Protein kinase p38α signaling in dendritic cells regulates colon inflammation and tumorigenesis

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Dendritic cells (DCs) play pivotal roles in maintaining intestinal homeostasis, but how the DCs regulate diverse immune networks on homeostasis breakdown remains largely unknown. Here, we report that, in response to epithelial barrier disruption, colonic DCs regulate the differentiation of type 1 regulatory T (Tr1) cells through p38α-dependent IL-27 production to initiate an effective immune response. Deletion of p38α in DCs, but not in T cells, led to increased Tr1 and protected mice from dextran sodium sulfate-induced acute colitis and chronic colitis-associated colorectal cancer. We show that higher levels of IL-27 in p38α-deficient colonic cDC1s, but not cDC2s, were responsible for the increase of Tr1 cells. Moreover, p38α-dependent IL-27 enhanced IL-22 secretion from intestinal group 3 innate lymphoid cells and protected epithelial barrier function. In p38α-deficient DCs, the TAK1–MKK4/7–JNK–c-Jun axis was hyperactivated, leading to high IL-27 levels, and inhibition of the JNK–c-Jun axis suppressed IL-27 expression. ChIP assay revealed direct binding of c-Jun to the promoter of *Il27p28*, which was further enhanced in p38α-deficient DCs. In summary, here we identify a key role for p38α signaling in DCs in regulating intestinal inflammatory response and tumorigenesis, and our finding may provide targets for the treatment of inflammatory intestinal diseases.

dendritic cells | p38α MAPK | T cells | innate lymphoid cells | inflammatory bowel disease

The intestine is one of the largest organs of the human body, and it represents a major entry site for potential pathogens but also, contains diverse microbiota (1). The maintenance of intestinal homeostasis relies on coordinated interactions between the microbiota, the intestinal epithelium, and the host immune system (2–4). The immune system plays an essential role in mediating the interaction between the host and the microbiota under normal conditions and during intestinal barrier dysfunction (5, 6). Different types of immune cells work together to keep a dynamic balance between the host's protective immunity and immune tolerance (2, 6). Breakdown of these immune regulatory networks in the intestine can lead to chronic inflammatory diseases, such as inflammatory bowel disease (IBD), as well as colorectal cancer (3, 7–9). IBD, including Crohn's disease and ulcerative colitis, is associated with production of proinflammatory cytokines, such as IL-17, TNF-α, IL-6, and IL-22, and the cells for producing those cytokines, including T cells and innate lymphoid cells (ILCs) (10–12). Although much progress has been made to understand the roles of diverse T cells and ILCs in controlling immune responsiveness in the gut (13, 14), how innate immune systems regulate their function in colitis pathogenesis remains largely unknown.

Dendritic cells (DCs) are the most important cell type that bridges innate and adaptive immunity (15). DCs are a highly heterogeneous population and can be broadly divided into two major branches: the classical (conventional) dendritic cells (cDCs) and the plasmacytoid DCs (16). In the intestine, by using an unsupervised analysis of flow cytometry and mass cytometry analysis, lamina propria (LP) cDCs can be further subdivided into cDC1s ($CD11c^+MHCII^+CD26^+CD64^-F4/80^-XCR1^+$) and cDC2s ($CD11c^+MHCII^+CD26^+CD64^-F4/80^-CD172a^+$) (17). Despite this well-accepted molecular characterization, their relative contributions to intestinal immune function have not been well defined (18, 19). Under steady state, the function of intestinal DCs may vary according to their locations (20). LP DCs continuously take up antigen from the intestine and migrate to the draining mesenteric lymph nodes (MLNs), where they present antigens to T cells to further drive different T cell fates (20). This process has been shown to be regulated by Wnt/β-catenin signaling (21) and by integrin αvβ8 expressed on DCs (22). Our published results also show that intestinal DCs use p38α-dependent TGF-β2 to reciprocally regulate the differentiation of induced Treg (iTreg) cells and Th1 cells, thereby maintaining intestinal homeostasis (23). However, on intestinal homeostasis breakdown during injury and pathogenesis, intestinal

Significance

Dendritic cells (DCs) are known to mediate immune regulatory networks in response to intestinal epithelial barrier disruption, but underlying signaling pathways and molecular mechanisms are not well understood. Here, we show that genetic disruption of p38α in DCs leads to higher levels of differentiated type 1 regulatory T cells and group 3 innate lymphoid cells. Specifically, p38α signaling in intestinal cDC1s, but not cDC2s or T cells, mediates colitis pathogenesis. Together, our results suggest that p38α MAPK represents a major signaling network in DCs that regulates inflammation and contributes to epithelial barrier function in colitis and associated colorectal cancer. Furthermore, our finding suggests that p38α signaling in DCs may represent a target for the treatment of inflammatory intestinal diseases.

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inflammation compromises the tolerogenic properties of intestinal DCs but enhances the inflammatory features (24). In the dextran sodium sulfate (DSS)-induced colitis mouse model (25), DCs have been shown to play both protective and harmful roles depending on the timing of DC ablation (26, 27). However, how these processes are regulated and what intracellular signaling pathways are involved during colitis pathogenesis are largely unknown.

The p38α MAPK signaling pathway is known to regulate the inflammatory immune response (28); p38 has been shown to play an essential role in IBD pathogenesis, and inhibition of p38 activity attenuates the disease severity of IBD (29). However, other groups have reported contradictory results (30). Mouse studies showed that the distinct roles of p38 in the pathogenesis of IBD or colitis-associated colorectal cancer (CACRC) depend on both targeted cell types and disease phases (31, 32). Our previous studies identified a key mechanism of DC p38α-mediated control of T cell fates and immune tolerance toward harmless intestinal antigens (23), but how p38α in DCs elicits the protective immune response on intestinal barrier breakdown remains to be elucidated.

In this study, we leveraged the DSS-induced colitis and azoxymethane (AOM)/DSS-induced CACRC model (25, 33), and we showed that p38α signaling in DCs regulated DSS-induced colitis and CACRC through IL-27–mediated type 1 regulatory T (Tr1) differentiation. Deletion of p38α in DCs, but not in T cells, protected mice from DSS-induced acute colitis and CACRC. Moreover, p38α-dependent IL-27 production in colonic DCs led to increased IL-22 secretion from colonic ILC3s and protected epithelial barrier function. Mechanistic study suggests that regulation of IL-27 expression in colonic DCs is mediated via the JNK–c-Jun axis. We reason that modulating p38α activity in DCs represents a promising strategy for the treatment of inflammatory intestinal diseases, such as IBD and CACRC.

Results

Deletion of p38α in DCs Protects Mice from DSS-Induced Colitis. In DSS-induced acute colitis, we observed significantly increased p38 activity in colonic and MLN DCs compared with water-treated control (Fig. 1*A*), indicating that p38 signaling in DCs is involved in the immune response during colitis development. To delineate the specific role of DC-intrinsic p38α signaling in colitis pathogenesis, we generated *Mapk14*$^{fl/fl}$CD11c-Cre mice (34), referred to in this study as p38α$^{\Delta DC}$ mice. We observed an efficient deletion of p38α in colonic DCs and significantly less efficient deletion in CD11c-expressing macrophages and other cells (*SI Appendix*, Fig. S1*A*); p38α deletion in DCs did not affect DC development, activation status, the subsets in the colon, or the neutrophil cell infiltration in the colon (*SI Appendix*, Fig. S1 *B–E*). In DSS-induced acute colitis, we found that p38α$^{\Delta DC}$ mice had less body weight loss (Fig. 1*B*) and clinical scores indicated as diarrhea and rectal bleeding (Fig. 1*C*) than wild-type control. At day 9, colons of p38α$^{\Delta DC}$ mice were significantly longer than those of wild-type mice (Fig. 1*D*). Miniendoscopic view of mouse colon showed that p38α$^{\Delta DC}$ mice exhibited less colonic bleeding than wild-type mice (Fig. 1*E*). Histological analysis showed that p38α$^{\Delta DC}$ mice had substantially less inflammation in the colon than wild-type mice (Fig. 1*F*). Flow cytometry analysis revealed that p38α$^{\Delta DC}$ mice showed diminished infiltration of neutrophils in the colon and spleen (Fig. 1 *G* and *H*). Collectively, these findings suggest

