PI3K p85α Subunit-deficient Macrophages Protect Mice from Acute Colitis due to the Enhancement of IL-10 Production

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We investigated the role of the PI3K p85α subunit in the development of acute colitis with a focus on intestinal macrophages. Experimental acute colitis was induced using 3% dextran sulfate sodium (DSS) in drinking water for 7 days. The severity of DSS-induced acute colitis was significantly attenuated in p85α hetero-deficient (p85α+/−) mice compared with WT mice. The expression of proinflammatory mediators in intestinal macrophages isolated from the inflamed colonic mucosa was significantly suppressed in p85α+/− colitis mice compared with WT colitis mice. Interestingly, we found that bone marrow-derived macrophages (BMDMs) from p85α+/− mice produced a significantly higher amount of IL-10 than BMDMs from WT mice. The adoptive transfer of p85α+/− BMDMs, but not WT BMDMs, significantly improved the severity in WT colitis mice, and this effect was reversed by anti-IL-10 antibody. Furthermore, the expression of IL-10 in the intestinal macrophages of p85α+/− normal colonic mucosa was significantly higher than that in the intestinal macrophages of WT normal colonic mucosa. The present results demonstrate that p85α+/− mice exhibit a reduced susceptibility to DSS-induced acute colitis. Our study suggests that a deficiency of PI3K p85α enhances the production of IL-10 in intestinal macrophages, thereby suppressing the development of DSS-induced acute colitis.

Inflammatory bowel disease (IBD), Crohn’s disease and ulcerative colitis are chronic inflammatory disorders. IBD has long been recognized to have a genetic basis, and likely involves a response of the immune system to some environmental agents. Abnormalities of intestinal innate immune functions and their relationship to the microbiota have been identified as key properties that characterize the immunogenetic profile of IBD and animal IBD models. PI3Ks have important functions in the innate immune system. Class IA PI3Ks are a family of heterodimeric enzymes consisting of a regulatory subunit (p85α, p55α, p50α, p85β or p55γ) and a catalytic subunit (p110α, p110β or p110δ). PI3K p85α is the most abundantly expressed among the regulatory subunits and is crucial for the development and functions of various innate immune cells such as dendritic cells and macrophages and mast cells. Thus, p85α-deficient mice showed impaired bacterial clearance in response to acute septic peritonitis and failed to develop a food allergy, which were attributed to a deficiency of mast cells in the intestine. However, the role of the p85α subunit in IBD remains unclear, although several reports have revealed that PI3K plays important roles in the pathogenesis of colitis in humans or mice.

Recently, intestinal macrophages and dendritic cells have been identified as key regulators of immune homeostasis and inflammation in the intestine. Resident intestinal macrophages can regulate themselves and other immune cells primarily through the spontaneous secretion of IL-10 that ultimately contributes to the prevention of pathological intestinal inflammation. In contrast, intestinal macrophages in the inflamed mucosa respond to microbial stimulation and produce large amounts of proinflammatory cytokines that further induce inflammation and damage in the intestine.

In the present study, we investigated the role of PI3K p85α in murine acute colitis, focusing on the cytokine production of macrophages. We demonstrated that p85α hetero-deficient (p85α+/−) mice exhibited a reduced susceptibility to dextran sulfate sodium (DSS)-induced acute colitis through an enhancement of IL-10 production.

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in the intestinal macrophages, and high IL-10-producing macrophages protected the mice from the development of DSS-induced acute colitis.

