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

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

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The mammalian target of rapamycin (mTOR) controls cell growth and survival through two distinct complexes called mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2). Although several reports have suggested the involvement of mTORC1 in development and function of dendritic cells (DCs), its physiological roles remain obscure. We therefore established mTORC1 signal-deficient mice lacking Raptor, an essential component of mTORC1 signal, specifically in DC lineage (referred to here as RaptorDC−/−). RaptorDC−/− mice exhibited cell expansion in specific subsets of DCs such as splenic CD8⁺ DCs and intestinal CD11c⁺CD11b⁺ DCs. We also found that impaired mTORC1 signal resulted in the suppression of IL-10 production along with enhanced CD86 expression in intestinal CD11c⁺CD11b⁺ DCs and that RaptorDC−/− mice were highly susceptible to dextran sodium sulfate-induced colitis. Our results uncover mTORC1-mediated anti-inflammatory programs in intestinal CD11c⁺CD11b⁺ DCs to limit the intestinal inflammation. The Journal of Immunology, 2012, 188: 4736–4740.

The mammalian target of rapamycin (mTOR) is a Ser/Thr kinase that affects broad aspects of cellular functions including immunity. mTOR is the core component of mTOR complex 1 (mTORC1), which contains the adapter protein Raptor, whereas Rictor and SIN1 classify mTOR complex 2 (mTORC2). mTORC1 acts downstream of the PI3K–Akt pathway to phosphorylate p70 S6 kinase (p70S6K) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1) in a rapamycin-sensitive fashion (1, 2). By contrast, mTORC2 induces Akt at Ser473 phosphorylation (2, 3).

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 RaptorDC−/−). RaptorDC−/− mice had increased numbers of splenic CD8⁺ DGs and intestinal lamina propria (LP)-CD11c⁺CD11b⁺ 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 LP-DCs and that RaptorDC−/− mice display increased susceptibility to dextran sodium sulfate (DSS)-induced colitis.

Materials and Methods

Mice

Raptorfl/fl mice on a C57BL/6 × CBA mixed background (details will be described elsewhere) were back-crossed ≥8 generations onto the C57BL/6 background and then crossed with CD11c-BAC-Cre transgenic mice.

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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; LP, lamina propria; LPMC, lamina propria mononuclear cell; MHCII, MHC class II; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; pDC, plasmacytoid dendritic cell; p70S6K, p70 S6 kinase; Pten, phosphatase and tensin homologue deleted on chromosome 10; SI, small intestine.

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(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 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 FACS Canto II by using FACS Diva 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.

FIGURE 1. mTORC1 signal is suppressed in RaptorDC−/− mice. (A) Sorted splenic cDCs from Raptorfl/fl or RaptorDC−/− 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 Raptorfl/fl or RaptorDC−/− mice were stimulated as in (A). The expression levels of phospho-S6 in cDCs (CD11bhighPDCA1−) and pDCs (CD11c+PDCA1+) were assessed by FACS. Data are representative of three independent experiments.

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.
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

DC−/− mice compared with RaptoR

mice (Fig. 1A). In contrast, RaptoR

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

PGFCA-1− conventional dendritic cells (cDCs) and CD11c+PGFCA-1+ plasmacytoid dendritic cells (pDCs) in RaptoR

DC−/− mice (Fig. 1B).

RaptoR

DC−/− mice showed increased proportion of CD11c

PGDCA-1+ pDCs (Fig. 2A) and increased numbers of CD11c

PGDCA-1− cDCs (5.33 ± 0.37 × 105 cells versus 3.33 ± 0.28 × 105 cells in RaptoR

mice, p < 0.01) and CD11c

PGDCA-1+ pDCs (11.79 ± 0.94 × 105 cells versus 7.53 ± 2.92 × 105 cells in RaptoR

mice, 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 RaptoR

DC−/− mice induced by TLR ligands, such as CpG-A and R848, was unaffected compared to induction of mTORC1 activation. In splenic CD11c+ DCs, including nutrients and sera, well-known activators of mTORC1, we cannot, however, formally rule out the possibility that residual mTORC1 signal, due to inefficient deletion of RaptoR in pDCs of RaptoR

DC−/− 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 RaptoR

DC−/− mice revealed that the CD8+ cDC population was specifically expanded (2.82 ± 0.66 × 105 cells versus 1.62 ± 0.67 × 105 cells in RaptoR

mice, 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 RaptoR

DC−/− mice (Fig. 2B). Expansion of RaptoR

DC−/−-derived splenic CD8+ cDCs was confirmed by analysis of mixed BM chimera mice (Supplemental Fig. 1B). In contrast, the intestinal CD103+ cDC subset, which is a tissue counterpart of CD8+ cDCs and corresponds to LP-CD11c+CD11b− DCs (data not shown), was not affected in RaptoR

DC−/− mice (Fig. 2C).

