic MLR

Bone marrow-derived DC from WT or aP2^{-/-} mice were used as stimulator cells, and BALB/c splenocytes were used as responder cells. DCs were isolated at day 8 of culture and cell division was inactivated by incubating cells with 50 μ g/ml mitomycin C (Sigma-Aldrich) for 20 min at 37°C in the dark. After inactivation, stimulator cells were washed three times with cell culture medium to remove any excess mitomycin C. Titrated numbers of mitomycin C-treated DC were cocultured with single-cell suspensions of CD3⁺ BALB/c splenocytes (2×10^5 cells/well) in 96-well U-bottom plates (Nunc) for 72 h. For the last 18 h of culture, cells were incubated with 1 μ Ci/well [³H]thymidine (Amersham Biosciences). Cells were then harvested onto a glass fiber filter (Packard Instrument) with a Filtermate 196 harvester (Packard Instrument), and the filter was dried overnight at 37°C. A TopCount microplate liquid scintillation counter (Packard Instrument) was used to measure [³H]thymidine incorporation. Levels of IL-2 and IFN- γ in culture supernatants were measured using commercially available ELISA kits (BD Pharmingen).

cDNA synthesis and real-time RT-PCR

Total RNA was isolated from BMDCs using TRIzol reagent (Invitrogen Life Technologies), according to the manufacturer's instructions. Reverse transcription was performed using either Reverse-IT RTase (ABgene House) or alfalfa mosaic virus RTase (Promega), according to each manufacturer's instructions, from 100 ng or 2 μ g of starting total RNA, respectively. Following cDNA synthesis, semiquantitative real-time RT-PCR was performed using FastStart DNA Master SYBR Green I Reagent by LightCycler (Roche Molecular Biochemicals), as previously described (25). All primers were designed using Primer3 software, with the following sequences: aP2, 5'-GATGCCTTTGTGGGAACCTG-3' and 5'-GAATTCCACGCCAGTTTGA-3'; mal1, 5'-ACGGTCAAAACCGAGAGAC-3' and 5'-CCACGATCATCTTCCCATCC-3'; FABP3, 5'-CATCGAGAAGAACGGGGATA-3' and 5'-AGGATGAGTTTCCCGTCAAC-3'; IL-12p35, 5'-CACCCCTTGCCTCCTAAACC-3' and 5'-GGTTTGGTCCCGTGTGATGT-3'; IL-12 p40, 5'-TGGAGCACTCCCATTCCTA-3' and 5'-TGAGCTTGCACGCAGACATT-3'; TNF- α 5'-AGCCCCAGTCTGTATCCTT-3' and 5'-CCACTCTCCCTTGCAGAAC-3'; GAPDH, 5'-CTCATGACCACAGTCCATGC-3' and 5'-CACATTGGGGGTAGGAACAC-3'.

Keyhole limpet hemocyanin (KLH) immunization

Mice were immunized s.c. at the tail base with 100 μ g of KLH in CFA. After 7 days, the draining lymph nodes were collected and single-cell suspensions were prepared. The cells were cultured at 5×10^5 CD3⁺ cells/ml

in RPMI 1640 supplemented with 10% FCS and stimulated with 0–10 $\mu\text{g/ml}$ KLH for 72 h. The cells were pulsed with 1 $\mu\text{Ci/well}$ [^3H]thymidine for 14 h before harvesting as described for the MLR assay above.

Virus infection

Mice were infected with 10^3 PFU ectromelia virus (EV) Moscow strain. On day 5 following infection, the mice were killed; the spleen was collected for analysis of CD8⁺ T cell function by ELISPOT and tetramer staining, and the liver and lung was collected for determination of viral load. Virus titration was performed on B-SC-1 cells with an overlay of MEM-1% methylcellulose. After 4 days, plaques were visualized by staining with 0.1% crystal violet in 70% ethanol.

IFN- γ ELISPOT

Spleen cells were collected from infected mice, and peptide reactivity was determined by IFN- γ ELISPOT. ELISPOT plates (Millipore) were coated with 4 $\mu\text{g/ml}$ anti-IFN- γ Ab (R4-6A2; BD Pharmingen) in PBS at 4°C overnight. The plates were washed once and blocked with 10% RPMI 1640 FCS for 1 h. After one wash, 2×10^5 spleen cells were added to the ELISPOT plate together with 0.5 $\mu\text{g/ml}$ TSYKFESV peptide (26) for 24 h. The cells were removed and any residual cells were lysed by addition of ice-cold H₂O. After extensive washing with PBS-0.5% Tween 20, 0.25 $\mu\text{g/ml}$ biotinylated anti-IFN- γ Ab (XMG1.2; BD Pharmingen) in PBS-1% BSA was added. After a 2-h incubation at room temperature, the plates were washed and alkaline phosphatase-conjugated streptavidin (Amersham Biosciences) was added for a further 90-min incubation. The plates were washed and color was developed for 15 min by addition of 5-bromo-4-chloro 3-indolylphosphate/nitro blue tetrazolium alkaline phosphatase substrate (Sigma-Aldrich). Spots were counted using an automated plate reader.