**Results**

**p85α+/- mice show a reduced susceptibility to DSS-induced acute colitis.** To clarify the role of the p85α subunit in colitis, we examined the development of DSS-induced acute colitis in WT and p85α+/- mice. Body weight loss was observed on day 4 after the start of DSS treatment in WT colitis mice and was significantly attenuated in p85α+/- colitis mice (Fig. 1a; 86.6 ± 1.6% in WT, 98.6 ± 0.9% in p85α+/- colitis at day 7). The score of the disease activity index, which was a combination of diarrhea and rectal bleeding, was significantly alleviated in p85α+/- colitis mice compared with WT colitis mice (Fig. 1b; 3.9 ± 0.3 in WT, 1.5 ± 0.1 in p85α+/- at day 7). Macroscopic observations showed that the shortening of the colon caused by DSS treatment was significantly attenuated in p85α+/- colitis mice compared with WT colitis mice (Fig. 1c and d), although the colon length under normal conditions was similar between WT and p85α+/- mice (Fig. 1d). As shown in Fig. 1e and f, H&E staining of the colon from DSS-treated WT colitis mice revealed a loss of epithelial integrity and crypt architecture as well as submucosal edema, which were significantly improved in p85α+/- colitis mice. The MPO activity in the colons of WT colitis mice was markedly elevated on day 7 (Fig. 1g). The elevated MPO activity was significantly suppressed in the p85α+/- colitis mice. Furthermore, the expression of proinflammatory mediators such as TNF-α, IL-1β, IL-6 and iNOS mRNA in the colons of WT colitis mice was markedly upregulated on day 7 and significantly higher than those of p85α+/- colitis mice (Fig. 1h). We observed that the expression of IL-10 mRNA in the colons of p85α+/- mice was higher than those of WT mice in both normal and colitis states (Fig. 1h). These results clearly demonstrate that the development of DSS-induced colitis is suppressed in p85α+/- mice compared with WT mice.

To examine the involvement of PI3K activation in the colitis, we evaluated the effects of the PI3K inhibitors LY294002 and wortmannin on the development of DSS-induced colitis. LY294002 (1 mg/kg) and wortmannin (0.1 mg/kg) aggravated the body weight loss and disease activity index caused by DSS treatment in WT mice (Figure S1).

**Intestinal macrophages of p85α+/- mice express low levels of proinflammatory mediators.** We examined the proportion of F4/80+CD11b+ macrophages in the colonic lamina propria. There was no difference in the proportion of the intestinal macrophages between WT and p85α+/- normal mice (Fig. 2a and b). The proportion of the intestinal macrophages was increased equivalently in both WT and p85α+/- mice in response to DSS treatment (Fig. 2a and b).

To investigate the expression levels of proinflammatory mediators in the intestinal macrophages, we isolated F4/80+ macrophages from the colonic lamina propria of mice on day 0 (normal) or 7 (colitis) after the start of DSS treatment. The intestinal macrophages of WT colitis mice expressed high levels of TNF-α, IL-1β, IL-6 and iNOS mRNA compared with the intestinal macrophages of WT normal mice (Fig. 2c). In contrast, the intestinal macrophages of p85α+/- colitis mice expressed similar levels of TNF-α, IL-1β, IL-6 and iNOS mRNA compared with intestinal macrophages of p85α+/- normal mice (Fig. 2c). Furthermore, the expression levels of these mediators in the intestinal macrophages of p85α+/- colitis mice were significantly lower than those in the intestinal macrophages of WT colitis mice (Fig. 2c). These results suggest that the p85α subunit is involved in the development of DSS-induced acute colitis by regulating the production of proinflammatory mediators in the intestinal macrophages of the inflamed colonic mucosa.

We also examined the proportion of Ly6C+CD11b+ inflammatory monocytes and Gr1+CD11b+ neutrophils in the colonic lamina propria. There was no difference in the proportion of intestinal inflammatory monocytes and neutrophils between WT and p85α+/- colitis mice (Figure S2A and B).

**Bone marrow-derived macrophages from p85α+/- mice produce a large amount of IL-10.** To investigate the contribution of the p85α subunit to the cytokine production of macrophages, we screened the expression levels of cytokine mRNA in BMDMs from WT or p85α+/- mice. We observed no morphological changes between WT and p85α+/- BMDMs (Fig. 3a). Furthermore, there was no difference in the proportion of F4/80+, CD11b+ or MHC-II+ cells between WT and p85α+/- BMDMs (Fig. 3b). The protein expression of PI3K p85α in p85α+/- BMDMs was less than 50% compared to WT BMDMs (Fig. 3c). The mRNA expression of proinflammatory cytokines in p85α+/- BMDMs was similar to WT BMDMs (Fig. 3d). To examine the mRNA expression of proinflammatory cytokines in inflammatory (M1) macrophages, BMDMs were further stimulated with IFN-γ and LPS for 24 h and were polarized to M1BMDMs. However, there was no difference in the expression of proinflammatory cytokine mRNAs between WT and p85α+/- M1BMDMs, although both M1BMDMs showed marked upregulation of proinflammatory cytokine expression compared with BMDMs (Fig. 3e). Interestingly, a significant threefold increase in IL-10 mRNA expression was observed in p85α+/- BMDMs compared with WT BMDMs (Fig. 3f). Furthermore, p85α+/- BMDMs secreted a significantly higher amount of IL-10 in response to LPS stimulation than WT BMDMs (Fig. 3g). The BMDMs from p85α KO mice also showed a significant threefold increase in IL-10 mRNA expression compared with those from WT mice (Figure S3).

