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

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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\textsuperscript{DC−/−}). Raptor\textsuperscript{DC−/−} mice had increased numbers of splenic CD8\textsuperscript{+} DCs and intestinal lamina propria (LP)-CD11c\textsuperscript{+}CD11b\textsuperscript{+} 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\textsuperscript{+}CD11b\textsuperscript{+} DCs and that Raptor\textsuperscript{DC−/−} mice were highly susceptible to dextran sodium sulfate-induced colitis. Our results uncover mTORC1-mediated anti-inflammatory programs in intestinal CD11c\textsuperscript{+}CD11b\textsuperscript{+} DCs to limit the intestinal inflammation. The Journal of Immunology, 2012, 188: 4736–4740.

Abbreviations used in this article: BM, bone marrow; cDC, conventional dendritic cell; DC, dendritic cell; DSS, dextran sodium sulfate; 4E-BP1, eukaryotic initiation factor 4E-binding protein 1; 4E-BP1, phosphatase and tensin homolog deleted on chromosome 10; SI, small intestine.

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To clarify the role of mTORC1 in DC function in vivo, we established DC-specific Raptor knockout mice (referred to here as Raptor\textsuperscript{DC−/−}). Raptor\textsuperscript{DC−/−} mice had increased numbers of splenic CD8\textsuperscript{+} DGs and intestinal lamina propria (LP)-CD11c\textsuperscript{+}CD11b\textsuperscript{+} DGs, 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 LP-DCs and that Raptor\textsuperscript{DC−/−} mice display increased susceptibility to dextran sodium sulfate (DSS)-induced colitis.
(C57BL/6; Jax no. 008608). C57BL/6-Rag-2−/− (CD45.2) mice and B6.SJL-Rag-2−/− (CD45.5) 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, 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, BioLegend, or eBioscience) after Fc receptor blockade. For the analysis of intracellular phospho-S6 levels, cells stained for cell surface marker were incubated in complete medium and then fixed and permeabilized with IntraPrep (Beckman Coulter). Fixed cells were stained for phospho-S6 (Cell Signaling Technology). For the analysis of intracellular IL-10, cells were incubated 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 (Raptor^{B/B}) with transgenic mice (CD11c-Cre). In Raptor^{B/B} × CD11c-Cre mice, referred to here as Raptor^{DC−/−}, 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 Raptor^{DC−/−} 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

[FIGURE 1. mTORC1 signal is suppressed in Raptor^{DC−/−} DCs. (A) Sorted splenic cDCs from Raptor^{R/R} or Raptor^{DC−/−} mice were incubated in PBS or complete medium for 30 min. Cell lysates were analyzed for the expression levels of the indicated proteins by Western blotting. Note that the mobility shifts of p70S6K and 4E-BP1 caused by multiple phosphorylation sites were observed. (B) Splenic DCs from Raptor^{R/R} or Raptor^{DC−/−} mice were stimulated as in (A). The expression levels of phospho-S6 in cDCs (CD11b^{high}PDCA1+) and pDCs (CD11c^{PDCA1+}) were assessed by FACS. Data are representative of three independent experiments.](http://www.jimmunol.org/)

[FIGURE 2. DC populations in Raptor^{DC−/−} mice. FACS analysis of BM cells (A), splenocytes (B), and LI-LPMCs (C) isolated from Raptor^{R/R} (n = 5) or Raptor^{DC−/−} (n = 5) mice. (A) Expression levels of MHC II and CD86 were analyzed in CD11c+ PDCA1+ cells and CD11c+PDCA1−pDCs. Raptor deficiency had marginal effect on expression levels of MHC II and CD86. (B) CD11b^{high}PDCA1+ cDCs and CD11c^{PDCA1+} pDCs (upper dot plots) were analyzed for MHC II and CD86 expression. cDCs were further subdivided by CD8 and CD11b expressions (lower dot plots). (C) LI-LPMCs were divided into CD11c^{CD11b+}, CD11c^{CD11b−}, and CD11c^{CD11b+} populations, and expression levels of CD103, F4/80, MHC II, and CD86 were compared. Numbers adjacent to outlined areas or in quadrants indicate percent cells in each as means ± SD. All data are representative of five mice per group. Similar results were obtained with three independent experiments. *p < 0.05, **p < 0.01.](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 Raptor<sup>DC⁻/⁻</sup> mice compared with Raptor<sup>B/B</sup> mice (Fig. 1A). In contrast, Raptor<sup>DC⁻/⁻</sup>-derived cells exhibited enhanced phosphorylation of Akt at Ser<sup>473</sup> and Thr<sup>308</sup> irrespective of stimulation (Fig. 1A), suggesting constitutive activation of PI3K signal (12). Intracellular phosphorylation of S6 revealed the attenuation of the mTORC1 activity in both CD11c<sup>hib</sup>PDCA-1<sup>⁻</sup> conventional dendritic cells (cDCs) and CD11c<sup>PDCA-1⁺</sup> plasmacytoid dendritic cells (pDCs) in Raptor<sup>DC⁻/⁻</sup> mice (Fig. 1B).