Tetramer staining

Tetramer staining was used to identify CD8⁺ T cells with specificity for the H2-K^b-restricted EV epitope TSYKFESV (26). PE-labeled tetramers were prepared by the Biomolecular Resource Facility (John Curtin School of Medical Research, Canberra, Australia). Spleen cells from EV-infected mice were stained with tetramer and CD8-FITC for 45 min at room temperature. The cells were analyzed on a FACSCalibur flow cytometer, and analyzed using CellQuest software (BD Biosciences).

Results

FABP expression across multiple leukocyte subsets

We have developed a library of gene profiling data from all the major human leukocyte subsets (14, 15). Because recent data suggests that FABPs are involved in inflammatory signaling pathways (27), we mined our array dataset for expression of FABP family members across all leukocyte subsets (Fig. 1). mal1 (FABP5) mRNA was widely expressed across numerous leukocyte subsets. aP2 (FABP4) and FABP3 expression was largely restricted to macrophages and myeloid DC, and little or no expression of other FABP family members was identified across the range of leukocyte subsets profiled. In macrophages, aP2 regulates the activity of PPAR γ - and NF- κ B-signaling pathways, most likely by regulating availability of key lipid signaling intermediaries (13). Because PPAR γ and NF- κ B play key roles in DC differentiation and maturation (18–21), we focused our studies on the role of aP2 in DC function. It is interesting to note that plasmacytoid DC expressed mal1 but not aP2 or FABP3 (Fig. 1), suggesting that fatty acid-binding mechanisms in this cell type are distinct from those in conventional myeloid DC.

FABP mRNA expression increases during BMDC differentiation

The DCs used in Fig. 1 were derived from human monocytes after differentiation in the presence of GM-CSF and IL-4. Monocytes express little or no aP2 (11), indicating that aP2 expression increases during the course of DC differentiation. We hypothesized that aP2 might contribute to DC differentiation. To test this, we adopted the murine system due to the availability of aP2-deficient mice. Initially, we monitored expression of aP2 and other FABPs during the differentiation of purified bone marrow precursors to

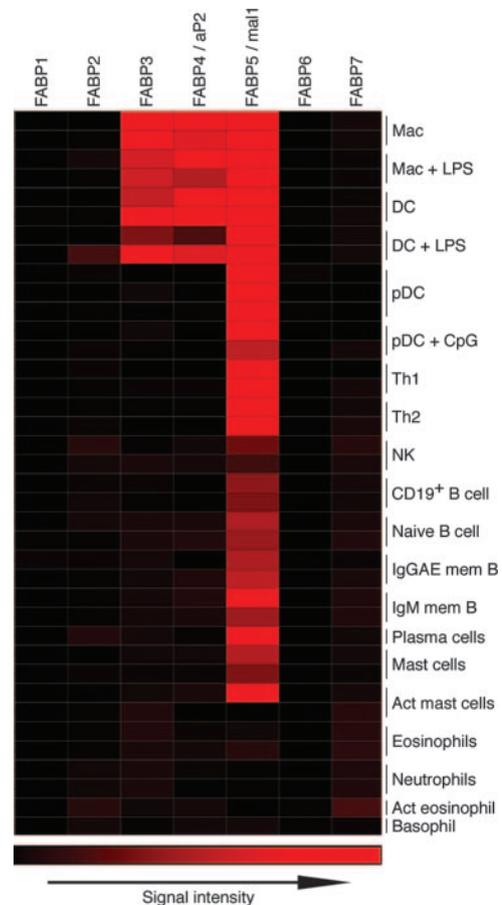


FIGURE 1. Selective expression of aP2 by DC and macrophages. cRNA was generated from a number of purified human leukocyte cell types and gene expression profiles were generated using Affymetrix U133A+B Gene Chips (14, 15). Data were analyzed using SpotFire software. Members of the FABP family are designated FABP1–7; aP2 and mal1 are indicated by FABP4 and FABP5, respectively. Mac, macrophage; pDC, plasmacytoid DC; mem B, memory B cell; Act, activated.