Next, we examined the expression of phosphorylated Akt in WT and p85α+/- BMDMs. Immunoblot analysis revealed that phosphorylated Akt was equivalently upregulated by LPS stimulation in both WT and p85α+/- BMDMs (Fig. 4a and b). Furthermore, there was no difference in the level of phosphorylated GSK-3β between WT and p85α+/- BMDMs (Fig. 4a and b). To clarify the involvement of Akt activation in the regulation of IL-10 production, we examined the effects of LY294002 and wortmannin on IL-10 secretion in WT BMDMs. LY294002 (1–3 μM) and wortmannin (100–300 nM) significantly inhibited the LPS-stimulated IL-10 secretion of WT BMDMs in a concentration-dependent manner (Fig. 4c). The expression of phosphorylated Akt induced by
LPS stimulation was significantly suppressed by treatment with LY294002 and wortmannin (Fig. 4d and e). These results suggest that p85α+− BMDMs produce a large amount of IL-10 through an Akt-independent pathway.

Furthermore, we investigated the IL-10 mRNA expression of intestinal macrophages in the normal colonic mucosa. In the steady state, the intestinal macrophages of p85α+− mice showed a significant increase in IL-10 mRNA expression compared with those of WT mice (Fig. 5). These findings suggest that the intestinal

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**Figure 1.** p85α+− mice show reduced susceptibility to DSS-induced acute colitis. Colitis was induced in WT or p85α+− mice through daily treatment with a 3% DSS solution in drinking water for 7 days. (a) Body weight, (b) disease activity index, (c) macroscopic observations of colons and (d) colon length are shown. (e) Representative images of H&E staining are shown. Scale bar is 300 μm. (f) Histological scoring of DSS-induced colitis. (g) Myeloperoxidase activity induced by DSS treatment in the mouse colons of WT or p85α+− mice. (h) The changes in the mRNA expression of proinflammatory mediators and IL-10 induced by DSS treatment in the mouse colons of WT or p85α+− mice. The data are presented as the mean ± SE of 4–6 mice and are representative of 1 out of 3 independent experiments. *p < 0.05; **p < 0.01, compared with WT normal mice. †p < 0.05; ††p < 0.01, compared with WT colitis mice. *p < 0.05; ††p < 0.05, compared with p85α+− normal mice.
macrophages in p85α+/- mice suppress the development of acute colitis through a high production of IL-10 in colonic mucosa.

**High IL-10-producing bone marrow-derived macrophages suppress the development of DSS-induced acute colitis.** To investigate whether high IL-10-producing macrophages can suppress the...
development of acute colitis, BMDMs from WT or p85α+/− mice were injected into WT mice in a DSS-induced colitis model (Fig. 6a). Adoptively transferred BMDMs from each genotype were equivalently located in the
colonic lamina propria at 2 days after the injection (Fig. 6b; 4.2 ± 0.2% in WT BMDM, 4.6 ± 0.3% in p85α−/− BMDM). Adoptive transfer of p85α−/− BMDMs, but not WT BMDMs, significantly suppressed the body weight loss and disease activity index caused by DSS treatment in WT mice (Fig. 6c and d). Histological analysis also showed that pathological abnormalities were improved in p85α−/− BMDM-transferred colitis mice compared with WT BMDM-transferred colitis mice (Fig. 6c and f). The expression of IL-10 mRNA was significantly higher in the colons of p85α−/− BMDM-transferred colitis mice than those of WT BMDM-transferred colitis mice (Fig. 6g). Furthermore, the ameliorative effect of p85α−/− BMDM transfer was significantly reversed by the administration of anti-IL-10 antibody (Fig. 6h and i). These results demonstrate that high IL-10-producing p85α−/− BMDMs suppress the development of DSS-induced acute colitis.