Rapton<sup>DC⁻/⁻</sup> mice showed increased proportion of CD11c<sup>PDCA-1⁺</sup> pDCs (Fig. 2A) and increased numbers of CD11c<sup>PDCA-1⁺</sup> cells (5.33 ± 0.37 × 10<sup>5</sup> cells versus 3.33 ± 0.28 × 10<sup>5</sup> cells in Rapton<sup>B/B</sup> p < 0.01) and CD11c<sup>PDCA-1⁺</sup> pDCs (11.79 ± 0.94 × 10<sup>5</sup> cells versus 7.53 ± 2.92 × 10<sup>5</sup> cells in Rapton<sup>B/B</sup> p < 0.05) in the bone marrow (BM). Although mTORC1 signal is reported positively to regulate type I IFN production in pDCs (13), IFN-α production by pDCs of Rapton<sup>DC⁻/⁻</sup> mice induced by TLR ligands, such as CpG-A and R848, was unaffected compared with IFN-α production by pDCs of Rapton<sup>B/B</sup> mice in vitro (Supplemental Fig. 1A). We cannot, however, formally rule out the possibility that residual mTORC1 signal, due to inefficient deletion of Rapton in pDCs of Rapton<sup>DC⁻/⁻</sup> 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 Rapton<sup>DC⁻/⁻</sup> mice revealed that the CD8<sup⁺</sup> cDC population was specifically expanded (2.82 ± 0.66 × 10<sup>5</sup> cells versus 1.62 ± 0.67 × 10<sup>5</sup> cells in Rapton<sup>B/B</sup> 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 Rapton<sup>DC⁻/⁻</sup> mice (Fig. 2B). Expansion of Rapton<sup>DC⁻/⁻</sup>-derived splenic CD8<sup⁺</sup> cDCs was confirmed by analysis of mixed BM chimera mice (Supplemental Fig. 1B). In contrast, the intestinal CD103<sup⁺</sup> cDC subset, which is a tissue counterpart of CD8<sup⁺</sup> cDCs and corresponds to LP-CD11c<sup⁺</sup>CD11b<sup⁻</sup> cDCs (data not shown), was not affected in Rapton<sup>DC⁻/⁻</sup> mice (Fig. 2C). Intriguingly, DC-specific Pten deletion leads to expansion of both splenic CD8<sup⁺</sup> cDCs and intestinal CD103<sup⁺</sup> cDCs (9). Future studies would reveal the molecular mechanism underlying the difference between DC-specific Pten deleted mice and Rapton<sup>DC⁻/⁻</sup> mice.

