Cutting Edge: mTORC1 in Intestinal CD11c⁺ CD11b⁺ Dendritic Cells Regulates Intestinal Homeostasis by Promoting IL-10 Production

Masashi Ohtani, Takayuki Hoshii, Hideki Fujii, Shigeo Koyasu, Atsushi Hirao and Satoshi Matsuda

J Immunol 2012; 188:4736-4740; Prepublished online 13 April 2012;
doi: 10.4049/jimmunol.1200069
http://www.jimmunol.org/content/188/10/4736

Supplementary Material
http://www.jimmunol.org/content/suppl/2012/04/12/jimmunol.1200069.DC1

References
This article cites 22 articles, 9 of which you can access for free at:
http://www.jimmunol.org/content/188/10/4736.full#ref-list-1

Subscription
Information about subscribing to The Journal of Immunology is online at:
http://jimmunol.org/subscription

Permissions
Submit copyright permission requests at:
http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts
Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/alerts
Dendritic cells (DCs) are specialized APCs that are critically involved in the initiation and control of innate and adaptive immunity (4). DCs recognize pathogens via pattern-recognition receptors, such as TLRs, and produce various cytokines, including IL-10 and IL-12. We have previously demonstrated that mTORC1 positively regulates LPS-induced IL-10 production and suppresses IL-12 production via an autocrine action of IL-10 in mouse DCs in vitro (5). Other groups also reported involvement of mTORC1 in cytokine production in human monocytes and DCs (6, 7). The PI3K–mTORC1 pathway is involved in DC development as well (8, 9). Although the physiological roles of mTORC1 in DC monocyte function have been addressed via systemic administration of rapamycin (6) or DC-specific deletion of phosphatase and tensin homolog deleted on chromosome 10 (Pten), a negative regulator of PI3K (9), these approaches cannot exclude the possible involvement of other signaling pathways such as mTORC2 (10).

To clarify the role of mTORC1 in DC function in vivo, we established DC-specific Raptor knockout mice (referred to here as Raptor<sup>DC<sup>−/−</sup></sup>). Raptor<sup>DC<sup>−/−</sup></sup> mice had increased numbers of splenic CD8<sup>+</sup> DCs and intestinal laminae propria (LP)-CD11c<sup>+</sup>CD11b<sup>+</sup> DCs, suggesting that mTORC1 rather attenuates cell expansion in specific subsets of DCs. We also show that impaired mTORC1 signal results in the suppression of IL-10 production along with enhanced CD86 expression in intestinal CD11c<sup>+</sup>CD11b<sup>+</sup> DCs and that Raptor<sup>DC<sup>−/−</sup></sup> mice were highly susceptible to dextran sodium sulfate-induced colitis. Our results uncover mTORC1-mediated anti-inflammatory programs in intestinal CD11c<sup>+</sup>CD11b<sup>+</sup> DCs to limit the intestinal inflammation. The Journal of Immunology, 2012, 188: 4736–4740.
(C57BL/6; Jax mice no. 008608). C57BL/6-Rag-2−/− (CD45.2) mice and B6.SJL-Rag-2−/− (CD45.1) mice, which are congenic to C57BL/6 mice, were obtained from Taconic Farms. All mice were maintained in a specific pathogen-free facility and used according to our institutional guidelines.

Western blotting
Western blotting for mouse Raptor, phospho-Akt, 4E-BP1, phospho-S6 (Cell Signaling Technology), p70S6K, and ERK2 (Santa Cruz Biotechnology) was carried out as described (5).

Cell preparation
Lamina propria mononuclear cells (LPMCs) were isolated according to Murai et al. (11) with slight modifications. In brief, colons were cut into small pieces, resuspended in HBSS with 5% FCS and 2 mM EDTA, and shaken for 20 min three times at 37°C. The cells were then resuspended in HBSS with 5% FCS, 5 mM CaCl2, and 1.5 mg/ml collagenase VIII (Sigma-Aldrich) and shaken for 20 min at 37°C. Pellet was washed and passed through a 108-μm nylon mesh. LPMCs were isolated by centrifugation on a 40/80% Percoll gradient for 20 min at 1000 × g. In some experiments, cells were incubated in RPMI 1640 containing 10% FCS supplemented with 10 mM HEPES, 1 mM sodium pyruvate, and 50 μM 2-mercaptoethanol (referred to here as a complete medium).

Flow cytometry
Cells were stained with the appropriately labeled Abs (Beckman Coulter, BD Biosciences, BioLegend, or eBioscience) after Fc receptor blockade. For the analysis of intracellular phospho-S6 levels, cells stained for cell surface marker with GolgiPlug (BD Biosciences) and then fixed and permeabilized, followed by staining with surface markers and IL-10 (BD Biosciences). Samples were analyzed on a FACSCantoII by using FACSDiva software (BD Biosciences).

