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Control of T Cell Fates and Immune Tolerance by p38α Signaling in Mucosal CD103+ Dendritic Cells

Gonghua Huang, Yanyan Wang, and Hongbo Chi

Dendritic cells (DCs) play a crucial role in launching protective adaptive immunity against pathogens while maintaining immune tolerance to self-Ags. However, how intracellular signaling pathways program DCs to mediate tolerogenic responses remains largely unexplored. In this study, we describe that p38α signaling in CD103+ mesenteric lymph node DCs reciprocally regulates the differentiation of anti-inflammatory induced regulatory T cells and proinflammatory Th1 cells from naive precursors and promotes mucosal tolerance. Deficiency of p38α in CD103+ DCs inhibited the generation of induced regulatory T cells while promoting Th1 cell development in a TGF-β2–dependent manner. Consequently, loss of p38α in DCs prevented induction of oral tolerance in vivo. Moreover, p38α in CD103+ DCs was required for optimal expression of retinaldehyde dehydrogenase, a key enzyme for retinoic acid synthesis, which in turn imprinted gut-homing receptors on responding T cells. Consistent with a crucial role of p38α to program the tolerogenic activity of CD103+ DCs, such DC subset contained constitutive activity of p38α and abundant expression of TGF-β2 and retinaldehyde dehydrogenase. Our studies identify a key mechanism of DC-mediated coupling of T cell differentiation and trafficking that orchestrates mucosal immune tolerance. The Journal of Immunology, 2013, 191: 650–659.

The primary function of the immune system, comprised of the interconnected innate and adaptive arms, is to launch a robust immune response toward pathogens while maintaining tolerance to self-Ags. Although much progress has been made in understanding the central role of innate immunity in engaging adaptive immune reactions for defense against pathogens, we are just beginning to appreciate how innate signals contribute to immune tolerance. The most important cell type that bridges innate and adaptive immunity is dendritic cells (DCs). DCs serve as potent APCs to mediate the activation and differentiation of naive T cells (1, 2). Recent studies have also established a crucial role of DCs in maintaining immune tolerance (3, 4), and depletion of DCs disrupts self-tolerance and results in myeloid inflammation and autoimmunity (5, 6). However, the underlying mechanisms by which DCs dictate protective immunity versus immune tolerance remain incompletely understood.

The GALT is the largest immune organ in the body. Because the intestine is constantly exposed to copious amounts of food Ags, foreign pathogens, and commensal microbes, intestinal homeostasis arises from a dynamic balance between protective immunity and regulatory mechanisms (7). One of the major regulatory mechanisms in the GALT is the de novo induction of Foxp3 expression from conventional CD4 T cells in response to intestinal Ags (8, 9). These induced regulatory T cells (iTregs) play an important role in intestinal immune homeostasis under steady-state (10, 11), and they contribute to tolerance induced by ingested Ags, namely oral tolerance (12). Induction of oral tolerance also relies on mesenteric lymph nodes (MLNs) and Ag carriage by DCs (13). A major DC subset in the intestinal lamina propria (LP) is CD103+ DCs, which constitutively traffic to MLNs where they promote tolerogenic responses (14, 15). Specifically, these DCs produce high levels of retinoic acid (RA), TGF-β, and other immunoregulatory molecules to induce iTreg generation and imprint gut-homing receptors, thereby facilitating intestinal immune tolerance (16–18). Despite these exciting advances on the role of DCs in intestinal tolerance, the intracellular signaling networks that program DCs to become tolerogenic are largely unexplored.

MAPKs, including ERK, JNK, and p38, constitute one of the central pathways activated by innate immune signals (19, 20). Excessive activation of MAPKs is associated with many autoimmune and inflammatory diseases. Negative regulation of MAPK activities is effected mainly through a group of phosphatases known as MAPK phosphatases (MKPs). Our recent studies have established that an intracellular signaling axis comprised of p38α and MKP-1 acts in DCs to dictate T cell fates, especially Th17 differentiation, and thus program effector T cell–mediated inflammatory and autoimmune diseases (21, 22). In contrast, the roles of this signaling pathway in DC-mediated tolerogenic responses are poorly defined.

To investigate the function of p38 signaling in DC-mediated intestinal immune tolerance, we used a genetic model with DC-specific ablation of p38α (p38αΔDC). Loss of p38α signaling in DCs impaired induction of oral tolerance and generation of Ag-specific iTregs in vivo. Mechanistically, p38 signaling is constitutively activated in CD103+ MLN DCs to mediate the generation of iTregs while inhibiting Th1 cell development in a TGF-β2–dependent manner. Moreover, p38α signaling in CD103+ DCs was important for the induction of gut-homing molecules on responding T cells, partly by regulating the expression of a rate-limiting enzyme in RA synthesis. Therefore, DC-derived p38α signaling coordinates T cell differentiation and trafficking by af-
fecting the expression of distinct downstream effector molecules. Altogether, our studies identify a key mechanism of DC-mediated control of T cell fates and immune tolerance in intestinal immune homeostasis.