BMDCs. aP2 expression showed little change during the first 2 days of culture, but increased dramatically between days 2 and 4 (Fig. 2A), a period shortly preceding the appearance of significant numbers of CD11c⁺ BMDCs (Fig. 2B). The expression of aP2 protein in differentiated BMDCs was confirmed by Western blotting, with levels similar to macrophages, but markedly lower than adipose tissue (Fig. 2C). Of the other FABPs, mal1 mRNA also showed a substantial increase during differentiation, while levels of FABP3 showed little change.

aP2 is not required for differentiation of BMDC

To test the involvement of aP2 in DC differentiation, we established cultures from aP2^{-/-} and WT bone marrow cells and monitored the course of DC differentiation. At the end of the 8-day culture period, there was no difference in the percentage or total number of CD11c⁺ DCs (data not shown). Thus, aP2 is not essential for DC differentiation. There is considerable functional overlap between some members of the FABP family, and in some instances, one FABP can compensate for the absence of another (22, 28, 29). However, there was no evidence for compensation in DCs, as the mRNA levels of mal1 and FABP3 in the aP2^{-/-} DCs were no different to WT (Fig. 2D).

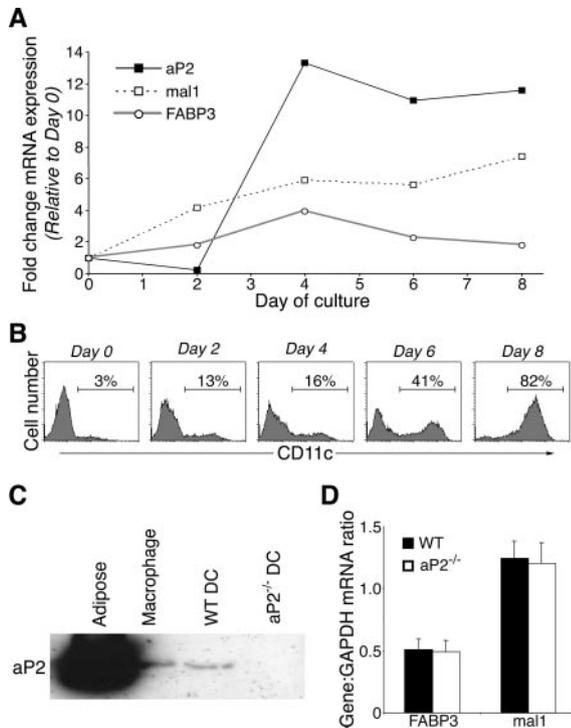


FIGURE 2. aP2 and mal1 are expressed during differentiation of BMDCs. DCs were cultured from bone marrow precursors as described in *Materials and Methods*. *A*, RNA was isolated at indicated time points after initiation of culture, and relative mRNA expression of aP2, mal1, and FABP3 was determined using quantitative real-time RT-PCR. Values indicate fold change in mRNA expression compared with day 0 levels, and are the mean of two independent cultures. *B*, Cells were harvested at indicated time points, and the percentage of CD11c⁺ DCs was determined using flow cytometry. *C*, Western blot showing aP2 expression in mouse white adipose tissue, thioglycolate-elicited macrophages, and BMDC from WT and aP2^{-/-} mice. *D*, Expression of FABP3 and mal1 mRNA in WT and aP2^{-/-} BMDC at day 8 of culture, measured using real-time PCR and normalized to GAPDH. Data are mean \pm SEM from four independent experiments.

Cytokine production, but not the expression of costimulatory molecules, is reduced in aP2-deficient BMDCs

At day 8 of culture, BMDCs are at an “immature” stage, characterized by high uptake of Ag and relatively low expression of costimulatory molecules. Following activation, for example through TLR signaling, BMDCs undergo a major phenotypic change leading to increased expression of costimulatory molecules and production of cytokines and chemokines. In response to TLR4 signaling, there was a significant increase in aP2 mRNA in BMDCs (Fig. 3A), suggesting that aP2 might be involved in the function of “mature” DCs. To test this, we conducted a systematic analysis of major DC functional parameters regulated by TLR4 signaling, namely Ag uptake, expression of MHC and costimulatory molecules, and production of cytokines and chemokines.

DC cytokine production plays an integral role in the ability of these cells to activate naive T cells. imDCs do not produce detectable amounts of cytokines such as IL-12 and TNF- α , but produce large amounts following stimulation with LPS. aP2^{-/-} BMDC produced substantially lower amounts of IL-12 p40, IL-12 p70, and TNF- α (Fig. 3, B–D). The expression of IL-12 p40 mRNA was also significantly reduced, indicating a possible role for aP2 in the transcriptional regulation of IL-12 (Fig. 3E). In contrast, TNF mRNA expression was the same in WT and aP2^{-/-} BMDCs (Fig.