DSS-induced colitis model
Mice were fed with 3% DSS (molecular mass 36–50 kDa; MP Biomedicals) in their drinking water for 7 d and sacrificed on day 8. H&E-stained colon sections were prepared by Swiss roll method and assessed for erosion value, which was calculated as the percentage of erosive area per leukocyte infiltrated area in submucosa.

Statistical analysis
Unless otherwise indicated, statistical analysis was performed using unpaired Student t test. A p value <0.05 was considered statistically significant.

Results and Discussion
Impairment of mTORC1 signal increases the proportion of certain DC subsets
To assess the role of mTORC1 in DC function specifically, we crossed mice with loxp-flanked Raptor alleles (Raptorfl/fl) with transgenic mice (CD11c-Cre). In Raptorfl/fl × CD11c-Cre mice, referred to here as RaptorDC−/−, Raptor expression was mostly abrogated in splenic CD11c+ DCs (Fig. 1A) but not significantly in CD11c+CD11b+ cells or T cells (data not shown). To evaluate the mTORC1 activity in DCs derived from RaptorDC−/− mice, we examined the phosphorylation status of p70S6K and 4E-BP1, as a measure of mTORC1 activity, as well as ribosomal protein S6, as a measure of p70S6K

![Image](http://www.jimmunol.org/)
activity. Note that cells were stimulated with complete medium including nutrients and sera, well-known activators of mTORC1, to induce mTORC1 activation. In splenic CD11c+ DCs, hypophosphorylation of p70S6K, 4E-BP1, and S6 was observed in RaptorDC−/− mice compared with Raptorfl/fl mice (Fig. 1A). In contrast, RaptorDC−/− mice-derived cells exhibited enhanced phosphorylation of Akt at Ser473 and Thr308 irrespective of stimulation (Fig. 1A), suggesting constitutive activation of PI3K signal (12). Intracellular phospho-S6 staining of splenocytes revealed the attenuation of the mTORC1 activity in both CD11c+hypPDCA-1− conventional dendritic cells (cDCs) and CD11c+PDCA-1+ plasmacytoid dendritic cells (pDCs) in RaptorDC−/− mice (Fig. 1B).

RaptorDC−/− mice showed increased proportion of CD11c+PDCA-1+ pDCs (Fig. 2A) and increased numbers of CD11c+PDCA-1− conventional dendritic cells (cDCs) and CD11c+PDCA-1− plasmacytoid dendritic cells (pDCs) in RaptorDC−/− mice (Fig. 1B).

RaptoRDC−/− mice showed increased proportion of CD11c+PDCA-1+ pDCs (Fig. 2A) and increased numbers of CD11c+PDCA-1− conventional dendritic cells (cDCs) and CD11c+PDCA-1− plasmacytoid dendritic cells (pDCs) in RaptoRDC−/− mice (Fig. 1B). Impaired IL-10 expression in RaptoR-deficient LP-DCs. (Fig. 2A) and increased numbers of CD11c+PDCA-1− conventional dendritic cells (cDCs) and CD11c+PDCA-1− plasmacytoid dendritic cells (pDCs) in RaptoRDC−/− mice (Fig. 1B).

Expansion of RaptoRDC−/− mice-derived splenic CD8+ cDCs was confirmed by analysis of mixed BM chimera mice (Supplemental Fig. 1B). In contrast, the intestinal CD103+ DC subset, which is a tissue counterpart of CD8+ cDCs and corresponds to LP-CD11c+CD11b− DCs (data not shown), was not affected in RaptoRDC−/− mice (Fig. 2C). Intriguingly, DC-specific Pten deletion leads to expansion of both splenic CD8+ cDCs and intestinal CD103+ DCs (9). Future studies would reveal the molecular mechanism underlying the difference between DC-specific Pten deleted mice and RaptoRDC−/− mice.

Intestinal APCs can be distinguished by the expression patterns of CD11c and CD11b (CD11c+CD11b− and CD11c+CD11b+ DCs, and CD11c+CD11b+ macrophages) (14). In RaptoRDC−/− mice, the number of CD11c+CD11b+ DCs that showed the CD103+F4/80− phenotype (22.8 ± 6.3 × 104 cells versus 11.4 ± 1.5 × 104 cells in RaptoRfl/fl, p < 0.01), but not CD11c+CD11b− DCs or CD11c+CD11b+ macrophages, was increased in the LP of large intestine (LI) and small intestine (SI) (Fig. 2C and data not shown). In addition, RaptoRDC−/− mice showed higher CD86 expression with IFN-α production by pDCs of RaptoRfl/fl mice in vitro (Supplemental Fig. 1A). We cannot, however, formally rule out the possibility that residual mTORC1 signal, due to inefficient deletion of RaptoR in pDCs of RaptoRDC−/− mice where CD11c expression is relatively low (Fig. 2A, 2B), is sufficient for TLR ligand-induced IFN-α production.