**Materials and Methods**

**Mice**

C57BL/6, Thy1.1*, OT-II, Rag1−/−, I-κB−/−, IκBα−/−, and CD4-dTGFβRII mice were from The Jackson Laboratory. CD11c-Cre, CD4-Cre, and p38αfl/fl mice have been described (22). Fox3pYFP-Cre mice were provided by Dr. Alexander Rudensky (23). All mice had been backcrossed to the C57BL/6 background for at least eight generations and were used at an age of 6–12 wk. Wild-type (WT) control mice were of the same genetic background and, where relevant, included Cre+ mice to account for the effects of Cre. All mice were kept in specific pathogen-free conditions in Animal Resource Center at St. Jude Children’s Research Hospital. Animal protocols were approved by the Institutional Animal Care and Use Committee of St. Jude Children’s Research Hospital.

**Oral tolerance**

OT-II or p38αΔD mice were administered intragastrically with OVA protein (2 mg/mouse) daily for 5 d. Control mice were given PBS alone. All mice were then immunized s.c. with 100 μg OVA protein emulsified in CFA. Seven days after immunization, splenocytes were isolated and stimulated with OVA for 3 d; proliferation was assayed after pulsing with [3H]thymidine for the final 8 h, and IFN-γ secretion was measured by Bio-Plex (Millipore).

**Adaptive transfer and in vivo challenges**

Naïve T cells (CD4+CD62L+CD44hiCD25−) were sorted from mice and transferred into recipient mice (donor and recipient cells were distinguished by the congenic markers Thy1.1 and Thy1.2). For oral Ag challenge, after 24 h, recipients were fed with OVA (20 mg/ml grade VI OVA; Sigma-Aldrich) in the drinking water for 5 d, followed by analysis of MLN cells by FACS. For Rag1−/− recipients, at 7 d after transfer, MLN cells were analyzed by FACS.

**Cell purification and culture**

Mouse spleen and MLNs were digested with collagenase D, and DCs (CD11c+MHC class II+TCR β+CD19−DX5−) for spleen DCs; CD11c+MHC class II+TCR β+CD19−DX5−CD103+ or CD103− for MLN DCs and, where indicated, CD11b+ DCs were further divided into CD11b+ and CD11b− subsets) were sorted on a Reflection (i-Cyt). Lymphocytes were sorted for naïve T cells and were labeled with CFSE (Invitrogen) where indicated. For DC:T cell cocultures, 2.5 × 10^5 DCs and 2.5 × 10^5 T cells were mixed in the presence of the cognate peptide (0.05 or 0.1 μg/ml OVA) or 0.1 or 10 μg/ml anti-CD3 (2C11; Bio X Cell). After 5 d of culture, live T cells were collected for Foxp3 staining (FJK-16S; eBioscience) or RNA analysis directly.

**Isolation of LP DCs**

The isolation of LP DCs was as described (24) with slight modifications. Briefly, after excising Peyer’s patches, the small and large intestines were opened longitudinally and washed twice in PBS. Epithelial cells were separated from the underlying LP by incubation in HBSS containing 5 mM EDTA for 15 min at 37°C with vigorous shaking. LP tissue was pulse-vortexed and washed twice in PBS. The remaining tissue was finely chopped with a razor blade and digested in a solution of 1 mg/ml collagenase type IV (Worthington Biochemical) and 5% PBS in HBSS for 20 min at 37°C. Tissue digestion was repeated twice. Leukocytes were isolated by underlayering with a Percoll (GE Healthcare) gradient separation method in which the cells were resuspended in 40% Percoll and underlayered with 80% Percoll followed by centrifugation at 2500 rpm for 20 min. The interface was collected for FACS analysis and sorting.

**Flow cytometry**

For analysis of surface markers, cells were stained in PBS containing 2% (v/v) BSA and surface Abs, as described (22). For intracellular phosphorylation assays, cells were stained with Abs for phosphorylated p38 (4552; Cell Signaling Technology) and ERK (612593; BD Biosciences) according to the manufacturers’ instructions. Flow cytometry data were acquired on an upgraded five-color FACScan or LSRII (BD Biosciences) and were analyzed with FlowJo software (Tree Star).

**RNA and protein analysis**

Primer and probe sets from Applied Biosystems were used for real-time PCR; results were normalized to those of the endogenous control gene encoding hypoxanthine phosphoribosyltransferase as described (25). For Bio-Plex assays, cytokines in supernatants were measured with Milliplex kits for mouse cytokines according to the manufacturer’s instructions (Millipore). Immunoblot analyses were performed as described (26), with Abs for p-Smad3 at Ser423 (ab51451; Abcam) and β-actin (AC-15; Sigma-Aldrich).