Intestinal APCs can be distinguished by the expression patterns of CD11c and CD11b (CD11c<sup⁻</sup>CD11b<sup⁻</sup> and CD11c<sup⁺</sup>CD11b<sup⁺</sup> DCs, and CD11c<sup⁻</sup>CD11b<sup⁺</sup> macrophages) (14). In Rapton<sup>DC⁻/⁻</sup> mice, the number of CD11c<sup⁺</sup>CD11b<sup⁺</sup> DCs that showed the CD103<sup⁺</sup>F4/80<sup⁻</sup> phenotype (22.8 ± 6.3 × 10<sup>4</sup> cells versus 11.4 ± 1.5 × 10<sup>4</sup> cells in Rapton<sup>B/B</sup>, p < 0.01), but not CD11c<sup⁻</sup>CD11b<sup⁻</sup> DCs or CD11c<sup⁻</sup>CD11b<sup⁺</sup> macrophages, was increased in the LP of large intestine (LI) and small intestine (SI) (Fig. 2C and data not shown). In addition, Rapton<sup>DC⁻/⁻</sup> mice showed higher CD86 expression compared with Rapton<sup>B/B</sup> mice.
in CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs in IL-1P and SI-LP compared with Raptor<sup><i>RGR</i></sup><sup><i>/mice</i></sup> (Fig. 2C and data not shown). The increased expression of CD86 in LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs of Raptor<sup><i>DC<sup><i>−</i></sup>/</i></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 Raptor<sup><i>RGR</i></sup> and Raptor<sup><i>DC<sup><i>−</i></sup>/</i></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><i>i</i></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><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs. The phosphorylation level of S6 in CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> and CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs but not CD11c<sup><i>c</i></sup>CD11b<sup><i>+</i></sup> macrophages of Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice was reduced, confirming the attenuation of mTORC1 (Fig. 3A). Because CX<sub>3</sub>CRI<sup><i>c</i></sup>CD11b<sup><i>i</i></sup>F<sup>4</sup>/80<sup>+</sup> cell DCs are major IL-10 producers (15), we checked IL-10 production by LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup>F<sup>4</sup>/80<sup>+</sup> DCs and found that IL-10 expression was impaired at both protein and mRNA levels in Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice (Fig. 3B). In addition, rapamycin suppressed IL-10 expression at both protein and mRNA levels in wild-type LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup>F<sup>4</sup>/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 LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs (16), it is likely that the increased expression of CD86 in LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs of Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice is caused by the reduction of IL-10 production by themselves. Consistently, we found higher CD86 expression in LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs of IL-10-deficient mice compared with wild-type mice (data not shown).

The lack of mTORC1 in DCs enhances DSS-induced colitis

The observation that Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs produced less IL-10, which is critically involved in the homeostasis of intestinal immune responses (17), prompted us to examine what impact the impaired mTORC1 signal in DCs has on the onset of inflammatory bowel disease. DSS treatment induced exaggerated body weight loss and reduction of colon length, which are a characteristic of intestinal inflammation, in Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice relative to Raptor<sup><i>RGR</i></sup> mice (Fig. 4A, 4B). Furthermore, histological analysis showed increased erosion value in the colon of Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice compared with Raptor<sup><i>RGR</i></sup> mice (Fig. 4C). Thus, the lack of mTORC1 signal in intestinal DCs likely induces a severe inflammatory response to enteric bacteria upon DSS treatment.

Given the general function of mTORC1 signal in promoting cell survival and proliferation, it may seem puzzling why the lack of mTORC1 signal in Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice does not lead to decrease of DC numbers compared with those of Raptor<sup><i>RGR</i></sup> mice. A recent report has demonstrated that rapamycin inhibits FLT3 ligand-driven BM development into cDCs and pDCs only when the drug is added to culture at the starting time point but not at a later time point (9). In addition, our Raptor-deficient DCs were obtained by a CD11c promoter-driven Cre-loxP system, which exhibits efficient recombination in all DC subsets but not in CD11c<sup><i>low</i></sup> DC progenitors (18). mTORC1 signal may thus be dispensable for survival of mature DC subsets.

We observed increased numbers of certain DC subsets, especially LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs, in Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice. Because Akt is a critical mediator of DC survival signal (19) and Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice showed enhanced Akt phosphorylation in splenic DCs (Fig. 1A) and LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs (Supplemental Fig. 2A), it is possible that increased numbers of DCs in Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice is caused by Akt-mediated survival signal. We failed, however, to find the differences in Bcl-2, Bcl-xL, and Bim mRNA levels, which are well-known targets of Akt signal, in LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs between Raptor<sup><i>RGR</i></sup> and Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice (Supplemental Fig. 2B). It still remains possible that Akt promotes survival through posttranslational regulation of other Bcl-2 family members like Bax and Bad. Alternatively, the lack of mTORC1 may directly promote survival of TLR ligand-stimulated LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs. Indeed, rapamycin inhibits apoptosis of LPS-stimulated but not unstimulated DCs (20). It could be possible that LP-CD11c<sup><i>c</i></sup>CD11b<sup><i>i</i></sup> DCs, a subpopulation of CX<sub>3</sub>CRI<sup><i>c</i></sup>CD11b<sup><i>i</i></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 Raptor<sup><i>DC<sup><i>−</i></sup>/</i></sup>mice (data not shown), exacerbation of DSS-induced colitis in Raptor<sup><i>DC<sup><i>−</i></sup>/</i></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.

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Disclosures

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