The analysis of splenic DC subsets in RaptoRDC−/− mice revealed that the CD8+ cDC population was specifically expanded (2.82 ± 0.66 × 105 cells versus 1.62 ± 0.67 × 105 cells in RaptoRfl/fl, p < 0.05), whereas total numbers of cDC and pDC populations remained intact (Fig. 2B). The expression levels of MHC class II (MHC II) as well as CD86 in splenic DCs were unaffected in RaptoRDC−/− mice (Fig. 2B).

FIGURE 3. Impaired IL-10 expression in RaptoR-deficient LP-DCs. (A) LILPMSCs from RaptoRfl/fl or RaptoRDC−/− mice were incubated in PBS or complete medium for 30 min. The expression levels of phospho-S6 in CD11c+CD11b−, CD11c+CD11b+, and CD11c+CD11b+ populations were assessed by FACS. (B) Left, LILPMSCs from RaptoRfl/fl (n = 3) or RaptoRDC−/− (n = 3) mice were incubated in complete medium with GolgiPlug for 4 h. The expression level of intracellular IL-10 in CD11c+CD11b−F4/80+ DCs was assessed by FACS. Indicated are mean fluorescence intensities of IL-10 expression. **p < 0.01. Right, Sorted LP-CD11c+CD11b−F4/80+ DCs from RaptoRfl/fl (n = 3) or RaptoRDC−/− (n = 3) mice were incubated in complete medium for 4 h. IL-10 mRNA levels were assessed by real-time PCR using cyclophilin A mRNA as a reference. *p < 0.05 (Mann–Whitney U test). (C) Sorted LP-CD11c+CD11b−F4/80+ DCs from wild-type mice were pretreated with or without 25 ng/ml rapamycin for 1 h, followed by stimulation with 1 μg/ml LPS for 20 h. IL-10 in culture supernatants (left) and IL-10 mRNA levels (right) were assessed by ELISA and real-time PCR using cyclophilin A mRNA as a reference, respectively. Data are representative of three (A, C) or two (B) independent experiments.

FIGURE 4. mTORC1 signal in LP-DCs regulates intestinal homeostasis. RaptoRfl/fl (n = 8) or RaptoRDC−/− (n = 7) mice were fed with 3% DSS for 7 d and analyzed for body weight loss [(A), *p < 0.05, Mann–Whitney U test], colon length [(B), **p < 0.01], and H&E staining of colonic sections (C) on day 8. (C) The dashed lines and arrows indicate the area of mucosal erosion and the start point observed in leukocyte infiltration of submucosa, respectively (left). Original magnification ×10. The erosion value was evaluated as described in Materials and Methods and summarized (right). Numbers in microphotographs indicate erosion value. *p < 0.05.
in CD11c<sup>+</sup>CD11b<sup>+</sup> DCs in IL-1P and SI-1P compared with wild-type mice (Fig. 2C and data not shown). The increased expression of CD86 in LP-CD11c<sup>+</sup>CD11b<sup>+</sup> DCs of Raport<sup>DC−/−</sup> mice was caused in a cell-intrinsic manner, which was confirmed by analysis of mixed BM chimera mice (Supplemental Fig. 1C). We failed to find significant differences in phenotypes of mesenteric lymph node-derived DCs between Raport<sup>fl/fl</sup> and Raport<sup>DC−/−</sup> mice (data not shown). These results indicate that the lack of mTORC1 signal promotes the accumulation of certain subsets of DCs in vivo.