**Statistical analysis**

The p values were calculated using a Student t test. A p value < 0.05 was considered significant. All error bars in graphs represent SEM calculated from at least three replicates.

**Results**

p38α activity in DCs is required for immune tolerance and iTreg generation in vivo

To determine the role of p38α signaling in DC-mediated immune tolerance, we crossed p38αΔD mice with OT-II TCR transgenic mice (a TCR transgenic model with T cells recognizing OVA amino acids 323–339). We administered OVA protein (or PBS control) intragastrically for 5 consecutive days to induce oral tolerance, followed by s.c. immunization with OVA emulsified in CFA. After 7 d, proliferation and cytokine production of splenic T cells were examined in an ex vivo recall response. As expected, tolerance induction by oral OVA treatment actively suppressed both proliferation and IFN-γ production in T cells from WT mice (Fig. 1A, 1B). Although mice with DC-specific deletion of p38α did not show defects under the PBS treatment conditions, they failed to respond to oral induction by suppressing T cell proliferation or IFN-γ production (Fig. 1A, 1B). These data indicate that p38α activity in DCs is important for oral tolerance induction in vivo.

Because Tregs are important regulators of oral tolerance (12), we next analyzed expression of Foxp3 in p38αΔD mice. Importantly, development and homeostasis of thymus-derived natural Tregs were undisturbed in these mice (Supplemental Fig. 1A). We therefore focused on the role of DC-mediated p38α signaling for the induction of Ag-specific iTregs in a model of oral Ag-induced generation of iTregs (27, 28). To this end, we transferred congenically marked naïve CD4+ T cells (CD62L+CD44hiCD25−) from OT-II mice into WT or p38αΔD mice that were subsequently fed with OVA protein in the drinking water for a total of 5 d. As compared with donor-derived T cells from MLNs of WT mice, those in p38αΔD mice developed into Foxp3+ iTregs at a third of the efficiency (Fig. 1C, 1D). Similar defects were observed in Peyer’s patches and spleen (Fig. 1D). These data collectively establish an important role of p38α activity in DCs to mediate oral tolerance and iTreg generation in vivo.

CD103+ DCs employ p38α signaling to drive iTreg generation

To explore the specific DC subset that depends on p38α-dependent mechanisms for iTreg induction, we first examined whether DCs from various immune compartments had different abilities to drive iTreg generation. Specifically, we purified CD103+ and CD103− MLN DCs using the gating strategy described in Supplemental Fig. 1B, as well as splenic DCs, and cocultured them with naïve OT-II CD4+ T cells in the presence of Ag but with no exogenous
cytokines to mimic the physiological interaction between DCs and T cells. After 5 d of culture, T cells activated with CD103+ MLN DCs developed into a Foxp3+ population at a 3- to 5-fold higher frequency than those activated with CD103− or splenic DCs (Fig. 2A). These results indicate a strong tolerogenic property of CD103+ MLN DCs for iTreg induction, in agreement with previous observations (27–29). We next examined signaling pathways in DC subsets. Importantly, CD103+ MLN DCs contained a higher p38 activity than did other DC populations under steady-state conditions (Fig. 2B). Furthermore, p38α was expressed at much higher levels than other family members, including p38β, p38γ, and p38δ in CD103+ DCs (Supplemental Fig. 1C). In contrast, activity of the related ERK pathway was reduced in these DC subsets. Importantly, CD103+ MLN DCs contained p38α activity (Fig. 2B). These results highlight a likely involvement of p38α in mediating the tolerogenic activity of CD103+ DCs.

To directly test this hypothesis, we analyzed MLN DCs from p38α−/− mice. Staining with surface markers CD103, CD11b, and CD8 did not reveal obvious defects in the development or homeostasis of DC subsets (Supplemental Fig. 1D, 1E). We next determined whether p38α mediated the tolerogenic activity of CD103+ DCs by coculturing naive OT-II T cells with CD103+ MLN DCs from WT or p38α−/− mice. Under these conditions, proliferation and apoptosis of T cells were comparable when stimulated with WT or p38α−/− DCs. We additionally examined whether the activity of p38α in CD103+ MLN DCs is selectively activated in CD103+ MLN DCs as an important mechanism to mediate iTreg induction in vitro. We explored the molecular mechanisms whereby p38α acted in CD103+ DCs to regulate iTreg differentiation. Arguably the most potent cytokine for iTreg generation mediated by p38α-deficient DCs (Fig. 2D). Mechanistically, the effect of DC-derived p38α signaling could be explained, at least partially, by its role in the maintenance of Foxp3 expression in mature iTregs, considering the emerging evidence that iTregs are more plastic than previously appreciated and may not stably maintain Foxp3 expression (30). To test this possibility, we first activated naive T cells isolated from Foxp3+/YFP Cre mice (23) with WT CD103+ DCs for 4 d and then sorted YFP+ cells and stimulated them with WT or p38α-deficient DCs in the second-round culture. We found that ∼50% of iTregs lost Foxp3 expression independent of p38α activity, indicating that p38α in CD103+ DCs promotes the induction but not maintenance of iTregs (Supplemental Fig. 2C).