Discussion
Several studies have reported a role for PI3K in colitis in humans and animal models. The activation of PI3K/ Akt signaling has been observed in the colonic mucosal biopsy specimens of ulcerative colitis patients and DSS-induced colitis model. A deficiency in the PI3K p110α subunit in mice develops spontaneous colitis because the p110α subunit controls homeostatic antigen presenting cell-T cell interactions by altering the balance

Figure 4. The production of a large amount of IL-10 in p85α−/− BMDMs is independent of Akt activation. BMDMs were prepared from the femurs and tibiae of WT or p85α−/− mice and cultured with M-CSF (100 ng/ml) for 7 days. (a) Representative images of pAkt and pGSK-3β protein level in WT or p85α−/− BMDMs as detected by western blotting. BMDMs were stimulated with LPS (100 ng/ml) for 1 h. (b) Relative pAkt and pGSK-3β levels were normalized by Akt signals. (c) Effect of LY294002 and wortmannin on the concentration of IL-10 in the culture supernatant of WT BMDMs. LY294002 (1–3 μM) and wortmannin (100–300 nM) was applied 30 min before LPS stimulation. (d) Representative images of pAkt protein level in LY294002 (LY) or wortmannin (Wor)-treated BMDMs as detected by western blotting. (e) Relative pAkt levels were normalized by Akt signals. The data are presented as the mean ± SE of 4 independent experiments. **p < 0.01, compared with non-stimulation (Non). ††p < 0.01, compared with vehicle (Veh).
between IL-10 and IL-12/23. Furthermore, p105α-deficient mice show a reduction in the number of arginase I-expressing M2 macrophages in the colonic mucosa, resulting in the development of more severe DSS-induced colitis than WT mice. Additionally, an inhibitor of PI3K has been reported to suppress the development of murine colitis. These studies indicate that PI3K subunits have roles in the pathogenesis of colitis. Here, we report that mice deficient in the p85α subunit exhibit a reduced susceptibility to DSS-induced acute colitis due to the augmentation of IL-10 production by intestinal macrophages in the colonic mucosa.

It is generally accepted that innate immunity largely contributes to the pathogenesis of DSS-induced acute colitis. In particular, enhanced infiltration and activation of macrophages in the colonic mucosa play a crucial role in the inflammatory response. Although we also found that the proportion of intestinal macrophages was increased in the inflamed colonic mucosa after DSS treatment as previously reported, there was no difference between WT and p85α−/− colitis mice. Furthermore, we did not observe a difference in the proportion of Ly6C+CD11b+ inflammatory monocytes and Gr1+CD11b+ neutrophils (Supplemental Figure S2A and B), which infiltrate the colonic mucosa after DSS treatment and are involved in the pathogenesis of DSS-induced colitis. Therefore, our findings suggest that a deficiency of p85α has no effect on the infiltration of proinflammatory innate immune cells in DSS-induced colitis.

Inflammatory macrophages in the inflamed colonic mucosa produce a large amount of proinflammatory mediators such as TNF-α, IL-1β, IL-6 or iNOS. In IBD patients, inflammatory macrophages in the inflamed colonic mucosa cause the expansion of further inflammation through a large amount of proinflammatory cytokine production. We demonstrated that the intestinal macrophages that were isolated from inflamed colonic mucosa of p85α−/− colitis mice expressed low levels of proinflammatory mediators compared with those of WT colitis mice. We assumed that the p85α subunit also contributed to the production of proinflammatory cytokines in macrophages because the p85α subunit in BMDMs is crucial for proliferation and migration.