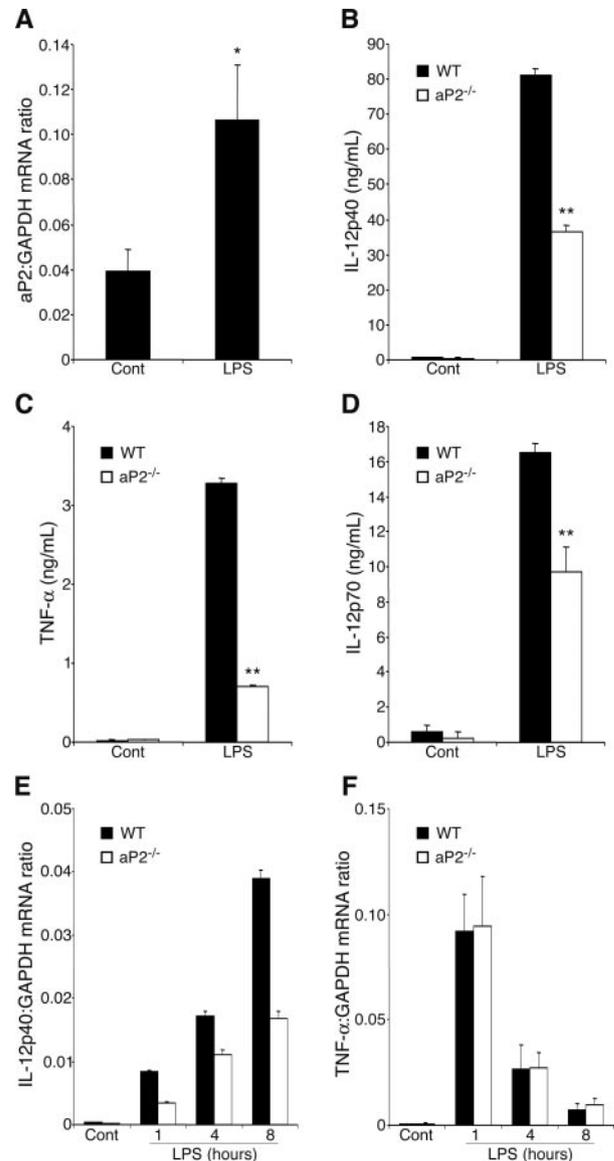


FIGURE 3. LPS-induced cytokine production is impaired in aP2-deficient DCs. *A*, imDCs were stimulated with 1 μ g/ml LPS and RNA was isolated after 24 h. Relative aP2 mRNA expression was determined using semiquantitative real-time RT-PCR, normalized to GAPDH expression levels. Data shown represent the mean \pm SEM of three independent experiments. *, $p < 0.05$ using a two-tailed Student *t* test. *B–D*, WT and aP2^{-/-} DCs were stimulated with 1 μ g/ml LPS for 24 h and (*B*) IL-12p40, (*C*) IL-12p70, and (*D*) TNF- α in culture supernatants was determined by ELISA. Data are representative of two independent experiments. **, $p < 0.01$ using a two-tailed Student *t* test. *E* and *F*, cDNA was generated from DC stimulated with LPS for 0, 1, 4, or 8 h, and relative mRNA expression of IL-12 p40 and TNF- α was determined by quantitative real-time RT-PCR. For IL-12 p40, data are mean \pm SEM of triplicate PCR assays, and are representative of three independent experiments. For TNF, data represent the mean \pm SEM of three independent experiments.

3F), suggesting that aP2 regulates TNF release by a posttranslational mechanism in these cells.

To examine the requirement for aP2 in the function of mature DCs, regulation of a number of key DC cell surface markers was examined in control and LPS-stimulated BMDC. There was no difference between WT and aP2^{-/-} DCs in expression of MHC II, CD40, CD80, CD86, CD36, CD1d, and ICAM-1, both before and after LPS stimulation (data not shown).