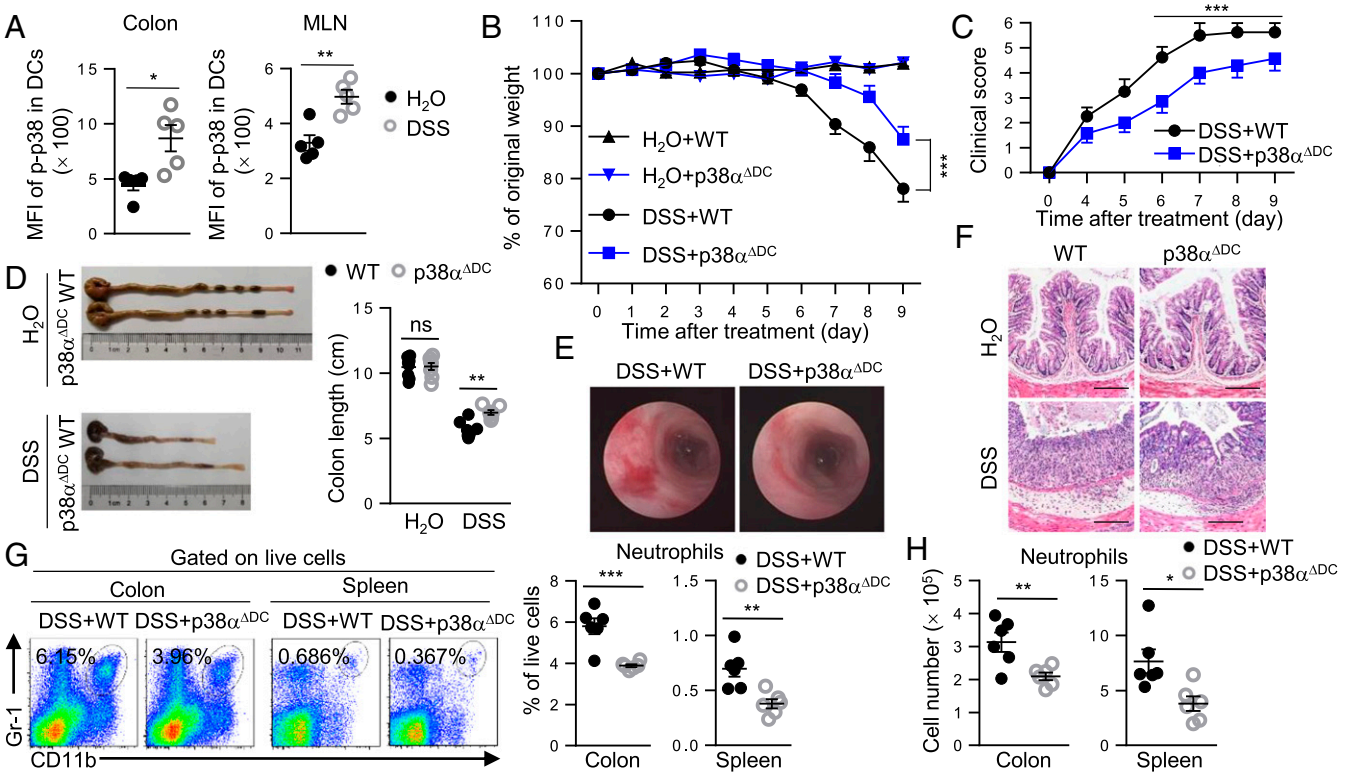

Fig. 1. Deletion of p38α in DCs protects mice from DSS-induced colitis. (*A*) Wild-type (WT) mice were supplied with 3% DSS solution or normal drinking water for 3 d, and the phosphorylation of p38 (p-p38) in DCs of colon and MLNs was examined ($n = 5$). MFI, mean fluorescence intensity. (*B–H*) WT and p38α$^{\Delta DC}$ mice were supplied with 3% DSS solution for 7 d followed with normal drinking water. In control groups, mice were supplied with normal drinking water. Body weight (*B*) and clinical score (*C*) were recorded daily ($n = 7–8$). At day 9, colon length was measured ($n = 7–8$; *D*). Representative images of miniendoscopic colon (*E*) and hematoxylin and eosin staining of colon section (*F*) are shown ($n = 5$). (Scale bars: *F*, 100 μm.) The percentages (*G*) and cell numbers (*H*) of neutrophils in colon tissue and spleen were analyzed ($n = 6$). Data represent mean ± SEM. Two-tailed Student's *t* test (*A*, *G*, and *H*) and two-way ANOVA with Bonferroni posttest (*B–D*). Results were replicated ($n = 3$ experiments). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

a key role for the colonic DC p38α signaling in DSS-induced acute colitis.

p38α$^{\Delta DC}$ Mice Are Less Susceptible to Colitis-Associated Tumorigenesis. The reduced colitis severity in p38α$^{\Delta DC}$ mice prompted us to investigate the role of p38α in DCs in colitis-associated tumorigenesis. To this end, we used the well-established AOM/DSS-induced colon tumorigenesis mouse model (33). We found that p38α$^{\Delta DC}$ mice had less body weight loss than wild-type mice in the DSS-treated phase after the first cycle and in the normal recovery phase after the second cycle (Fig. 2*A*). In addition, p38α$^{\Delta DC}$ mice had longer colons than wild-type mice (Fig. 2*B*). We also observed that p38α$^{\Delta DC}$ mice had significantly lower tumor number, tumor size, and tumor load in the colon than wild-type mice (Fig. 2 *C*–*G*). These results indicate an important role for p38α signaling in DCs in promoting colitis-associated tumorigenesis.

DC p38α Promotes DSS-Induced Intestinal Inflammatory Responses by Suppressing Tr1 Differentiation. Next, we examined the cytokine production in the colon on DSS treatment. While the transcript levels of cytokines and chemokines in colon tissues and colon LP cells of wild-type mice were comparable with those in p38α$^{\Delta DC}$ mice under normal water conditions, their levels of secretion were dramatically increased on DSS treatment (Fig. 3*A* and *SI Appendix*, Fig. S2*A*). Moreover, compared with wild-type mice, p38α$^{\Delta DC}$ mice had lower transcript levels of *Tnfa*, *Il1b*, *Il6*, *Il17a*, *Cxcl1*, and *Cxcl2* but higher levels of *Ifng* and *Il10* in colon tissues (Fig. 3*A* and *SI Appendix*, Fig. S2*A*). *Il12a*, *Il4*, *Foxp3*, and *Tgfb1* levels were comparable between wild-type and p38α$^{\Delta DC}$ mice (*SI Appendix*, Fig. S2*A*). Consistent with mRNA expression, the secreted proinflammatory cytokines, such as TNF-α, IL-1β, IL-6, and IL-17A, were significantly lower in the colon of p38α$^{\Delta DC}$ mice compared with those in wild-type mice, whereas IFN-γ and antiinflammatory cytokine IL-10 were higher in p38α$^{\Delta DC}$ mice (Fig. 3*B*). These results demonstrate that p38α signaling in DCs promotes the expression of proinflammatory cytokines and chemokines and suppresses the expression of antiinflammatory cytokine in the colon on DSS treatment.

To further characterize the cellular origin of these cytokines and chemokines, we purified CD4$^+$ T cells from the isolated colonic lamina propria leukocytes (LPLs) by flow cytometry and analyzed their mRNA expression. The results showed that the differential expressions of *Il17a*, *Ifng*, and *Il10* were from CD4$^+$ T cells, whereas *Tnfa*, *Il1b*, *Il6*, *Cxcl1*, and *Cxcl2* were mainly from non-CD4$^+$ cells (*SI Appendix*, Fig. S2 *B* and *C*). We further confirmed the cellular source of IL-17, IFN-γ, and Foxp3 both in the colon and in the MLNs from wild-type and p38α$^{\Delta DC}$ mice (*SI Appendix*, Fig. S3 *A* and *B*). Although CD4$^+$ Foxp3$^+$ T (Treg) cells express large amounts of IL-10, our results showed that most of the IL-10$^+$CD4$^+$ T cells are Foxp3$^-$ cells after DSS treatment (*SI Appendix*, Fig. S3*C*). Interestingly, while IL-10 from Treg cells was comparable between wild-type and p38α$^{\Delta DC}$ mice, IL-10 from CD4$^+$ Foxp3$^-$ T cells was significantly higher in the colon and MLNs of p38α$^{\Delta DC}$ mice than those of wild-type mice (Fig. 3*C* and *SI Appendix*, Fig. S3 *D*–*G*). The increased Tr1 cells in p38α$^{\Delta DC}$ mice could be due to the enhanced T cell proliferation or survival. For this, we used BrdU and active caspase-3 analyses to explore whether p38α deficiency in DCs would affect T cell proliferation and survival. We found that wild-type and p38α$^{\Delta DC}$ mice had comparable BrdU incorporation and active caspase-3 staining in CD4$^+$ T cells isolated from the colon and MLNs on DSS treatment (*SI Appendix*, Fig. S3 *H* and *I*), suggesting that deletion of p38α in DCs only affects T cell cytokine secretion on DSS treatment but not T cell proliferation and survival.