In contrast to the role of p38α in CD103+ MLN DCs, T cells activated with CD103− DCs from WT or p38α−/− mice expressed low but comparable levels of Foxp3 (Fig. 2E). Thus, consistent with a lower p38 activity in CD103− DCs (Fig. 2B), p38α in these cells is dispensable for iTreg generation. We therefore conclude that p38α is selectively activated in CD103+ MLN DCs as an important mechanism to mediate iTreg induction in vitro.

p38α-dependent TGF-β2 expression in CD103+ DCs promotes iTreg generation

We explored the molecular mechanisms whereby p38α acted in CD103+ DCs to regulate iTreg differentiation. Arguably the most potent cytokine for iTreg differentiation is TGF-β (31). Among the three TGF-β isoforms, Tgfb2 was expressed at a much higher level in CD103+ MLN DCs as compared with other DC subsets, as previously reported (27), whereas Tgfb1 or Tgfb3 was not selectively expressed by CD103+ DCs (Fig. 3A). Notably, TGF-β...
activity in CD103+ and CD103- T cells (Fig. 3C). Interestingly, whereas mediated by CD103+ DCs from either WT or p38- deficient CD103+ MLN DCs, expression of Foxp3 in OT-II T cells activated with WT or p38- deficient CD103+ MLN DCs, in the presence of the indicated doses of anti-CD3, for 5 d. (E) Expression of Foxp3 in polyclonal T cells activated with WT or p38α-deficient CD103+ MLN DCs, in the presence of the indicated doses of OVA, for 5 d. Data are representative of two [(A, D, E), n = 4 mice/group], three [(B), n = 4 mice/group], and four [(C), n ≥ 5 mice/group] independent experiments.

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FIGURE 2. p38α is selectively activated in CD103+ MLN DCs and is required for these DCs to mediate iTreg generation in vitro. (A) Expression of Foxp3 in OT-II T cells activated with CD103+ and CD103- MLN DCs and splenic DCs, in the presence of OVA (0.05 μg/ml), for 5 d. (B) p38 and ERK activity in CD103+ and CD103- MLN DCs and splenic DCs. Pooled samples of all three DC populations were used for isotype staining. (C) Expression of Foxp3 in OT-II T cells activated with WT or p38α-deficient CD103+ MLN DCs, in the presence of the indicated doses of anti-CD3, for 5 d. (D) Expression of Foxp3 in polyclonal T cells activated with WT or p38α-deficient CD103+ MLN DCs, in the presence of the indicated doses of anti-CD3, for 5 d. Data are representative of two [(A, D, E), n = 4 mice/group], three [(B), n = 4 mice/group], and four [(C), n ≥ 5 mice/group] independent experiments.

proteins are synthesized as latent precursors that require integrin-dependent activation, in a process dependent on the integrin αvβ8, expressed by DCs (32, 33). We found that integrin β8 (encoded by Itgb8), but not αv (Itgav), was selectively expressed by CD103+ MLN DCs (Fig. 3A). Importantly, deletion of p38α in CD103+ DCs considerably diminished the expression of Tgfb2 and Itgb8, but not Tgfb1, Tgfb3, or Itgav (Fig. 3B), further supporting a model that p38α endows such DCs with a selective tolerogenic activity. Consistent with the requirement of p38α in TGF-β2 mRNA expression and functional activation, CD103+ DCs lacking p38α were impaired to induce Smad3 activation in cocultured T cells (Fig. 3C). Interestingly, whereas Foxp3 mRNA induction was defective in T cells activated with p38α-deficient CD103+ DCs, expression of other TGF-β target genes, including Gfi1 and Smad7, was unaltered (Fig. 3D). These results are in agreement with a crucial and selective role of Smad3 in transducing TGF-β signals for iTreg generation (34). Therefore, p38α activates the TGF-β2-Smad3 axis at the DC/T cell interface.