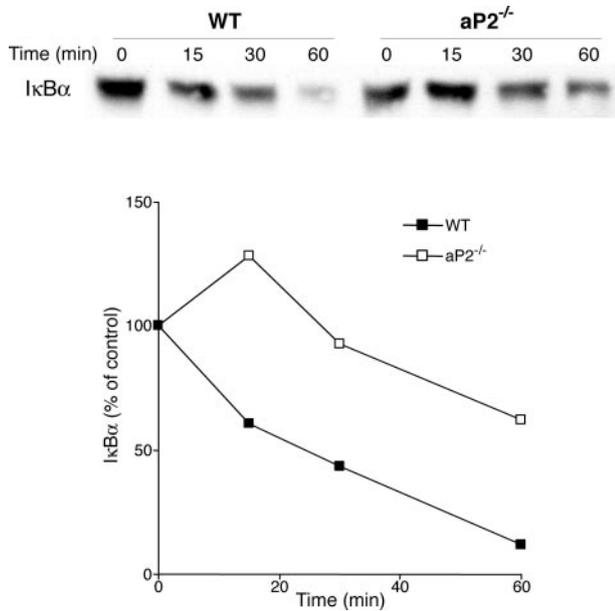


FIGURE 4. Reduced LPS-mediated IκBα degradation in aP2^{-/-} BMDCs. WT and aP2^{-/-} BMDCs were stimulated for the indicated times with 1 μg/ml LPS. Lysates were prepared and levels of IκBα were determined by Western blotting. Lower panel shows densitometry values for the upper panel. Data are representative of two independent experiments.

DCs use two distinct mechanisms to capture Ag, macropinocytosis and receptor-mediated endocytosis. Macropinocytosis is a constitutive process in DCs (30), and can be assessed by measuring uptake of FITC-OVA. In contrast, FITC-dextran can be used to measure mannose receptor-mediated endocytosis, although it is also taken up by macropinocytosis. In immature BMDC, Ag uptake by either mechanism was not regulated by aP2 (data not shown). Similarly, aP2 did not affect the ability of BMDCs to down-regulate Ag uptake in response to LPS (data not shown).

Reduced degradation of IκBα in aP2-deficient BMDCs

To identify potential biochemical mechanisms underlying the reduction in cytokine production in aP2-deficient DCs, we focused on the NF-κB pathway. NF-κB regulates production of IL-12 in DCs (31), and previous studies have described regulation of the IKK-NF-κB pathway by aP2 in macrophages (13). In response to stimuli such as LPS, IKK becomes activated leading to phosphorylation and degradation of IκB subunits, which in turn allows migration of the NF-κB dimer to the nucleus, leading to transcriptional activation (32). In WT DCs, substantial IκBα degradation occurred within 15 min after LPS stimulation, and IκBα levels were reduced by 80–90% at 60 min (Fig. 4). In contrast, in aP2^{-/-} DCs, no IκBα degradation was observed by 15 min, and the levels were reduced by <50% at 60 min (Fig. 4). Thus, these data are consistent with a role for aP2 in the regulation of the IKK-NF-κB pathway, and suggest that perturbation of this pathway contributes to the cytokine defect in aP2^{-/-} BMDCs.

aP2 deficiency impairs the ability of DCs to activate T cells in MLR

The principal function of DCs is thought to be Ag presentation and activation of T cells. An MLR assay was used to test the requirement for aP2 in DCs for T cell activation. Using WT and aP2^{-/-} DC as stimulators, the proliferation of BALB/c spleen cells was measured. A marked reduction in proliferation was observed at most DC concentrations (Fig. 5A). The major factor driving T cell

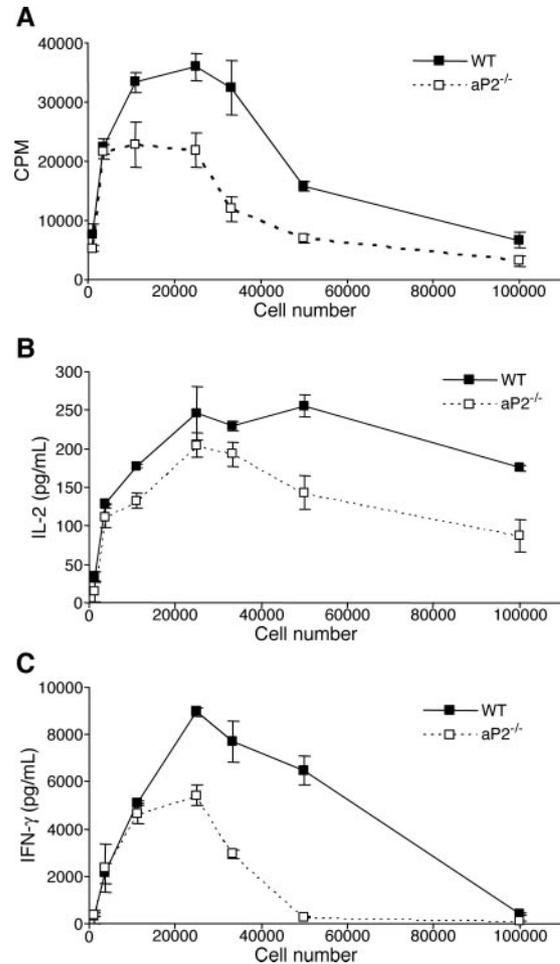


FIGURE 5. aP2 deficiency in DCs results in impaired T cell priming in vitro. Allogeneic MLR was performed using titrated numbers of mitomycin C-treated WT or aP2^{-/-} DCs and cocultured with CD3⁺ BALB/c splenocytes (2×10^5) for 72 h. A, For the last 18 h of culture, 1 μCi/well [³H]thymidine was added and cellular proliferation was determined. B and C, Levels of IFN-γ and IL-2 in culture supernatants were determined by ELISA. Data shown represent mean ± SEM and are representative of three independent experiments.