Monocytes recruited by CCR2 differentiate locally into intestinal macrophages through stimulation of M-CSF. Thus, M-CSF-induced BMDMs are widely used as an in vitro model for intestinal macrophages. Furthermore, the stimulation of BMDMs in vitro with INF-γ and LPS generates M1BMDMs, which exhibit the features of proinflammatory macrophages. Our results indicated that p85α deficiency did not influence the expression of proinflammatory mediators in both BMDMs and M1BMDMs. Interestingly, p85α-deficient BMDMs produced a higher amount of IL-10 than WT BMDMs. Resident intestinal macrophages express low levels of Toll-like receptors and do not produce proinflammatory cytokines after exposure to bacterial signals. In mice, this state of inflammatory anergy is largely attributable to IL-10 that is constitutively expressed by intestinal macrophages. A lack of intestinal macrophages resulted in the enhanced severity of DSS-induced acute colitis, which was reversed by the adoptive transfer of BMDMs. Intestinal macrophages have also been reported to suppress the development of DSS-induced colitis due to the enhanced release of IL-10 from intestinal macrophages by treatment with Clostridium butyricum. These reports suggest that p85α deficiency augments IL-10 production in intestinal macrophages, thereby suppressing the development of DSS-induced acute colitis in p85α−/− mice. Indeed, intestinal macrophages of p85α−/− mice expressed a high level of IL-10 in the steady state. A noteworthy finding in the current study was that the adoptive transfer of high IL-10-producing macrophages (p85α−/− BMDMs) significantly suppressed the development of DSS-induced acute colitis, and this effect was impaired by anti-IL-10 antibody. The injection of anti-IL-10 antibody did not affect the development of DSS-induced colitis in WT BMDM-transferred WT mice (Figure S4A and B), suggesting that anti-IL-10 antibody has no profound pro-inflammatory effect. We also verified that the intraperitoneally injected BMDMs were
Figure 6. High IL-10-producing macrophages suppress the development of DSS-induced acute colitis in WT mice. (a) Schematic drawing of the experimental design for the evaluation of BMDM adoptive transfer in the DSS-induced acute colitis model. BMDMs from WT or p85α+/− mice were intraperitoneally injected into WT mice at 2 days before and 1 day after the start of DSS treatment. (b) Representative flow cytometry plots show the proportion of CD11b+ F4/80+ CM-Dil+ intestinal macrophages in cLP cells. Plots were representative of the results from 3 independent experiments. Body weight (c), disease activity index (d) and representative images of H&E staining (e) are shown. Scale bar is 300 μm. (f) Histological scoring of DSS-induced colitis. (g) IL-10 mRNA expression in the mouse colons of WT BMDM or p85α+/− BMDM-transferred colitis mice. The data are presented as the mean ± SE of 4 mice and are representative of 1 out of 3 independent experiments. †p < 0.05; ††p < 0.01, compared with WT BMDM. (h,i) Effect of anti-IL-10 antibody on the ameliorative action of p85α+/− BMDM against the development of DSS-induced colitis in WT mice. Anti-IL-10 antibody or control IgG was injected intraperitoneally into p85α+/− BMDM-transferred WT mice 2 h before the start of DSS treatment and then every other day. Body weight (h) and disease activity index (i) are shown. The data are presented as the mean ± SE of 5 mice and are representative of 1 out of 2 independent experiments. †p < 0.05, compared with Control.
H&E-stained sections were scored for inflammation and crypt damage as described previously47. To exclude bias, each tissue sample was embedded in Tissue Freezing Medium. Frozen sections (10 μm) were cut at 20 °C using a cryostat microtome (Leica Microsystems, Nussloch, Germany). The sections were then routinely stained with H&E. Histological study.

The distal part of the colon was removed, washed with ice-cold phosphate-buffered saline, and immersed in 4% paraformaldehyde for 24 h at 4 °C. After treatment with a 30% sucrose solution, the tissue sample was embedded in Tissue Freezing Medium. Frozen sections (10 μm) were cut at −20 °C using a cryostat microtome (Leica Microsystems, Nussloch, Germany). The sections were then routinely stained with H&E. H&E-stained sections were scored for inflammation and crypt damage as described previously47. To exclude bias, histological scores were determined in a masked manner.

Determination of myeloperoxidase activity.

Myeloperoxidase (MPO) activity was measured in the mouse colon as described previously47. Briefly, the animals were sacrificed 7 days after DSS treatment, and the colons were excised. After the tissue was rinsed with ice-cold saline, the whole colon was weighed and homogenized in 50 mM phosphate buffer containing 0.5% hexadecyltrimethylammonium bromide (pH 6.0). The homogenized samples were subjected to 3 cycles of freeze-thawing and then centrifuged at 2,000 g for 10 min at 4 °C. The MPO activity in the supernatant was determined by adding the supernatant to 0.5 M o-dianisidine hydrochloride in 10 mM phosphate buffer (pH 6.0) containing 0.00005% (wt/vol) hydrogen peroxide. The changes in the absorbance of each sample were recorded at 460 nm using a spectrophotometer (UV160A; Shimadzu, Kyoto, Japan). MPO activity was expressed as units per wet weight of colonic tissue in grams.

Determination of mRNA expression.

Cytokine mRNA expression was measured in the mouse colon as described previously47. Briefly, total RNA was extracted from the colon using Sepasol RNA I Super
Flow cytometry analysis. The cells were incubated with FcR Blocking Reagent (Miltenyi Biotec, Auburn, CA) and Via-Probe (BD Biosciences) for 5 min followed by staining with specific antibodies for 30 min at 4 °C. Flow cytometry analyses were conducted on a FACSComp II flow cytometer.