Tr1 cells are particularly important in suppressing aberrant innate and adaptive immune responses in the intestine (13). To explore whether the less colitis severity in p38α$^{\Delta DC}$ mice was due to the increased Tr1 cells, we treated wild-type and p38α$^{\Delta DC}$ mice with DSS to induce colitis and then, intraperitoneally injected IL-10R neutralization antibody (αIL-10R) or isotype controls. As expected, neutralization of IL-10 signaling dramatically increased the colitis severity in both wild-type and p38α$^{\Delta DC}$ mice as indicated by body weight loss, colon length, inflammatory cell infiltration, and proinflammatory cytokine expression (Fig. 3 *D* and *E* and *SI Appendix*, Fig. S4 *A*–*D*). Importantly, neutralization of IL-10 signaling restored colitis severity and proinflammatory cytokine expression in p38α$^{\Delta DC}$ mice (Fig. 3 *D* and *E* and *SI Appendix*, Fig. S4 *A*–*D*). Notably, the CD11c-expressing macrophages and other cells were less competent to drive IL-10$^+$ T cell differentiation than DCs, and deletion of p38α in these macrophages did not affect the differentiation of IL-10–expressing T cells (*SI Appendix*, Fig. S4*E*). Taken together,

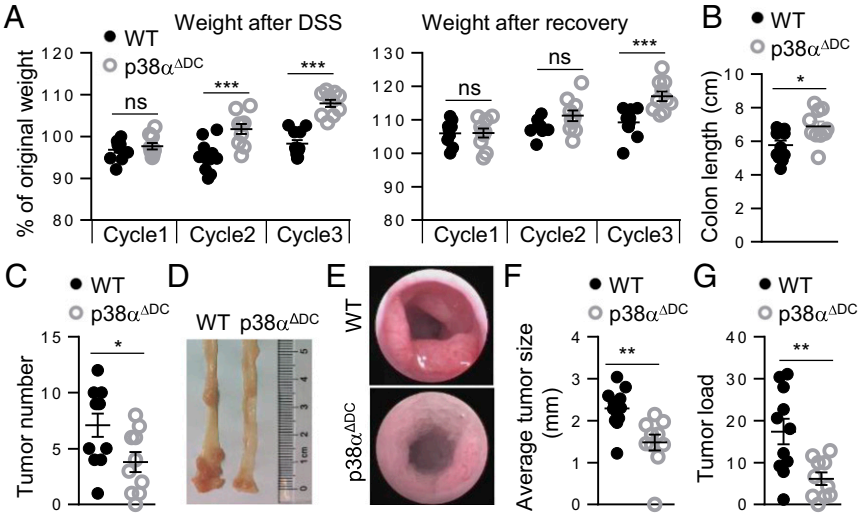

Fig. 2. Mice with p38α deletion in DCs have less susceptibility to chronic colitis-induced colon tumorigenesis. Wild-type (WT) and p38α$^{\Delta DC}$ mice were injected with 10 mg/kg AOM at day 0 and then given three cycles of 5 d of 2% DSS solution (n = 10–11). (*A*) Weight changes at the end of each DSS cycle and recovery period were recorded. (*B*–*G*) Mice were killed at day 80. They were analyzed for colon length (*B*) and tumor numbers in the whole colons (*C*), were photographed (*D*), had miniendoscopy of colon tumors (*E*), and were analyzed for tumor diameters (*F*) and tumor load (which indicates the sum of tumor diameter per colon; *G*). Data represent mean ± SEM. Two-way ANOVA with Bonferroni posttest (*A*) and two-tailed Student's t test (*B*, *C*, *F*, and *G*). Results were replicated (n = 3 experiments). *P < 0.05; **P < 0.01; ***P < 0.001; ns, not significant.

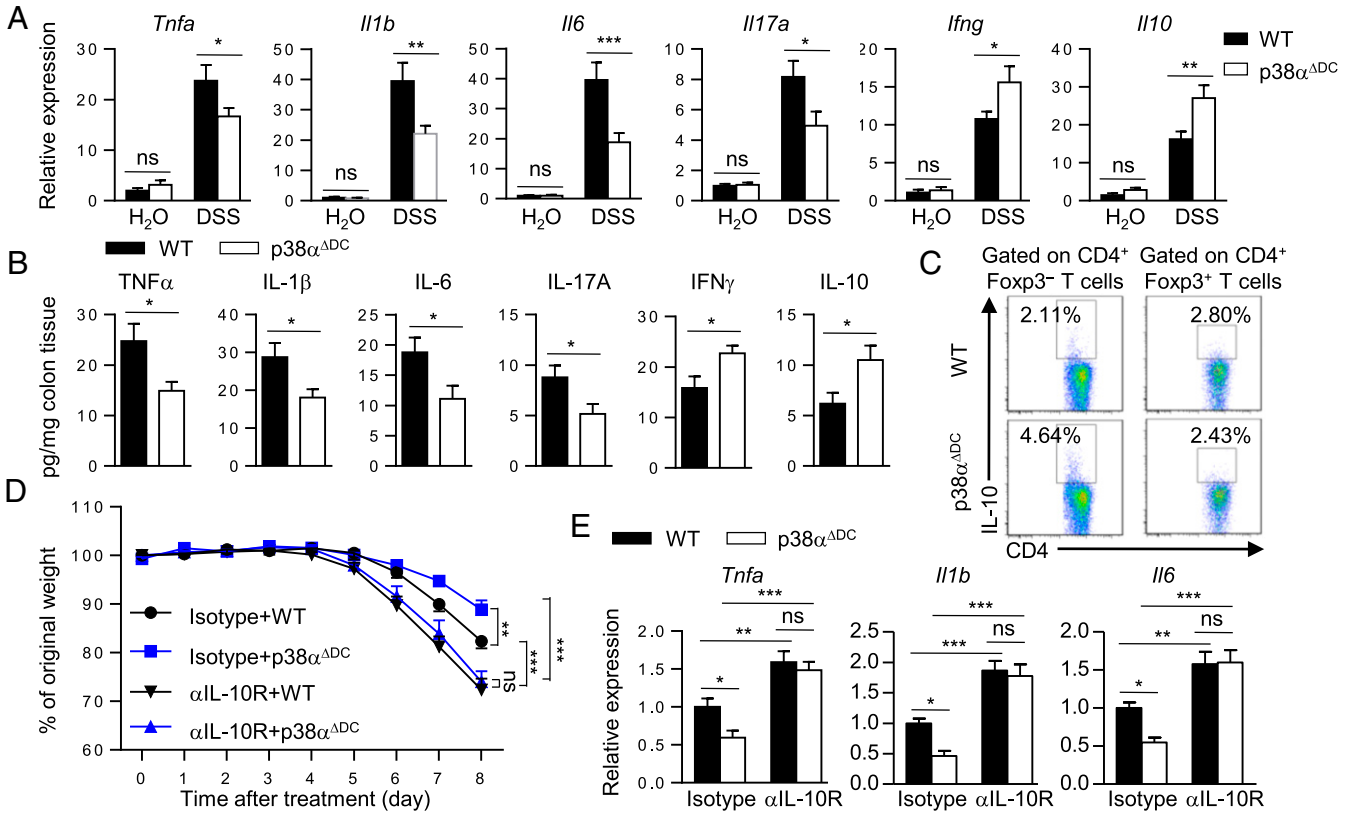

Fig. 3. p38α deficiency in DCs suppresses inflammatory responses by promoting Tr1 differentiation. (*A–C*) Wild-type (WT) and p38α$^{\Delta DC}$ mice were supplied with 7 d of 3% DSS solution followed with 2 d of normal water or 9 d of normal water. Relative expression of inflammation-related genes in colon tissue was examined ($n = 5$; *A*). Cytokine secretion in colon tissue was determined in DSS-treated mice ($n = 7$–8; *B*). The percentages of Tr1 cells and IL-10$^+$ Treg cells in colon tissue of DSS-treated mice were analyzed (*C*). (*D* and *E*) WT and p38α$^{\Delta DC}$ mice were supplied with 7 d of 2.5% DSS solution followed with normal drinking water and injected with αIL-10R or isotype at days 3 and 5 for analyses of body weight (*D*) and relative expression of inflammation-related genes in colon tissue (*E*; $n = 6$). Data represent mean ± SEM. Two-way ANOVA with Bonferroni posttest (*A*, *D*, and *E*) and two-tailed Student's *t* test (*B*). Results were replicated ($n = 3$ experiments). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

DC p38α promotes DSS-induced intestinal inflammatory responses at least in part by suppressing Tr1 differentiation and reducing antiinflammatory cytokine secretion.