proliferation in the MLR is IL-2 production by the T cells. T cells responding to aP2^{-/-} DCs produced markedly less IL-2 (Fig. 5B). The effector cytokine IFN-γ is produced at high levels in an MLR, and the production of this cytokine was markedly reduced in cultures stimulated by aP2^{-/-} BMDC (Fig. 5C), consistent with the reduced IL-12 production by these cells.

It is interesting to note that the response of T cells to low numbers of DCs was not affected by aP2. We speculate that at low DC densities, T cell activation is largely driven by cell surface expression of MHC and costimulatory molecules, which are equivalent in WT and aP2^{-/-} BMDCs (data not shown). At higher DC densities, DC-derived cytokines also contribute to T cell activation, conditions in which the effects of aP2 deficiency would be expected to become apparent. In support of this, DCs rendered IL-12 deficient by RNA interference had impaired ability to stimulate an MLR at high, but not at low, DC densities (33).

Impairment of T cell priming in aP2^{-/-} mice

DCs are thought to be essential for priming naive T cells. To test the requirements for aP2 in T cell priming, WT and aP2^{-/-} mice were immunized s.c with KLH in CFA, and recall responses in draining lymph node cells were measured 7 days later. There was

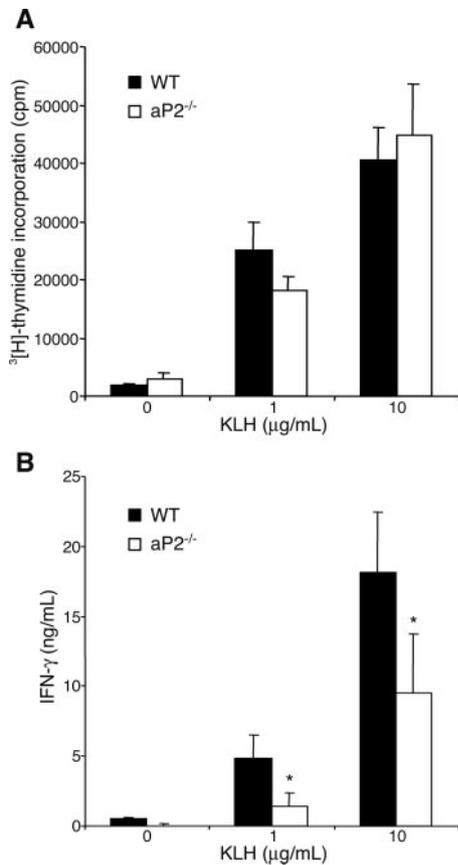


FIGURE 6. Impaired T cell priming in aP2^{-/-} mice. Mice were immunized s.c with KLH/CFA. After 7 days, the draining lymph node cells were collected and cultured for 72 h with indicated concentrations of KLH. Proliferation (A) and IFN-γ production (B) were measured. A value of $p < 0.05$ using a two-way factorial ANOVA on log₁₀-transformed values.

no difference in Ag-specific T cell proliferation in WT and aP2^{-/-} mice (Fig. 6A). However, the production of IFN-γ was substantially lower in activated cells from aP2^{-/-} mice (Fig. 6B). These data indicate a clear requirement for aP2 for differentiation of naive T cells into activated effector T cells. The finding that KLH-specific proliferation was unimpaired in aP2^{-/-} mice suggests that DC migration from the site of immunization is not affected by aP2 deficiency. This was confirmed in experiments analyzing migration of FITC-positive, CD11c-positive cells to the draining lymph node from the skin of mice painted with FITC. In this experimental system, there was no difference in DC migration between WT and aP2^{-/-} mice (data not shown). Finally, the unimpaired KLH-specific proliferation contrasts with the results obtained in the MLR, reflecting undefined differences between these assays.