**Isolation of lamina propria macrophages.** Colonic lamina propria (cLP) cells were isolated from the mouse colons as described previously. Briefly, the colons were then cut into small pieces that were stirred at 37 °C for 20 min in RPMI-1640 containing 2% FBS (GIBCO, Carlsbad, CA) and 0.5 mM EDTA and washed twice with RPMI-1640. This process was repeated without EDTA. The pieces were incubated at 37 °C for 20 min in RPMI-1640 containing 200 U/ml collagenase (Wako), and the digested tissues were collected and washed with RPMI-1640. This process was repeated 3 times, and cells were pooled. The pooled cell suspension was passed through a strainer (70 μm), and washed with RPMI-1640. Isolated cells were suspended in RPMI-1640 containing 100 ng/ml M-CSF (Sigma-Aldrich, St. Louis, MO), layered onto 75% Percoll, and centrifuged at 770 g for 20 min. cLP cells were recovered from the Percoll interphase and washed twice with RPMI-1640. cLP cells were stained with APC-conjugated anti-F4/80 antibody (eBioscience, San Diego, CA), and F4/80 positive cLP macrophages were purified using the BD IMag APC Magnetic Particles (BD Biosciences, San Diego, CA). For determination of cytokine mRNA expression, isolated cLP macrophages (2×10⁶) were stimulated with 100 ng/ml LPS (Sigma-Aldrich) for 4 h, and total RNA was extracted from the intestinal macrophages using an RNeasy Mini kit (Qiagen, Valencia, CA). RT-PCR was performed as described above.

**Bone marrow-derived macrophage culture.** Bone marrow-derived macrophages (BMDMs) were prepared from the femurs and tibiae of p85α−/− or WT mice and cultured with M-CSF (R&D systems, Minneapolis, MN). Bone marrow cells were cultured in RPMI-1640 medium supplemented with 100 ng/ml M-CSF, 10% heat-inactivated FBS, 55 μM 2-mercaptoethanol (GIBCO), 50 U/ml penicillin, and 50 μg/ml streptomycin (Sigma-Aldrich) at 37 °C in a humidified 5% CO₂ atmosphere. After 7 days, macrophage purity was examined using flow cytometry (FACSCanto II), and more than 90% of the adherent cells were CD11b and F4/80 positive. For determination of cytokine mRNA expression, BMDMs (5×10⁵) were stimulated with LPS (100 ng/ml) for 4 h, and total RNA was extracted from BMDMs using an RNeasy Mini kit. RT-PCR was performed as described above.

**Measurement of IL-10 protein.** BMDMs (5×10⁵) were seeded on 24-well culture plates (BD Biosciences) in RPMI-1640 supplemented with 10% FBS, 50 U/ml penicillin, and 50 μg/ml streptomycin (Sigma-Aldrich), and stimulated with LPS (100 ng/ml) for 24 h at 37 °C in a humidified incubator with 5% CO₂. In some experiments, LY-294002 (1–3 μM; Sigma-Aldrich) or wortmannin (100–300 nM; Sigma-Aldrich) was applied 30 min before the LPS stimulation. Culture supernatants were collected and stored at −80 °C until IL-10 measurement was performed. IL-10 concentrations in culture supernatants were detected using a Cytometric Beads Array Kit (BD Biosciences) according to the manufacturer’s instructions. Samples were analyzed with a FACSComp II flow cytometer.

**Determination of protein level using western blotting.** BMDMs were stimulated with 100 ng/ml LPS for 1 h at 37 °C. In some cases, LY-294002 (3 μM) or wortmannin (300 nM) was applied 30 min before the LPS stimulation. BMDMs were homogenized in lysis buffer (pH 7.4) as described previously. The samples (20 μg/lane) were then subjected to electrophoresis on 10% SDS-polyacrylamide gels and transferred electrophotically to PVDF membranes (Millipore, Billerica, MA). The membranes were incubated with rabbit anti-p-Akt (Ser473) antibody, rabbit anti-PI3 Kinase p85 antibody or rabbit anti-PI3 Kinase p85 antibody (Cell Signaling Technology, Denvers, MA) and treated with horseradish peroxidase-conjugated goat anti-rabbit IgG (Cell Signaling Technology). Immune complexes were visualized using an enhanced chemiluminescence detection system (GE Healthcare Japan, Tokyo, Japan) and photographed (ImageQuant LAS4000; GE Healthcare Japan). Akt and p-Akt protein expression levels were determined densitometrically with ImageJ (NIH, Bethesda, MD).