DC p38α-Dependent IL-27 Regulates Tr1 Differentiation and Colitis Severity. The results reported above prompted us to assess whether p38α directly mediates DC–Tr1 cell cross-talk. For this, we cocultured naïve OT-II CD4$^+$ T cells (transgenic expression of a T cell receptor specific for OVA$_{323–329}$ peptide) with colonic DCs in the presence of antigen OVA$_{323–329}$ and LPS but without any exogenous cytokines. T cells activated by p38α$^{\Delta DC}$ DCs produced significantly higher levels of IL-10 on αCD3ε restimulation than those activated by wild-type DCs (Fig. 4*A*). In line with this finding, we found that, compared with those activated by wild-type DCs, significantly more CD4$^+$Foxp3$^-$ T cells activated by p38α$^{\Delta DC}$ DCs developed into Tr1 cells (Fig. 4*B*). This correlated with higher *Il10* mRNA and Tr1-related transcription factors, such as *Maf* and *Ahr*, and cytokine, such as *Il21*, but similar *Il10ra* expression in p38α$^{\Delta DC}$ DC-activated T cells (*SI Appendix*, Fig. S5). Furthermore, DC subset assay showed that deletion of p38α in cDC1, but not in cDC2, led to increased Tr1 differentiation and *Maf*, *Ahr*, and *Il21* expression (Fig. 4*C*). Collectively, these results indicate that deletion of p38α in cDC1 promotes Tr1 differentiation in vitro.

Next, we explored the potential mechanisms by which p38α acted in DCs to regulate Tr1 differentiation. Deletion of p38α did not affect the DC numbers or the subsets in the colon on DSS treatment (*SI Appendix*, Fig. S6 *A* and *B*). Moreover, colonic DCs from wild-type and p38α$^{\Delta DC}$ mice expressed comparable costimulatory molecules, such as CD40, CD80, and CD86 (*SI Appendix*, Fig. S6*C*). We examined whether p38α deletion in DCs would affect their proliferation and survival by doing the BrdU incorporation and active caspase-3 assay. Our results showed that wild-type and p38α$^{\Delta DC}$ mice had comparable BrdU incorporation and active caspase-3 staining in DCs isolated from the colon and MLN on DSS treatment (*SI Appendix*, Fig. S6 *D* and *E*). These results indicate that p38α is not required for colonic DC development or costimulatory molecule expression in DSS-treated mice and that deletion of p38α does not affect DC proliferation and survival.

We next examined whether p38α signaling in colonic DCs regulates the expression of cytokines that facilitate Tr1 differentiation. Deletion of p38α in DCs significantly decreased *Il10* expression (*SI Appendix*, Fig. S7*A*), excluding a role of DC p38α-dependent IL-10 in the initiation of Tr1 differentiation. IL-27 is another cytokine to drive Tr1 differentiation (35). Our results showed that *Il27p28* expression was higher in p38α$^{\Delta DC}$ DCs than wild-type DCs, while other cytokines, such as *Il23a*, *Il1b*, *Tnfa*, *Tgfb1*, and *Tgfb2*, were comparable, but *Il12a* was increased in p38α$^{\Delta DC}$ DCs (*SI Appendix*, Fig. S7*A*). We then isolated the colonic DC subsets from wild-type and p38α$^{\Delta DC}$ mice and stimulated them with LPS for 24 h. Our results showed that the increased IL-27 was from p38α$^{\Delta DC}$ cDC1s but not cDC2s (Fig. 4*D*). Correlated with higher IL-27 expression in p38α$^{\Delta DC}$ cDC1s, phosphorylation of STAT1 and STAT3, two transcription factors that directly regulate Tr1 differentiation (36), and *Il27ra* expression in T cells activated by p38α$^{\Delta DC}$ cDC1s were higher than those activated by wild-type cDC1s (*SI Appendix*, Fig. S7 *B–D*).

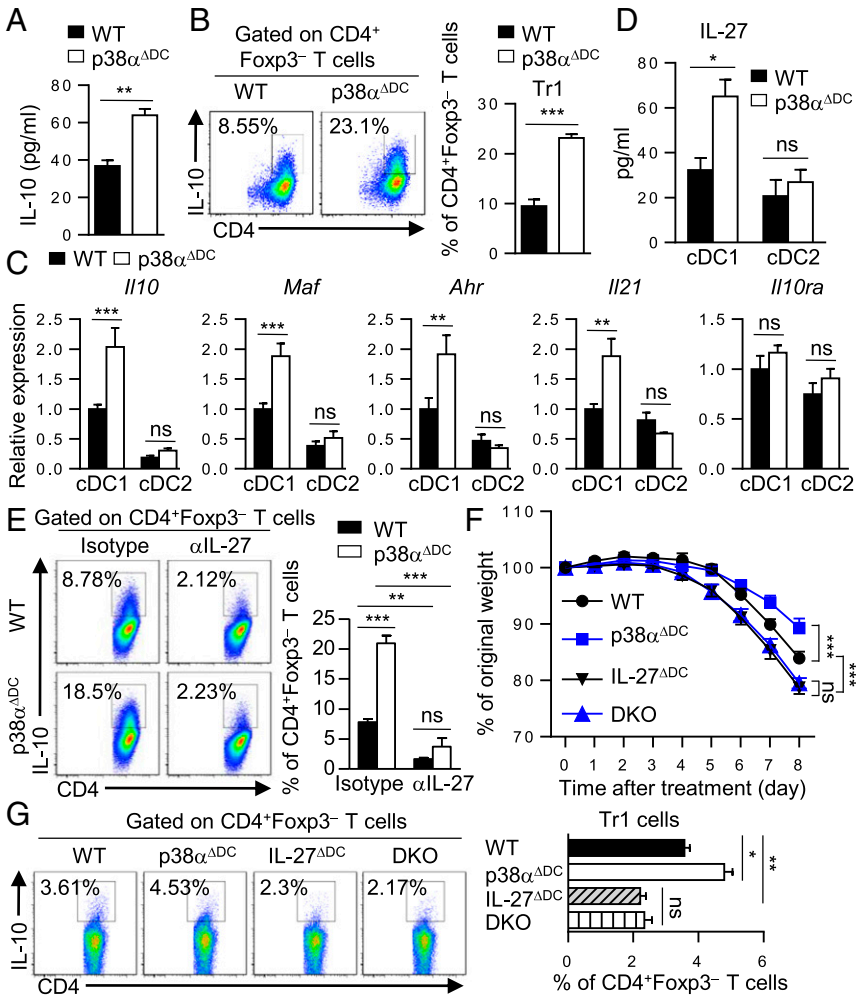

Fig. 4. p38α deficiency in cDC1s, but not cDC2s, promotes Tr1 generation by up-regulating IL-27 expression. (*A* and *B*) Colonic DCs of wild-type (WT) and p38α$^{\Delta DC}$ mice were cocultured with naïve CD4$^+$ T cells for 6 d, and then, IL-10 secretion ($n = 3$; *A*) and Tr1 differentiation ($n = 3$; *B*) were analyzed. (*C* and *D*) Colonic cDC1s and cDC2s of WT and p38α$^{\Delta DC}$ mice were cocultured with naïve CD4$^+$ T cells for 6 d to examine Tr1-related gene expression ($n = 5$; *C*) or IL-27 secretion ($n = 3$; *D*). (*E*) Colonic cDC1s of WT and p38α$^{\Delta DC}$ mice were cocultured with naïve CD4$^+$ T cells with αIL-27 or isotype for 6 d to check Tr1 differentiation ($n = 3$). (*F* and *G*) WT, p38α$^{\Delta DC}$, IL-27$^{\Delta DC}$, and DKO (both p38α and IL-27 were deleted in DCs) mice were supplied with 7 d of 3% DSS solution followed with normal drinking water ($n = 6$). Body weight (*F*) and Tr1 differentiation in colon tissue (*G*) were examined. Data represent mean ± SEM. Two-tailed Student's *t* test (*A* and *B*), two-way ANOVA with Bonferroni posttest (*C*–*F*), and one-way ANOVA with Tukey's posttest (*G*). Results were replicated ($n \geq 2$ experiments). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant.