Regulation of CD8 T cell effector function by aP2

A virus infection model was adopted as a rigorous test of the requirement for aP2 in T cell priming and effector function in vivo. Mice were infected with EV, a natural mouse pathogen, and CD8⁺ T cell function in the spleen was measured 5 days later. The percentage of CD8⁺ T cells with specificity for the immunodominant epitope TSYKFESV was marginally increased in the aP2^{-/-} mice (Fig. 7A). In contrast, TSYKFESV-specific IFN-γ production, measured by ELSIPOT, was completely defective in spleen cells from aP2^{-/-} mice (Fig. 7B). Virus load was significantly increased in lungs of aP2^{-/-} mice (Fig. 7C), with a similar, but not significant, trend in the liver (Fig. 7C). The increased viral load is most likely directly related

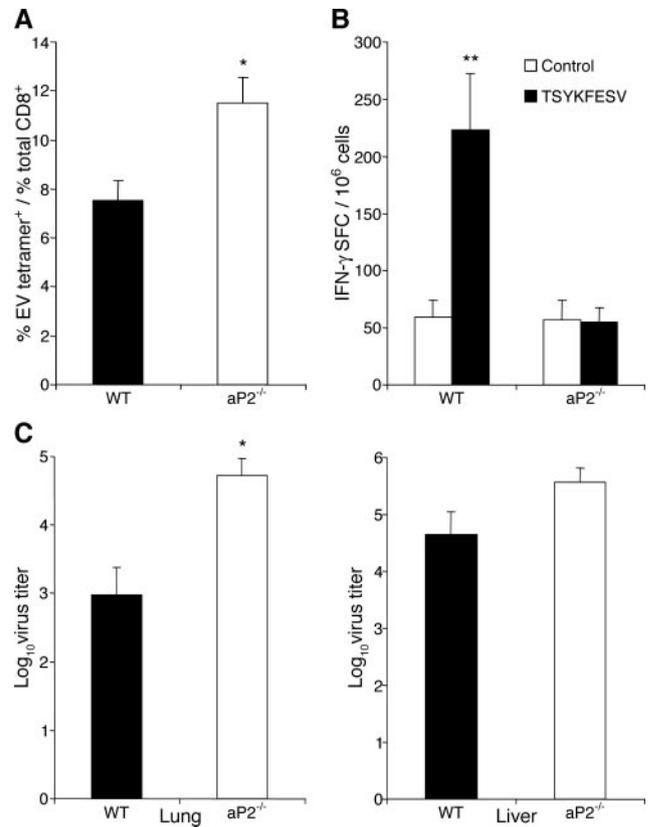


FIGURE 7. Impaired IFN-γ production and delayed virus clearance in aP2^{-/-} mice. WT and aP2^{-/-} mice were infected in the footpad with 10³ PFU EV. On day 5 following infection, the mice were killed and their spleens collected for (A) identification of TSYKFESV-specific CD8⁺ T cells and (B) enumeration of TSYKFESV-specific, IFN-γ-producing cells. Viral load in the (C, left panel) lung and (C, right panel) liver was measured. Data are mean ± SEM, $n = 5$ mice/group. *, $p < 0.05$, **, $p < 0.01$ using a Student two-tailed t test.

to impaired IFN-γ production because this cytokine is an essential effector in immune defense against EV (34).

Discussion

To facilitate identification of novel inflammatory disease gene targets, we conducted a comprehensive gene expression analysis in all the major human leukocyte subsets (14, 15). By mining the transcript database, strong expression of the adipocyte FABP aP2 was identified in human monocyte-derived DCs. In this study, we describe an essential role for aP2 in DC function; it regulates cytokine production and the ability of DCs to activate T cells, both in vitro and in vivo.

FABPs are a family of cytoplasmic proteins with affinity for hydrophobic ligands. Despite 30 years of research, it has proved difficult to define the cellular and molecular activity of FABPs. On the cellular level, they are thought to participate in lipid uptake, transport, storage and metabolism (6, 7). Cellular levels of FABPs generally reflect intracellular lipid levels, and in cells such as adipocytes, hepatocytes, and cardiac myocytes, FABPs constitute up to 1–5% of all cytosolic proteins (6). In contrast, we found that aP2 levels in macrophages and DCs were several orders of magnitude lower than in adipose tissue. Similarly, macrophages and DCs express ~10,000-fold less aP2 mRNA than adipocytes (24). Thus, there are likely to be differences in the role of FABPs in DCs and macrophages compared with cell types with higher intracellular lipid loads. Recent evidence implicates FABPs in the control of

intracellular signaling pathways such as PPAR γ and NF- κ B, most likely by regulating availability of lipid ligands (13, 35–37). Indeed, our study found that degradation of I κ B α was impaired in aP2-deficient DCs, a mechanism likely to contribute to the impaired immune function of these cells (31). The mechanism by which aP2 regulates NF- κ B signaling is not known, although there is considerable evidence that fatty acids can regulate the activity of this pathway (38–40). Given the highly pleiotropic role of fatty acids in cellular physiology, it is probable that additional signaling pathways and/or distinct mechanisms of action also contribute to the phenotype of aP2-deficient DCs.