We next assessed the functional importance of p38α-dependent TGF-β2 expression. Addition of TGF-β2 to the DC/T cell cocultures restored the defective Foxp3 induction, as well as the decreased phosphorylation of Smad3, in T cells activated by p38α-deficient CD103+ DCs (Fig. 3E, 3F). Conversely, neutralizing TGF-β activity in the cocultures abrogated iTreg development mediated by CD103+ DCs from either WT or p38αΔDC mice (Fig. 3G). Similar findings were obtained when we used T cells expressing the dominant negative TGF-βR2 molecule to block TGF-β signaling (Fig. 3H) (35). Therefore, p38α-dependent TGF-β2 expression in CD103+ DCs plays a crucial role in mediating iTreg generation.

p38α in CD103+ DCs mediates iTreg induction independently of proinflammatory cytokines

The differentiation of iTregs is not only driven by tolerogenic cytokines such as TGF-β, but is also shaped by proinflammatory cytokines. IL-27 is a potent cytokine limiting iTreg differentiation (36). IL-27 expression was increased in p38α-deficient CD103+ DCs (Fig. 4A), similar to our recent observations in splenic DCs (22). However, blockade of IL-27 had little effect on restoring the defective iTreg differentiation mediated by the mutant DCs (Fig. 4B). IL-6 is another proinflammatory cytokine for inhibiting iTreg differentiation (30), and its secretion by splenic DCs is affected by p38α deficiency (22). To test the role of IL-6 in p38α-dependent DC/T cell crosstalk, we crossed p38αΔDC mice with mice deficient in IL-6. Compared with CD103+ DCs deficient in IL-6, those lacking both IL-6 and p38α showed a decreased ability to drive iTreg differentiation, similar to the effects of p38α deficiency on an IL-6–sufficient background (Fig. 4C). Similarly, we did not find a role of IL-12 produced from p38α-deficient DCs in this process (Fig. 4C). Altogether, p38α signaling in DCs regulates iTreg generation largely independently of the proinflammatory cytokines IL-27, IL-6, and IL-12, further highlighting a key role for p38α in specifically programming the tolerogenic activity of CD103+ DCs.

DC-derived p38α signaling reciprocally regulates iTreg and Th1 differentiation

We recently described that naive T cells can engage reciprocal developmental pathways to generate iTreg and Th1 cells (25, 37). Indeed, associated with enhanced ability to drive iTreg generation (Fig. 2A), CD103+ MLN DCs were less potent at mediating Th1 differentiation as compared with CD103- MLN or splenic DCs (Fig. 5A). We next determined whether CD103+ DCs employed p38α signaling to actively suppress the differentiation of Th1 cells. First, we adoptively transferred naive T cells from C57BL/6 mice into Rag1−/− recipients as compared with CD103− MLN or splenic DCs (Fig. 5B), indicating that p38α activity in DCs suppresses IFN-γ production in T cells in vivo. Second, in the
CD103+ DC/T cell cocultures, T cells activated with p38α-deficient DCs showed a greater degree of Th1 differentiation (Fig. 5C), associated with higher Tbx21 (encoding T-bet) and Ifng expression (Fig. 5D). Therefore, p38α activity in CD103+ DCs exerts reciprocal effects on Th1 and iTreg differentiation.

Role of p38α in distinct subsets and origins of intestinal CD103+ DCs

The intestinal CD103+ DCs can be further divided into two subsets on the basis of CD11b expression (38, 39). To determine the roles of p38α signaling in these two DC subsets in mediating T cell...
demonstrated), we purified CD103+CD11b− and CD103+CD11b− MLN DCs and cocultured them with naive OT-II CD4+ T cells. Compared with T cells activated by CD103+CD11b+ DCs, those activated by CD103+CD11b− DCs developed into more Foxp3+ but less IFN-γ+ cells, indicating a stronger tolerogenic activity of CD103+CD11b− DCs (Fig. 5F, 5G). Importantly, loss of p38α in both DC subsets diminished iTreg but enhanced Th1 differentiation, thereby reinforcing the key role of p38α in mediating DC-dependent reciprocal differentiation of these T cell lineages (Fig. 5F, 5G). Under these conditions, IL-17+ cells were barely detectable by intracellular staining (data not shown). However, RNA analysis indicated that p38α signaling in both DC subsets was required for Th17 differentiation (Fig. 5H), in agreement with our recent finding for the requirement of p38α signaling in splenic DCs for driving Th17 differentiation (22).

Recent studies have identified discrete immune functions of DC subsets in LP. For example, the CD103+CD11b+ DC subset in LP plays an important role in mediating Th17 responses (24, 40). However, how intracellular signaling programs the tolerogenic activity of LP DC subsets remains poorly understood. We therefore purified CD103+CD11b+ and CD103+CD11b− LP DCs from WT and p38α−/− mice and examined their ability to drive iTreg differentiation. Similar to our observations in MLN DCs, CD103+CD11b− LP DCs had a stronger ability to induce T cell Foxp3 induction than did their CD103+CD11b+ counterpart, but both DC subsets depended on p38α activity for iTreg differentiation (Supplemental Fig. 3). We conclude that p38α signaling represents a crucial pathway to program the tolerogenic activity of mucosal CD103+ DCs of distinct subsets and origins.