To determine whether the increased expression of IL-27 in p38α$^{\Delta DC}$ cDC1s contributed to the enhanced Tr1 differentiation, we added IL-27 neutralization antibody (αIL-27) to the DC–CD4$^+$ T cell coculture systems and showed that blockade of IL-27 suppressed Tr1 differentiation and *Il10* expression but restored the high level of IL-10 production from T cells activated by p38α$^{\Delta DC}$ cDC1s (Fig. 4*E* and *SI Appendix*, Fig. S7*E*). Collectively, these data show that p38α-dependent IL-27 mediates the cross-talk between DCs and T cells to further regulate Tr1 differentiation.

To further assess the functional importance of p38α-dependent IL-27 in the regulation of Tr1 differentiation in vivo, we crossed *Mapk14*$^{fl/fl}$CD11c-Cre mice with *Il27p28*$^{fl/fl}$ mice to generate a mouse line in which both p38α and IL-27 were deleted in DCs, referred to as "DKO." We treated wild-type, p38α$^{\Delta DC}$, IL-27$^{\Delta DC}$ (*Il27p28*$^{fl/fl}$ crossed with CD11c-Cre mice), and DKO mice with DSS to induce colitis. Although deletion of IL-27 in DCs led to increased colitis severity compared with wild-type mice, DKO mice exhibited similar disease severity as IL-27$^{\Delta DC}$ mice, including body weight loss, colon length, and neutrophil infiltration in the colon and spleen (Fig. 4*F* and *SI Appendix*, Fig. S7 *F* and *G*). Moreover, deletion of IL-27 in p38α$^{\Delta DC}$ DCs also restored the increased Tr1 cells and *Il10* expression in p38α$^{\Delta DC}$ mice, where IL-27 is intact (Fig. 4*G* and *SI Appendix*, Fig. S7*H*). Other proinflammatory cytokines or chemokine, such as *Tnfa*, *Il1b*, *Il6*, *Il17a*, and *Cxcl1*, were also comparable in IL-27$^{\Delta DC}$ and DKO mice (*SI Appendix*, Fig. S7*H*). Although IL-27 has been shown to promote Th1 differentiation, DC p38α-dependent IL-27 was not required for *Ifng* expression in vivo and in vitro (*SI Appendix*, Fig. S7 *H* and *I*). Taken together, these results suggest that p38α-dependent IL-27 directs DC-mediated Tr1 differentiation and cytokine production and that it is responsible for the severity of colitis.

DC p38α-Dependent IL-27 Regulates IL-22–Producing ILC3 Generation and Epithelial Barrier Function on DSS Treatment. IL-22 plays a pivotal role in resolving tissue damage induced by DSS through enhanced epithelial cell proliferation, survival, and barrier function (37, 38). We observed an increased IL-22 expression at both protein and mRNA levels in colon tissues of p38α$^{\Delta DC}$ mice on DSS treatment compared with that of wild-type mice (Fig. 5*A* and *SI Appendix*, Fig. S8*A*). IL-22 can be produced by T cells and

other innate immune cells (39). Cellular source analysis showed that the increased *Il22* mRNA level in p38α$^{\Delta DC}$ mice was mainly from non-CD4$^+$ cells isolated from colonic LPLs (*SI Appendix*, Fig. S8*B*). Flow cytometry analysis showed that large amounts of IL-22–producing lineage$^-$colonic LPL cells were CD90$^+$RORγt$^+$ cells (*SI Appendix*, Fig. S8*C*). The lineage$^-$CD90$^+$ RORγt$^+$ cells are ILC3s (*SI Appendix*, Fig. S8*D*), and p38α$^{\Delta DC}$ mice had significantly higher IL-22–producing ILC3s than wild-type mice on DSS treatment (Fig. 5*B*). Accumulating data show that IL-22–producing ILC3s regulate mucosal immunity via the IL-22R signaling and that IL-22R is selectively expressed by intestinal epithelial cells (IECs) (39). Consistent with higher *Il22* expression in p38α$^{\Delta DC}$ mice on DSS treatment, the expression of *Il22ra1* was significantly higher in IECs sorted from p38α$^{\Delta DC}$ mice than those from wild-type mice (*SI Appendix*, Fig. S8*E*). IL-22–induced antimicrobial proteins from these IECs, such as *Reg3g* and *Reg3b*, were also significantly increased in p38α$^{\Delta DC}$ mice compared with wild-type mice, while the expression of *Lcn2* and *Nos2* was comparable after DSS treatment (*SI Appendix*, Fig. S8*E*). We also observed that genes regulating cell proliferation and survival, such as *Bcl2* and *Ccnd1*, were also up-regulated in IECs from p38α$^{\Delta DC}$ mice (*SI Appendix*, Fig. S8*E*). These results indicate that p38α activity in DCs regulates IL-22 signaling at the ILC3–epithelial interface to direct epithelial antimicrobial proteins secretion.

To examine whether DC p38α signaling modulates intestinal epithelial barrier function on DSS treatment, we performed BrdU and active caspase-3 assays to assess IEC proliferation and survival. Our results showed that IECs from p38α$^{\Delta DC}$ mice had higher BrdU incorporation and lower active caspase-3 staining than those from wild-type mice on DSS treatment (Fig. 5 *C* and *D*). Notably, in the AOM/DSS-induced CACRC model, IEC proliferation and survival were comparable between wild-type and p38α$^{\Delta DC}$ mice (*SI Appendix*, Fig. S8*F*). We then orally gavaged the DSS-treated mice with FITC-dextran to measure serum FITC-dextran concentration to assess intestinal permeability. Our results showed that p38α$^{\Delta DC}$ mice had significantly lower serum FITC-dextran concentration than wild-type mice (Fig. 5*E*), indicating that intestinal permeability was improved in p38α$^{\Delta DC}$ mice on DSS treatment compared with wild-type mice. These results demonstrate that p38α activity in DCs regulates epithelial barrier function during acute colitis.

Next, we explored the molecular mechanisms of how p38α regulated intestinal DC function to direct IL-22–producing ILC3 generation and intestinal barrier function. Given that p38α$^{\Delta DC}$ mice had higher IL-27 expression in DCs and more IL-22–producing ILC3 generation than wild-type mice, we reason that the increased IL-27 in p38α$^{\Delta DC}$ DCs might contribute to the higher IL-22–producing ILC3 generation. We reanalyzed the IL-22–producing ILC3s from DSS-treated wild-type, p38α$^{\Delta DC}$, and DKO mice and showed that deletion of IL-27 in p38α$^{\Delta DC}$ DCs restored IL-22–producing ILC3 generation in p38α$^{\Delta DC}$ mice to wild-type levels (Fig. 5*F*). Deletion of IL-27 in p38α$^{\Delta DC}$ DCs also restored *Il22* mRNA expression in colon tissue of p38α$^{\Delta DC}$ mouse (*SI Appendix*, Fig. S8*G*) as well as *Il22ra1* and IL-22–induced antimicrobial proteins, such as *Reg3g* and *Reg3b*, from these IECs (*SI Appendix*, Fig. S8*H*). The expression of *Bcl2* and *Ccnd1*in IECs was also restored by deletion of IL-27 in p38α$^{\Delta DC}$ DCs (*SI Appendix*, Fig. S8*H*). In addition, BrdU and active caspase-3 analyses showed that the increased IEC proliferation and survival were also restored on IL-27 deletion in p38α$^{\Delta DC}$ DCs (*SI Appendix*, Fig. S8 *I* and *J*). Moreover, the improved intestinal permeability, which was seen in p38α$^{\Delta DC}$ mice, was disrupted again after deletion of IL-27 in p38α$^{\Delta DC}$ DCs (Fig. 5*G*). Notably, although p38α$^{\Delta DC}$ mice had higher Tr1 development on DSS treatment and neutralization of IL-10R signaling could largely restore the colitis severity (Fig. 3 *D* and *E* and *SI Appendix*, Fig. S4 *A–D*), blockade of IL-10R signaling did not restore the higher expression of IL-22 in those mice (*SI Appendix*, Fig. S4*D*). Taken together, our results show that DC p38α-dependent IL-27 regulates IL-22–producing ILC3 generation and intestinal barrier function on DSS treatment.