We identified considerable overlap between macrophages and BMDCs in both FABP expression and function. Both cell types express the same FABPs, namely aP2, mal1, and FABP3 (9, 41). aP2 expression is enhanced in macrophages (13) and DCs following LPS stimulation, and in both cell types it regulates inflammatory responses. In addition, we found that aP2 regulates the ability of DCs to activate naive T cells, a function that is specific to DCs. It is noteworthy that in aP2-deficient mice, IFN- γ production by T cells was markedly reduced, but T cell proliferation was largely unaffected. We hypothesize that the ability of aP2-deficient BMDCs to stimulate expansion of Ag-specific T cells is unimpaired due to their expression of normal levels of costimulatory molecules. In contrast, aP2-dependent DC functions such as IL-12 production are required to induce full differentiation into IFN- γ -producing effector cells.

We have recently discovered that aP2 $^{-/-}$ mice are protected from allergic airway inflammation through the activity of aP2 in airway epithelial cells (24). In this experimental system, which is characterized by a highly polarized Th2 response, there was no difference in initial T cell priming between WT and aP2 $^{-/-}$ mice. Bone marrow chimera experiments confirmed that absence of aP2 in hemopoietic cells did not contribute to the protection from allergic airway inflammation. These results further highlight the selective activity of DC-expressed aP2 in regulating the development of IFN- γ -producing T cells.

Members of the FABP family show significant overlap in ligand affinity and, presumably, in function. In a number of FABP knockout models, functional compensation by other FABPs (22) or related molecules (42) has been described. For example, in aP2 $^{-/-}$ mice, a compensatory up-regulation of mal1 mRNA expression was observed in adipose tissue, which was thought to underlie the relatively mild metabolic phenotype of these mice (22). Although aP2 comprises 1–5% of total adipocyte protein, baseline metabolism in aP2 $^{-/-}$ mice is relative normal, and only in models of obesity does a metabolic phenotype emerge. In both diet and genetic models of obesity, aP2 $^{-/-}$ mice are largely protected from the development of insulin resistance (22, 43). The importance of the functional compensation by mal1 in aP2 $^{-/-}$ mice was subsequently confirmed by analysis of combined aP2/mal1 $^{-/-}$ mice which have a much stronger metabolic phenotype than mice deficient in either aP2 or mal1 alone (28). In aP2 $^{-/-}$ DCs, however, we did not observe compensatory up-regulation of transcripts for the other two FABP family members expressed by DCs, mal1, and FABP3. Similarly, aP2 deficiency in macrophages did not affect expression of mal1 (10). Our data do not exclude compensation mediated by posttranscriptional regulation of mal1 or FABP3, nor do they exclude effects mediated by other lipid-binding proteins. It will be very informative to investigate the phenotype of DCs deficient in multiple FABPs. However, the initial description of mal1 $^{-/-}$ splenic DCs, which produce elevated levels of IL-12 p70 in response to LPS (44), suggests that mal1 and aP2 have different roles in regulating DC responses.

In the apoE $^{-/-}$ mouse model of atherosclerosis, aP2 deficiency provides substantial protection (10). Using bone marrow chimeras, bone marrow-derived cells were identified as the major contributors to the atherosclerosis phenotype in aP2 $^{-/-}$ mice. This experiment most likely reflects macrophage activity, because aP2 regulates cytokine production and cholesterol metabolism in this cell, both of which are intimately involved in the pathogenesis of atherosclerosis (45). However, there is clear evidence for an involvement of both T cells and DCs in atherosclerosis (45), and we speculate that impaired DC function might also contribute to the protection of aP2 $^{-/-}$ mice in the ApoE mouse model.

There is a growing awareness of the intimate, bidirectional link between inflammatory and metabolic systems (46). FABPs are thought to be at the interface of these two systems, regulating, and perhaps integrating, both inflammatory and metabolic signals (8). For example, it is well-established that dietary lipid intake can have a major effect on immune function and the development of inflammatory disease (47, 48). The molecular mechanisms underlying this phenomenon are largely uncharacterized, but are thought to include effects on membrane composition, eicosanoid production, and regulation of transcription (49–51). aP2 binds many common dietary long chain fatty acids (52), and we speculate that FABPs may contribute to pathways linking dietary lipids with inflammatory disease and immunological responsiveness. Due to their key role in T cell activation, DCs would be very likely participants in such a scenario.

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Disclosures

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