**T cell–intrinsic p38α signaling is dispensable for iTreg or Th1 differentiation**

Published studies using p38 inhibitors have revealed an important role for T cell–intrinsic p38 activity in the generation of iTregs (41) and Th1 cells (19), but these effects have yet to be genetically defined. To this end, we crossed p38α−/− mice with CD4-Cre mice to achieve T cell–specific deletion of p38α (called p38αiV mice) (22). Naïve T cells from WT or p38αiV mice had a similar ability to develop into iTregs when stimulated with CD103+ or CD103− MLN DCs (Fig. 6A). Also, p38α-deficient T cells showed normal iTreg differentiation in response to in vitro polarizing conditions mediated by TGF-β (Fig. 6B). Furthermore, p38α-deficient T cells had no defects in Th1 differentiation in vitro (Fig. 6C). Collectively, our results exclude a T cell–intrinsic function of p38α in iTreg and Th1 generation, and they further highlight a selective requirement of p38α activity for DC-mediated T cell lineage differentiation.

p38α in CD103+ DCs imprints induction of gut-homing receptors on responding T cells

To perform immune functions, activated T cells in the GALT migrate preferentially to LP of the intestine, in a process dependent on induction of gut-homing receptors, including the integrin αβ7 and chemokine receptor CCR9. MLN DCs are essential for imprinting gut-homing specificity on T cells by producing RA to promote T cell expression of these trafficking molecules (16–18). Synthesis of RA from its precursor, vitamin A, depends on the enzyme retinaldehyde dehydrogenase, especially the major isoform retinaldehyde dehydrogenase (Aldh1a2) in intestinal DCs (16–18). Indeed, Aldh1a2 was highly expressed in CD103− MLN DCs compared with CD103+ DCs (Fig. 7A), but its level was considerably decreased following p38α deletion (Fig. 7B). To examine whether this defect alters trafficking molecules on responding T cells, we transferred naive OT-II T cells into WT or p38α−/− mice, followed by feeding with OVA protein in the drinking water. Expression of αβ7 and CCR9 on donor T cells from p38α−/− recipients was substantially reduced as compared with those from WT mice (Fig. 7C). Consistent with the in vivo findings, T cells stimulated with p38α-deficient CD103+ DCs expressed lower levels of αβ7 and CCR9 than did those stimulated with WT DCs (Fig. 7D). These results indicate that p38α is important for the expression of Aldh1a2 in CD103+ DCs and for DC-dependent induction of T cell trafficking molecules.

To identify the causative relationship between these two effects, we added exogenous RA into the cocultures. RA treatment restored the defective αβ7 and CCR9 expressions on T cells activated by p38α−/− DCs (Fig. 7E, 7F). In agreement with previous observations that RA acts as a cofactor for TGF-β–dependent Foxp3 induction (16–18), RA alone had minimal effects at promoting iTreg generation and was unable to rectify the diminished

![Image](http://www.jimmunol.org/DownloadedFrom/655)
iTreg generation mediated by p38α-deficient CD103+ DCs (Fig. 7G). Therefore, p38α-dependent Aldh1a2 expression in CD103+ DCs mainly contributes to the induction of gut-homing receptors but not Foxp3 expression from responding T cells (Fig. 7H).

Discussion
A fundamental question in immunology is how the decision between protective immunity and immune tolerance is made. DCs, the most potent cells for bridging innate and adaptive immunity, have emerged as a central regulator of this process. Much emphasis has been placed on DC-mediated cellular and molecular mechanisms that program T cell activation and effector differentiation (1, 2). Our recent identification of the p38α/MKP-1 axis for DC-dependent Th17 differentiation contributes to our understanding of the signaling mechanisms orchestrating this process (20–22). In contrast, we have little information on how intracellular signaling networks program DCs to become tolerogenic, a crucial function of DCs under steady-state (3, 4, 42). In this study, we describe that p38α is constitutively activated in CD103+ MLN DCs to program the tolerogenic activity of these DCs in the intestinal immune system. Loss of p38α activity in DCs prevents the induction of oral tolerance, highlighting the physiological significance of this novel signaling mechanism in vivo.