Intuitively, DC-specific Pten deletion leads to expansion of both splenic CD8+ cDCs and intestinal CD103+ cDCs (9). Future studies would reveal the molecular mechanism underlying the difference between DC-specific Pten deleted mice and RaptoR

DC−/− 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 RaptoR

DC−/− 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 RaptoR

mice, 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, RaptoR

DC−/− mice showed higher CD86 expression with IFN-α production by pDCs of RaptoR

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 RaptoR

DC−/− mice where CD11c expression is relatively low (Fig. 2A, 2B), is sufficient for TLR ligand-induced IFN-α production.

FIGURE 3. Impaired IL-10 expression in RaptoR-deficient LP-DCs. (A) LI-LPMCs from RaptoR

or RaptoR

DC−/− 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, LI-LPMCs from RaptoR

(n = 3) or RaptoR

DC−/− (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 RaptoR

(n = 3) or RaptoR

DC−/− (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 pre-treated 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. RaptoR

(n = 8) or RaptoR

DC−/− (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 (left) 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 LI-LP and SI-LP compared with Raptor<sup>fl/fl</sup> mice (Fig. 2C and data not shown). The increased expression of CD86 in LP-CD11c<sup>+</sup>CD11b<sup>+</sup> DCs of Raptor<sup>DC<sup>−/−</sup></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>fl/fl</sup> and Raptor<sup>DC<sup>−/−</sup></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 Raptor<sup>DC<sup>−/−</sup></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 Raptor<sup>DC<sup>−/−</sup></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 and LP-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 DCs in vitro (16), it is likely that the increased expression of CD86 in LP-CD11c<sup>+</sup>CD11b<sup>+</sup> DCs of Raptor<sup>DC<sup>−/−</sup></sup> mice is caused by the reduction of IL-10 production by themselves. Consistently, we found higher CD86 expression in LP-CD11c<sup>+</sup>CD11b<sup>+</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>DC<sup>−/−</sup></sup> LP-CD11c<sup>+</sup>CD11b<sup>+</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>DC<sup>−/−</sup></sup> mice relative to Raptor<sup>fl/fl</sup> mice (Fig. 4A, 4B). Furthermore, histological analysis showed increased erosion value in the colon of Raptor<sup>DC<sup>−/−</sup></sup> mice compared with Raptor<sup>fl/fl</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>DC<sup>−/−</sup></sup> mice does not lead to decrease of DC numbers compared with those of Raptor<sup>fl/fl</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<sup>+</sup> promoter-driven Cre-loxP system, which exhibits efficient recombination in all DC subsets but not in CD11c<sup>low</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>+</sup>CD11b<sup>+</sup> DCs, in Raptor<sup>DC<sup>−/−</sup></sup> mice. Because Akt is a critical mediator of DC survival signal (19) and Raptor<sup>DC<sup>−/−</sup></sup> mice showed enhanced Akt phosphorylation in splenic DCs (Fig. 1A) and LP-CD11c<sup>+</sup>CD11b<sup>+</sup> DCs (Supplemental Fig. 2A), it is possible that increased numbers of DCs in Raptor<sup>DC<sup>−/−</sup></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>+</sup>CD11b<sup>+</sup> DCs between Raptor<sup>fl/fl</sup> and Raptor<sup>DC<sup>−/−</sup></sup> mice (Supplemental Fig. 2B). It still remains possible that Akt promotes survival through protranslational 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>+</sup>CD11b<sup>+</sup> DCs. Indeed, rapamycin inhibits 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 differentation 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>DC<sup>−<sup>2</sup></sup></sup> mice (data not shown), exacerbation of DSS-induced colitis in Raptor<sup>DC<sup>−<sup>2</sup></sup></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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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<sup>DC-/-</sup> mice-derived LP-CD11c<sup>+</sup>CD11b<sup>+</sup> DCs. (A) Sorted LP-CD11c<sup>+</sup>CD11b<sup>+</sup> DCs from Raptor<sup>fl/fl</sup> or Raptor<sup>DC-/-</sup> 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<sup>+</sup>CD11b<sup>+</sup> DCs from Raptor<sup>fl/fl</sup> or Raptor<sup>DC-/-</sup> 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.