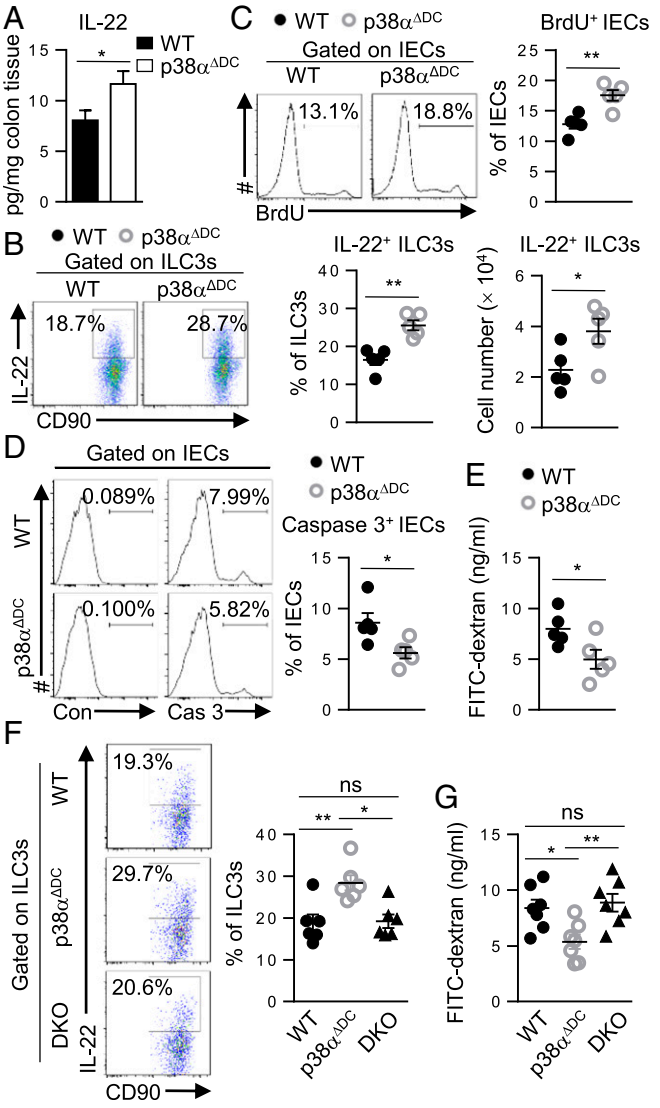

Fig. 5. p38α-dependent IL-27 in DCs regulates IL-22–producing ILC3 generation and IEC barrier function on DSS treatment. (*A–E*) Wild-type (WT) and p38α$^{\Delta DC}$ mice were supplied with 7 d of 3% DSS solution followed with 2 d of normal drinking water (n = 5). IL-22 secretion in colon tissue (*A*), the percentages and cell numbers of IL-22$^+$ ILC3s in colon tissue (*B*), the BrdU (*C*) and active caspase-3 (Cas 3) staining (*D*) of IECs, and FITC-dextran concentration in serum in intestinal permeability analysis (*E*) were examined. Isotype antibody was used as control (Con) of active Cas 3 staining. (*F* and *G*) WT, p38α$^{\Delta DC}$, and DKO mice were treated with DSS to check the percentage of IL-22$^+$ ILC3s in colon tissue (n = 6; *F*) and FITC-dextran concentration in serum (n = 7; *G*). Data represent mean ± SEM. Two-tailed Student's t test (*A–E*) and one-way ANOVA with Tukey's post-test (*F* and *G*). Results were replicated ($n \geq 2$ experiments). $^*P < 0.05$; $^{**}P < 0.01$; ns, not significant.

DC p38α-Dependent IL-27 Regulates the Susceptibility to Colitis-Associated Tumorigenesis. Having shown that p38α signaling in DCs promotes colitis-associated tumorigenesis, to further examine whether this susceptibility depends on IL-27, we treated wild-type, p38α$^{\Delta DC}$, IL-27$^{\Delta DC}$, and DKO mice with AOM/DSS to induce colon tumorigenesis. Deletion of IL-27 in p38α$^{\Delta DC}$ DCs restored the colon length in p38α$^{\Delta DC}$ mice to the wild-type level (Fig. 6*A*). While p38α$^{\Delta DC}$ mice had significantly lower tumor

numbers, tumor size, and tumor load in the colon than wild-type mice, deletion of IL-27 in p38α$^{\Delta DC}$ DCs restored the tumor number, load, and size seen in p38α$^{\Delta DC}$ mice to wild-type levels (Fig. 6 *B*–*E*). Together, these results demonstrate that DC p38α-dependent IL-27 regulates the susceptibility to colitis-associated tumorigenesis.

p38α Regulates IL-27 Expression by Modulating the JNK–c-Jun Axis in DCs. Next, we determined the biochemical mechanism of p38α regulation of IL-27 expression in DCs. As *Il27p28* was also up-regulated in LPS-stimulated p38α-deficient bone marrow-derived dendritic cells (BMDCs) (*SI Appendix*, Fig. S9*A*), considering that the obtained colonic DC numbers are relatively low to use for Western blot study, we used BMDCs as a cellular model for this study. Western blot analysis showed that the activities of JNK and ERK were increased in p38α-deficient DCs compared with wild-type DCs (Fig. 7*A*). To explore whether the increased JNK or ERK activity in p38α-deficient DCs could contribute to the higher *Il27p28* expression, we pretreated wild-type and p38α-deficient DCs with JNK-specific inhibitor SP600125 or ERK-specific inhibitor U0126 and then stimulated them with LPS to measure *Il27p28* expression. While JNK-specific inhibitor SP600125 completely suppressed *Il27p28* expression in p38α-deficient DCs, ERK-specific inhibitor U0126 had only modest effect (Fig. 7*B*), indicating that p38α regulates IL-27 expression through modulating JNK activity in DCs.

We then explored the mechanism of how p38α regulates JNK activity in DCs on LPS stimulation. It was reported that LPS-induced p38α activation in macrophages down-regulates TAK1 (an MAP3K) via a negative feedback mechanism, resulting in decreased JNK1 and JNK2 activation (40). To this end, we measured the activities of MAPK signaling molecules that function upstream of JNK, including TAK1, MLK3 (MAP3K), MKK4, and MKK7 (MAP2Ks) (41). Our results showed that TAK1 and MKK4/MKK7 activities were significantly increased in p38α-deficient DCs compared with in wild-type DCs, while MLK3 activation was comparable in these two genotypes (Fig. 7 *C* and *D*). These results indicate that p38α regulates JNK activity via a negative feedback mechanism to modulate the TAK1–MKK4/MKK7–JNK cascade in DCs.

We further examined how p38α-mediated JNK activation regulates IL-27 at the transcriptional level. Consistent with increased JNK phosphorylation in p38α-deficient DCs, its downstream transcription factor c-Jun activity was increased, and inhibition of JNK activation decreased c-Jun activity in p38α-deficient DCs (Fig. 7 *D* and *E*). To explore whether the increased c-Jun activity in p38α-deficient DCs could contribute to the higher *Il27p28* expression, we pretreated DCs with AP-1–specific inhibitor SR 11302 and then stimulated DCs with LPS followed by measurements of *Il27p28* expression. SR 11302 dramatically inhibited *Il27p28* expression in both wild-type and p38α-deficient DCs and completely restored the increased *Il27p28* expression in p38α-deficient DCs to wild-type levels (Fig. 7*F*). We further used ChIP assay to find that c-Jun directly bound to an AP-1 binding site on the promoter of the *Il27p28* gene, and this binding activity was further enhanced in p38α-deficient DCs (Fig. 7*G* and *SI Appendix*, Fig. S9*B*). Luciferase reporter assay showed that overexpression of c-Jun increased reporter transcription mediated by the *Il27p28* promoter (852 bp upstream of the transcription initiation site) and the *Il27p28* promoter segment between –313 and –455 that harbors the AP-1 binding site, and reporter activation was in a dose-dependent manner (Fig. 7*H* and *SI Appendix*, Fig. S9 *C* and *D*). Taken together, these results indicate that p38α regulates IL-27 expression through modulating the JNK–c-Jun axis in DCs.