Recent studies have revealed specialized functions of CD103+ MLN DCs, relative to the CD103− counterpart and other DC subsets, in imprinting T cell gut-homing receptors and differentiative capacity toward iTregs (27, 29, 43, 44). Such tolerogenic properties of CD103+ DCs are mediated by the secretory factors,
especially RA and TGF-β, which can be further attributed to the exposure to the local conditioning factors in LP prior to their migration to MLNs (14). Despite the advances on the biology and origin of CD103+ DCs, what remains undefined is the molecular

FIGURE 6. T cell–intrinsic p38α activity is dispensable for Th1 or iTreg differentiation. (A) Expression of Foxp3+ in WT and p38αΔT naive T cells activated with CD103+ and CD103– MLN DCs, in the presence of anti-CD3 (0.5 μg/ml), for 5 d. (B) Expression of Foxp3 in WT and p38αΔT naive T cells differentiated under iTreg conditions for 5 d. (C) Expression of IFN-γ in WT and p38αΔT naive T cells differentiated under Th1 conditions for 5 d, followed by PMA and ionomycin stimulation and intracellular staining. Data are representative of two (n = 4 mice/group) independent experiments.

FIGURE 7. p38α is required for Aldh1a2 expression in CD103+ DCs and DC-mediated imprinting of gut-homing receptors on responding T cells. (A) RNA analysis of Aldh1a2 from CD103+ and CD103– MLN DCs and splenic DCs. (B) Expression of Aldh1a2 in CD103+ DCs from WT or p38αΔDC mice. (C) Naive OT-II T cells (Thy1.1+) were transferred into WT or p38αΔDC mice that were subsequently fed with OVA in the drinking water for 5 d, followed by analysis of α4β7 and CCR9 expression on the donor population in MLNs. Right, Proportions of α4β7+ and CCR9+ populations among donor CD4+ T cells. (D) Expression of α4β7 and CCR9 on OT-II T cells stimulated with WT or p38α-deficient CD103+ DCs, in the presence of OVA (0.05 μg/ml), for 5 d. (E and F) Expression of α4β7 (E) and CCR9 (F) on OT-II T cells stimulated with WT or p38α-deficient CD103+ DCs, in the presence of OVA (0.05 μg/ml), with or without RA for 5 d. (G) Expression of Foxp3 in OT-II T cells activated with WT or p38α-deficient CD103+ DCs, in the presence of OVA (0.05 μg/ml), with or without RA for 5 d. (H) Proposed model of p38α-dependent programming of CD103+ mucosal DCs to mediate reciprocal differentiation of Th1 and iTregs and the induction of gut-homing receptors on T cells. Data are representative of two [(A, C, E, F), n ≥ 5 mice/group; (G), n = 3 mice/group] and three [(B, D), n = 4 mice/group] independent experiments. Error bars indicate SEM.
mechanism orchestrating such unique profiles. We found that p38α is constitutively activated in CD103⁺ MLN DCs relative to other DC populations. Moreover, p38α activity in CD103⁺ DCs is required for the expression of Tgfb2 and Aldh1a2, two molecules crucial for mucosal DC functions (18, 27, 28). This synergistic effect shapes T cell differentiation and further couples it to the induction of gut-homing receptors on T cells. To our knowledge, p38α is the first signaling pathway identified that programs the tolerogenic properties of CD103⁺ MLN DCs.

Emerging evidence suggests a greater complexity in T cell lineage commitment and plasticity than previously appreciated (30). The most notable example is the shared circuitry between iTregs and Th17 cells, as the differentiation of both lineages relies on TGF-β and the balance between them is dictated by the inflammatory environment (45). Despite extensive progress in this area, it remains poorly understood how the differentiation of naive precursors into opposing lineages is regulated. Also, as many of the studies rely on the use of cytokine supplement or ablation, how T cell lineage commitment and plasticity are coordinated by physiological stimuli remains to be established. Using DC/T cell cocultures and in vivo DC-specific deletion systems to recapitulate the physiological interactions between these cells, we have identified that DC-derived p38α signaling directs the reciprocal differentiation of iTregs and Th17 cells by modulating the TGF-β2/Smad3 axis at the DC/T cell interface. This function is operative in mucosal CD103⁺ DCs of distinct subsets and origins, but not in CD103⁻ MLN DCs, highlighting the important role of p38α in mediating the tolerogenic activity of CD103⁺ DCs. This extrinsic control mechanism is reminiscent of our recent studies implicating TGF-β/Smad3 signaling in T cell–intrinsic regulation of these two related lineages (25, 37), as well as the dichotomy of iTreg and Th17 lineage decisions (45). Notably, because of the tolerogenic properties of CD103⁺ MLN DCs, these mucosal DCs do not polarize Th17 differentiation regardless of p38α activity, and this is distinct from the roles of p38α activity in programming DCs in the peripheral immune compartment to drive Th17 differentiation under inflammatory conditions (22). Furthermore, p38α is not required in CD103⁻ MLN DCs for iTreg generation, nor is it important for T cell–intrinsic regulation of iTreg and Th17 differentiation. Taken together, p38α activity in DCs integrates both inflammatory and anti-inflammatory signals in a context-dependent manner, with a dominant effect at mediating intestinal tolerogenic responses under steady-state.