**mTORC1 regulates IL-10 expression in LP-DCs**

LP-CD11b<sup>+</sup> cells spontaneously produce IL-10, a potent anti-inflammatory cytokine, and regulate intestinal immunity and tolerance (14, 15). Because we have previously shown that mTORC1 positively regulates IL-10 expression in BM-derived DCs (5), we examined whether mTORC1 is involved in IL-10 production by LP-CD11c<sup>+</sup>CD11b<sup>+</sup> DCs. The phosphorylation level of S6 in CD11c<sup>+</sup>CD11b<sup>+</sup> and CD11c<sup>+</sup>CD11b<sup>+</sup> DCs but not CD11c<sup>+</sup>CD11b<sup>+</sup> macrophages of Raport<sup>DC−/−</sup> mice was reduced, confirming the attenuation of mTORC1 signal (Fig. 3A). Because CX<sub>3</sub>CR1<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup> LP cells are major IL-10 producers (15), we checked IL-10 production by LP-CD11c<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup> DCs and found that IL-10 expression was impaired at both protein and mRNA levels in Raport<sup>DC−/−</sup> mice (Fig. 3B). In addition, rapamycin suppressed IL-10 expression at both protein and mRNA levels in wild-type LP-CD11c<sup>+</sup>CD11b<sup>+</sup>F4/80<sup>+</sup> DCs irrespective of LPS stimulation (Fig. 3C). These results clearly indicate that mTORC1 positively regulates IL-10 expression in LP-DCs. Because IL-10 is known to suppress CD86 expression in macrophages and CD11c<sup>+</sup>CD11b<sup>+</sup> DCs (5), we examined whether mTORC1 is involved in IL-10 production by LP-DCs and macrophages. Indeed, rapamycin inhibited apoptosis of LPS-stimulated but not unstimulated DCs (20). It could be possible that LP-CD11c<sup>+</sup>CD11b<sup>+</sup> DCs, a subpopulation of CX<sub>3</sub>CR1<sup>+</sup>CD11b<sup>+</sup> cells, are constitutively exposed to bacteria-derived TLR ligand under steady-state conditions (21).

In mouse inflammatory bowel disease models, IL-10 production by LP-DCs and macrophages contributes to the suppression of macrophage activation and Th1/Th17 differentiation and to the maintenance of regulatory T cell function (11, 14, 22). Acute DSS-induced colitis is accompanied by macrophage activation and subsequent secretion of inflammatory cytokines and is viewed as a T cell-independent model. Because proportions of Th1, Th17, and regulatory T cells in intestinal LP were normal in Raport<sup>DC−/−</sup> mice (data not shown), exacerbation of DSS-induced colitis in Raport<sup>DC−/−</sup> mice might be caused by attenuation of the IL-10 suppressive effect on macrophages. The role of mTORC1 signal in DC function to regulate acquired immunity will be elucidated in future studies.

**Acknowledgments**

We thank Dr. Tatsuo Kinashi and the staff of the laboratory at Kansai Medical University for valuable discussions.

**Disclosures**

S.K. is a consultant for Medical and Biological Laboratories Co., Ltd. The other authors have no financial conflicts of interest.

**References**


Supplementary figure 1. (A) TLR ligand-induced IFN-α production by pDCs of RaptorDC-/− mice is intact. Sorted BM-derived CD11c+PDCA-1+ pDCs were stimulated with the indicated concentrations of CpG-A (TLR9 ligand) or 1 μg/ml R848 (TLR7 ligand) for 24 h. IFN-α in culture supernatants were assessed by ELISA. N.D., not detected. (B, C) The expansion of splenic CD8+ cDCs and increased CD86 expression of LP-CD11c+CD11b+ DCs of RaptorDC-/− mice are caused in a cell-intrinsic manner. FACS analysis of splenocytes (B) or LI-LPMCs (C) in (CD45.1 x CD45.2)-Rag2−/− mice which were irradiated at 4 Gy and reconstituted with CD45.1-Raptorfl/fl and CD45.2-RaptorDC-/− mouse-derived BM cells (2.5 x 10⁶ cells each). (B) Indicated are the proportions of CD8+ cells in splenic CD11c highPDCA-1− cDCs derived from Raptorfl/fl or RaptorDC-/− BM in the same recipient mice. (C) The proportions of three APC subsets derived from Raptorfl/fl or RaptorDC-/− BM in the same recipient mice were evaluated (upper panel). *p<0.05, Paired t test. Expression levels of CD86 in three APC subsets in the same recipient mice were compared between Raptorfl/fl or RaptorDC-/−. Indicated are representative of three (lower panel).
**Supplementary figure 2.** Expression of apoptosis-related genes in $Raptor^{DC/-}$ mice-derived LP-CD11c$^+$CD11b$^+$ DCs. (A) Sorted LP-CD11c$^+$CD11b$^+$ DCs from $Raptor^{fl/fl}$ or $Raptor^{DC/-}$ mice were incubated in complete medium for 30 min. Cell lysates were analyzed for expression level of the indicated proteins by Western blotting. (B) RNAs of sorted LI-LP-CD11c$^+$CD11b$^+$ DCs from $Raptor^{fl/fl}$ or $Raptor^{DC/-}$ mice were isolated. mRNA levels of anti-apoptotic genes such as Bcl-2 and Bcl-xL as well as pro-apoptotic gene Bim were assessed by real-time PCR using cyclophilin A mRNA as a reference.