T Cell-Intrinsic p38α Signaling Is Not Required for DSS-Induced Colitis and Colitis-Associated Tumorigenesis. To study the role of p38α in T cells in colitis pathogenesis, we generated *Mapk14*$^{fl/fl}$CD4-Cre mice (referred to as "p38α$^{\Delta T}$ mice" here), in which p38α was deleted from CD4-expressing T cells or non-T cells. Notably, we found that most CD4-expressing non-T cells in the colon had very low levels of CD11c and MHC II (*SI Appendix*, Fig. S10*A*). T cell-specific deletion of p38α was not found to affect the severity of DSS-induced colitis as indicated by comparable weight loss or colon length changes in both wild-type and p38α$^{\Delta T}$ mice (*SI Appendix*, Fig. S10 *B* and *C*). Both wild-type and p38α$^{\Delta T}$ mice had comparable cytokine expression in colon tissue (*SI Appendix*, Fig. S10*D*). Th1, Th17, and Treg cells were also comparable in the MLNs in wild-type and p38α$^{\Delta T}$ mice (*SI Appendix*, Fig. S10*E*). In the AOM/DSS-induced CACRC, p38α$^{\Delta T}$ mice had comparable colon length, tumor number, and tumor size as well as tumor load compared with wild-type mice (*SI Appendix*, Fig. S10 *F*–*I*). Thus, our results demonstrate that p38α signaling in T cells does not affect DSS-induced colitis and colitis-associated tumorigenesis.

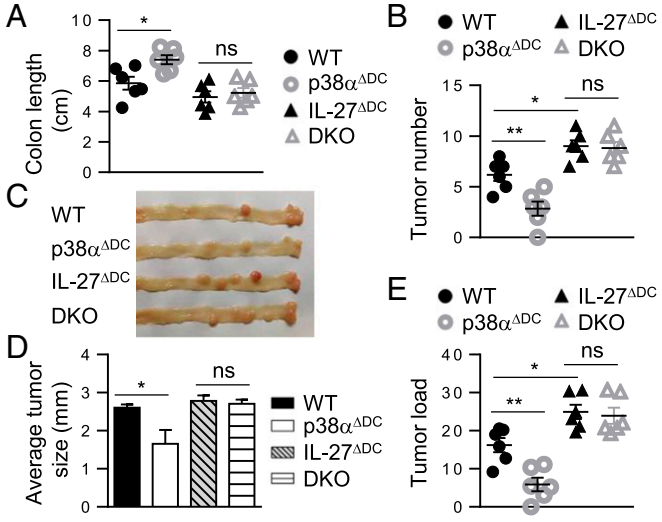

Fig. 6. The effect of DC p38α on colitis-associated tumorigenesis is in an IL-27–dependent manner. Wild-type (WT), p38α$^{\Delta DC}$, IL-27$^{\Delta DC}$, and DKO mice were injected with 10 mg/kg AOM at day 0 and then given three cycles of 5 d of 2% DSS solution ($n = 6$). Mice were killed at day 80. (*A*) Colon length. (*B*) Tumor numbers. (*C*) Photograph of colon tumors. (*D*) Tumor diameter. (*E*) Tumor load. Data represent mean ± SEM. One-way ANOVA with Tukey's posttest (*A*, *B*, *D*, and *E*). Results were replicated ($n = 3$ experiments). $^*P < 0.05$; $^{**}P < 0.01$; ns, not significant.

Discussion

There is growing experimental evidence that supports the notion that DCs play essential roles in maintaining the balance between immune tolerance to self-antigen or microbiota and protective immunity against pathogens in the intestine (16, 18, 19, 42). However, the mechanism of how DCs regulate the immune networks to mount an immune response to intestinal bacteria on intestinal barrier disruption remains largely unknown. Here, we show that DCs regulate Tr1 cell differentiation and IL-22–producing ILC3 generation through activation of p38α signaling during intestinal barrier disruption. Deletion of p38α in DCs, but not in T cells, protected mice from DSS-induced acute colitis and CACRC. p38α-dependent IL-27 mediated the cross-talk between DCs and Tr1 cells or ILC3s to further regulate colon inflammation, intestinal epithelial barrier function, and tumorigenesis. Our findings highlight a crucial role for the p38α–IL-27–IL-10/IL-22 axis in the pathogenesis of colitis and colitis-associated cancer. Modulation of p38α signaling in DCs may provide an attractive treatment for inflammatory intestinal diseases.

Under homeostatic conditions, IECs secrete factors, such as retinoic acid and TGF-β (2). DCs may sense these tolerogenic factors through p38α signaling to regulate TGF-β2 expression