Aside from lineage specification, proper trafficking of Ag-specific T cells is also crucial for the outcome of immune responses and homeostasis. In the intestinal immune system, naïve T cells traffic to MLNs where they are activated by DCs to differentiate into effector or Tregs, which then home to the LP to mediate immune reactions and maintain intestinal homeostasis. CD103⁺ MLN DCs metabolize vitamin A to produce RA, which facilitates both iTreg generation and gut-homing properties of T cells (18, 27, 28). RA has also been shown to suppress Th17 differentiation (46), although a recent study has described an obligatory role of RA in T cell activation and proinflammatory Th17 and Th1 responses (47). Consistent with a complex role of RA in T cell lineage decisions, supplement with RA did not affect iTreg differentiation mediated by p38α-deficient CD103⁺ DCs, but it completely restored the defective induction of gut-homing receptors on responding T cells. In contrast, exogenous TGF-β2 rectified the altered abilities of these DCs to mediate the differentiation of iTreg and Th1 cells. Therefore, p38α signaling in DCs coordinately regulates T cell differentiation and trafficking by orchestrating the expression of two distinct effector molecules, Tgfb2 and Aldh1a2, respectively (Fig. 5H).

Aside from DC-derived signals, T cell–intrinsic signaling pathways also contribute to cell fate decisions. In particular, the p38 pathway has been shown to contribute to the generation of iTregs (41) and Th1 cells (19). However, T cell–specific deletion of p38α did not impact the differentiation of these cells, at least under the in vitro polarizing conditions. We speculate that the apparent functions of p38 previously observed in T cells were likely due to nonspecific actions of pharmacological approaches, or the inability to distinguish the effects in T cells from those in APCs. Our results support a selective role of p38α in DC-mediated, but not T cell–intrinsic, iTreg and Th1 differentiation.

Collectively, our results identify p38α as a central pathway for programming the tolerogenic activity of mucosal CD103⁺ DCs. Although T cell responses can be influenced by the specific type of DC subsets, our results suggest that at the fundamental level, it is the intracellular signaling pathways, especially those mediated by p38α, that endow DC subsets with specific abilities to control T cell fates. Given the key roles of CD103⁺ DCs in intestinal tolerance (14), the identification of this novel control mechanism has important implications for therapeutic intervention of intestinal inflammation and other diseases. From this perspective, p38α is by far the most extensively investigated protein kinase target for the development of anti-inflammatory drugs in the pharmaceutical industry, but severe side effects have prevented clinical advancement of p38α inhibitors (48). Identification of the tolerogenic activity and downstream effectors mediated by p38α will be instrumental to the design of more selective strategies for therapeutic intervention.

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Disclosures

The authors have no financial conflicts of interest.

References


Supplementary Figure 1. Expression and deletion of p38α in mucosal DCs. (A) Expression of Foxp3 and proportion of Foxp3+ Treg cells among CD4+ T cells in the spleen, MLN and Peyer's patches (PP) of WT and p38α<sup>ΔDC</sup> mice. (B) Sorting strategy of CD103<sup>+</sup> and CD103<sup>-</sup> MLN DCs. (C) RNA analysis of p38α, p38β, p38γ and p38δ in CD103<sup>+</sup> MLN DCs. (D) Analysis of MLN DC subsets in WT and p38α<sup>ΔDC</sup> mice. (E) Analysis of MLN DC maturation markers in WT and p38α<sup>ΔDC</sup> mice. Data are representative of 2-3 independent experiments (n=3-4 mice per group). Error bars indicate S.E.M.
Supplementary Figure 2. Effects of p38α deletion on mucosal DC-mediated T cell responses. **(A)** Expression of Foxp3 in CFSE-labeled T cells activated by WT or p38α-deficient CD103+ MLN DCs for 5 days. **(B)** Analysis of viability (7-AAD–) in T cells activated with WT or p38α-deficient CD103+ MLN DCs for 5 days. **(C)** Naïve T cells from Foxp3<sup>YFP-Cre</sup> mice were activated with WT CD103+ DCs for 4 days, and then sorted YFP<sup>+</sup> cells were re-stimulated with WT or p38α-deficient CD103+ DCs in the second-round culture for 4 days, before analysis of Foxp3-YFP expression. Data are representative of 2 independent experiments (n=3 mice per group).
Supplementary Figure 3. Effect of p38α deletion on lamina propria (LP) CD103+CD11b+ and CD103+CD11b- DC functions. 
(A) Analysis of intestinal LP DC subsets in WT and p38αΔDC mice. (B) Expression of Foxp3 in OT-II T cells activated by WT or p38α-deficient CD103+CD11b+ or CD103+CD11b- LP DCs, in the presence of OVA (0.05 μg/ml), for 5 days. Data are representative of 2 independent experiments (n=3 mice per group).