**Adoptive transfer.** BMDMs (2×10⁶) from WT or p85α−/− mice were intraperitoneally injected into WT mice 2 days before and 1 day after the start of DSS treatment. To neutralize IL-10, LEAF Purified anti-mouse (Nacalai Tesque, Kyoto, Japan) according to the manufacturer’s instructions. Reverse transcription was performed using the PrimeScript RT reagent Kit (Takara Bio, Ohtsu, Japan) and random primers followed by real-time PCR. Real-time PCR amplification of TNF-α, IL-1β, IL-6, IL-10, IFN-γ, and GAPDH was performed using SYBR Premix Ex Taq (Takara Bio). The following primer pairs were used: TNF-α, (forward) 5′-AAGCCCTGTACCCAGCTCGTA-3′ and (reverse) 5′-GGCAACACCT-AGTGTGGTGTCTTGG-3′; IL-1β, (forward) 5′-CTGTGCTTCTTCCCCGAGCAC-3′ and (reverse) 5′-CAGCTCATATGGGTCCGACA-3′; IL-6, (forward) 5′-CCAACCT-CACAACTGCGAGCTTA-3′ and (reverse) 5′-GAAATGTCATCAGTGTGGTCTAAC-3′; IL-10, (forward) 5′-GGCCCTTTGATCTTGTCC-3′ and (reverse) 5′-AAGCGGCTGGGAGGATGAC-3′; IFN-γ, (forward) 5′-TCTGGAGGAATGGCCCGGAG-3′ and (reverse) 5′-CCCTAGCGGCTGCCTACTC-3′; GAPDH, (forward) 5′-TGACCCACATCCAGGCGTATC-3′ and (reverse) 5′-GAGCGGAC-ATTGGGGGTAG-3′. Real-time PCR was performed using the Takara TP800 (Takara Bio). The PCR reaction conditions consisted of 10 s at 95 °C followed by 40 cycles of 5 s at 95 °C and 20 s at 60–63 °C. Target mRNA levels were normalized to those of GAPDH as an internal control in each sample. The results are expressed as ratios relative to the average of the control group.
IL-10 or LEAF Purified Rat IgG1κ (250 μg/mouse; Biolegend, San Diego, CA) was injected intraperitoneally into p85α+/− BMDM-transferred WT mice 2 h before the start of DSS treatment and then every other day. To trace the transferred BMDMs in vivo, BMDMs were labeled with the fluorescent membrane marker CM-DiI (Molecular Probes, Eugene, OR) according to the manufacturer’s instructions and injected into mice. After 2 days, cLP cells were isolated from the mouse colons and the proportion of the CD11b+ F4/80+ CM-DiI+ intestinal macrophages was analyzed using a FACSCanto II flow cytometer.

Statistical analyses. The data are presented as the means ± SE. Statistical analyses were performed using repeated measures two-way ANOVA followed by Bonferroni’s multiple comparison test, a one-way ANOVA followed by a Dunnett’s multiple-comparison test, nonparametric Mann-Whitney test or an unpaired (two-tailed) t-test. Values of p < 0.05 were considered to be significant.

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**Acknowledgements**

We thank Dr. Yasuharu Watanabe for technical advice regarding the culture of BMDMs. This research received the support of JSPS KAKENHI grant numbers JP15K18873 (S.H.), JP25460891 (T.Y.), JP16H05276, and JP16K15408 (M.K.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan, Takeda Science Foundation (S.H.), Tamura Science Foundation (S.H.) and the Uehara Memorial Foundation (S.H.).

**Author Contributions**

S.H. study concept and design, acquisition of data, analysis and interpretation of data, statistical analysis, obtained funding and wrote the manuscript. T.H. acquisition of data, analysis and interpretation of data and statistical analysis. D.G.A.Z., M.O., and K.I. acquisition of data. T.Y. obtained funding and technical support. M.K. study supervision, obtained funding and wrote the manuscript. All authors reviewed the manuscript.

**Additional Information**

Supplementary information accompanies this paper at doi:10.1038/s41598-017-06464-w

**Competing Interests:** The authors declare that they have no competing interests.

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