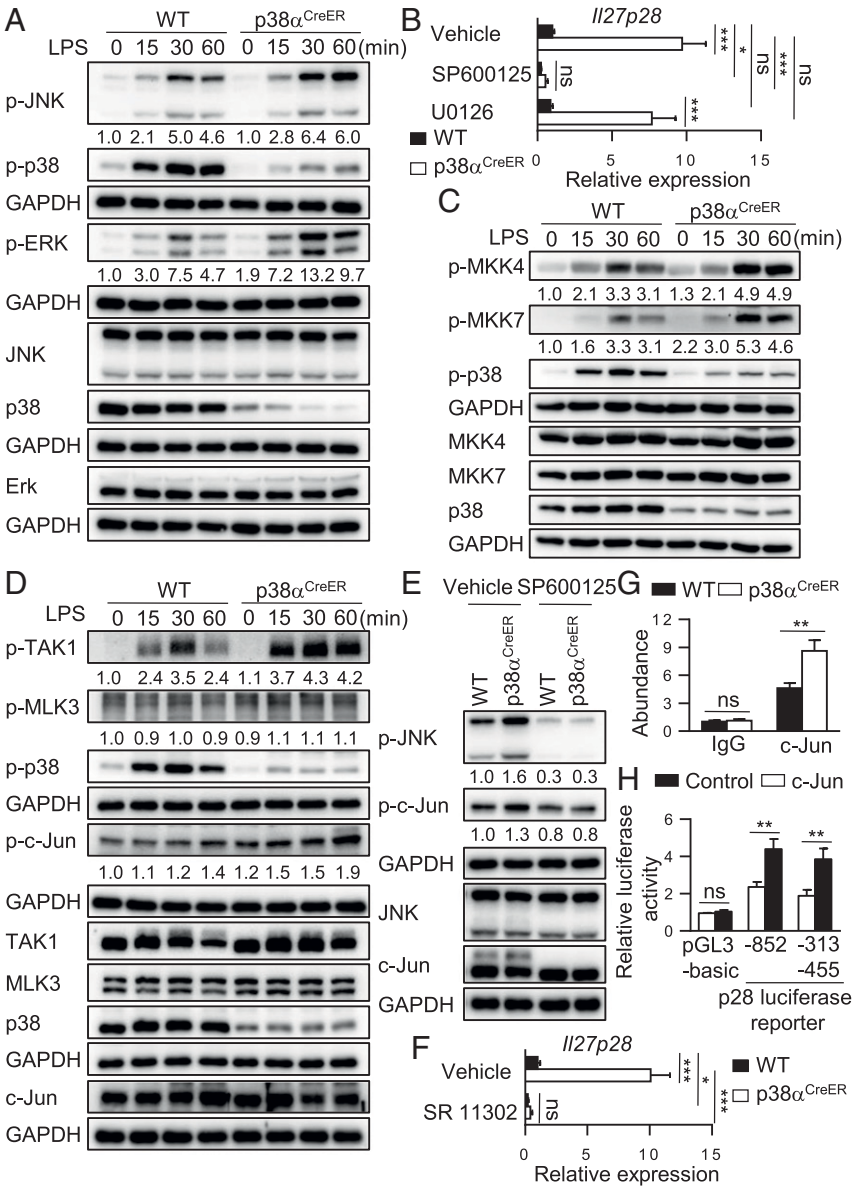

Fig. 7. p38α deficiency up-regulates IL-27 expression by enhancing JNK–c-Jun axis in DCs. (*A–G*) Bone marrow cells were isolated from wild-type (WT) and p38α^{CreER} mice to culture BMDCs. BMDCs were stimulated with LPS to examine the activities of JNK, ERK, and p38 (*A*). BMDCs were pretreated with JNK inhibitor SP600125, ERK inhibitor U0126, or vehicle and then stimulated with LPS for 5 h to analyze *Il27p28* expression ($n = 5$; *B*). BMDCs were stimulated with LPS to examine the activities of MKK4 and MKK7 (*C*) and the activities of TAK1, MLK3, and c-Jun (*D*). BMDCs were pretreated with AP-1 inhibitor SR 11302 or vehicle and then stimulated with LPS for 60 min to analyze the activities of JNK and c-Jun (*E*) or for 5 h to check *Il27p28* expression ($n = 5$; *F*). ChIP assays were performed with LPS-pretreated BMDCs, and the abundance of c-Jun bound to the AP-1 binding site on *Il27p28* promoter was determined ($n = 3$; *G*). (*H*) p28 luciferase reporter (−852 or −313–455) or pGL3-basic vector was cotransfected with Renilla and c-Jun expression plasmid or empty vector (control) into DC2.4 cells to examine luciferase activities ($n = 3$). The numbers below the lanes indicate the band intensity relative to that of the loading control GAPDH. Data represent mean ± SEM. Two-way ANOVA with Bonferroni posttest (*B*, *F*, and *H*). Results were replicated ($n = 3$ experiments). $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$; ns, not significant.

and further promote Foxp3$^+$ iTreg differentiation and T cell homing, thereby maintaining intestinal immune tolerance (23). In this study, we show that, after the intestinal barrier function is disrupted by DSS, the intestinal environment shifts to a more inflammatory condition. The inflammation could dampen the tolerogenic properties of DCs (24). Under this condition, DCs might use p38α signaling to respond to proinflammatory stimulation to initiate an effective immune response. In this study, we found that p38α signaling was not required for DCs to regulate tolerogenic TGF-β2 expression and TGF-β2–dependent Foxp3$^+$ iTreg differentiation. Instead, p38α signaling, specifically in cDC1s but not in cDC2s, suppresses IL-27 expression and IL-27–dependent Tr1 differentiation, leading to the pathogenesis of IBD. Notably, cDC1s have more potential to drive Tr1 differentiation than cDC2s in our study. This functional flexibility of DCs is attributable to their ability to use different DC subsets to sense the changes of local environment and to elicit p38α signaling to shape Treg differentiation. Considering that Foxp3$^+$ iTreg cells are essential for promoting tolerance of the microbiota in the intestine under steady state and that Tr1 cells are pivotal in controlling inflammatory conditions (13), this functional adaptation might be dynamically regulated by p38α signaling in colonic DCs. Thus, modulation of local DC p38 signaling may offer a strategy for treating intestinal inflammatory diseases.

In addition to inducing intestinal inflammation, DSS-induced colitis is involved in the host's repair of intestinal epithelium barrier (25). ILCs are a highly heterogeneous population residing in almost every tissue and have been shown to be pivotal in tissue remodeling on mucosal barrier function disruption (43, 44). Among all of the identified ILC subsets, ILC3s have been shown to play important roles in intestinal tissue repair during DSS-induced colitis, in which IL-22 promotes epithelial cell proliferation and survival (45). IL-22 induction by ILC3s is mediated by either positive regulators, such as IL-23, IL-1β, Ahr, IL-7, and Notch, or negative regulators, such as TGF-β, ICOS, cMaf, IL-22BP, IL-25, IL-33, and TSLP (46). Among them, IL-23 is the most potent inducer of IL-22 production of ILC3s. Although IL-27 has been reported as a negative regulator of human $CD4^+$ T cell-derived IL-22 (46) or ILC2s (47, 48), its role in regulating ILC3 function has never been reported. In this study, we report that DC p38α-dependent IL-27 is essential for ILC3s to produce IL-22, where deletion of IL-27 in $p38\alpha^{\Delta DC}$ DCs largely abolished the increased IL-22–producing ILC3 generation, which is pivotal in intestinal tissue repairing and antimicrobial protein secretion during the acute colitis development. Notably, deletion of IL-27 in DCs does not dramatically affect the generation of IL-22–producing ILC3s on DSS treatment. Therefore, our finding establishes a previously underappreciated, nonredundant role for p38α-dependent IL-27 in the generation of IL-22–producing ILC3s. Notably, DC p38α-dependent IL-27 is essential for both Tr1 differentiation and IL-22–producing ILC3 generation, which contribute to the severity of DSS-induced colitis. Although neutralization of IL-10R signaling could largely restore the colitis severity but not the higher IL-22 expression of $p38\alpha^{\Delta DC}$ mice to the wild-type level, the relative effects of p38α-dependent IL-27 on Tr1 development and ILC3/IL-22–dependent epithelial barrier function acting on the susceptibility to colitis and tumor growth still need to be further explored in a future study. In addition, how DC p38α-driving Tr1 and ILC3 interacting with each other in vivo also needs to be explored.

Chronic intestinal inflammation represents a high risk factor for CACRC development, although its exact role is not clear (49, 50). Proinflammatory cytokines, such as TNF-α, IL-1β, IL-6 (49), and the more recently identified IL-17 (51), are thought to play essential roles in the development of CACRC. Many cytokine-modulatory therapies for colorectal cancer are under clinical trials and have shown promise (49). However, the overall efficacy is not satisfactory, highlighting a need for elucidating the complex cytokine networks within specific cell types. Our study indicates that these tumor-promoting proinflammatory cytokines can be tightly regulated by p38α signaling and that p38α deletion in DCs could reduce the levels of these cytokine, thereby ameliorating disease severity. Thus, modulation of p38 activity in DCs may prove to be an effective therapeutic strategy for CACRC. In addition, CACRC is of epithelial origin, and defective intestinal epithelial barrier function is associated with IBD and CACRC (52, 53). IL-22 has been shown to play a crucial role in restoring the dysregulated epithelial barrier function and exerting a protective role in IBD via enhanced epithelial cell proliferation, survival, and barrier function (39, 46). IL-22 production is found to be increased in the blood and intestine of IBD patients (54). However, IL-22 has also been shown to promote chronic pathological inflammatory responses and tumor development (12, 39, 46, 55), indicating that targeting IL-22 in the treatment of inflammatory-related diseases or cancer should take disease phase into account. In this study, we show that DC p38α-dependent regulation of IL-22 production from ILC3s as well as the regulation of epithelial cell proliferation and survival occur at the early acute colitis phase. However, in the chronic colitis phase, p38α signaling in DCs is not required for IL-22 production, highlighting a critical role of DC p38α-dependent regulation of early intestinal inflammation in the pathogenesis of IBD and CACRC.

Single-nucleotide polymorphisms analysis of the *Il27p28* gene revealed an association with susceptibility to IBD (56, 57). IL-27 expression is up-regulated in human colitis and mouse DSS colitis (58). However, the role of IL-27 in the pathogenesis of IBD is controversial. Depending on the disease models or cell types, IL-27 could play a protective or pathogenic role in IBD development (59–61). Thus, targeting the IL-27 pathway for treatment should consider the cell types (62). In our model here, mice deficient in IL-27 in DCs are susceptible to DSS-induced colitis, which is associated with decreased Tr1 cells, highlighting a protective role of IL-27 in the pathogenesis of IBD. Moreover, the regulation of IL-27 is different in different cell types or under different stimuli (46). In *Mycobacterium tuberculosis*-infected macrophages, p38 reduces AP-1 (mainly targeting c-Fos) binding to *Il27p28* promoter, resulting in decreased IL-27 expression and decreased Th1 response, which facilitates the survival of *M. tuberculosis* in macrophages (63). However, in LPS-stimulated p38α-deficient DCs, the up-regulated JNK activity could further promote c-Jun (but not c-Fos) binding to the *Il27p28* promoter and promote the transcription of *Il27p28*, further highlighting the complex regulation of IL-27 in different cell types.

In this study, we explored the role of p38α signaling in DCs by using the generally used CD11c-Cre as a "DC"-specific approach. Notably, recent studies have shown that many other cells in the intestine also express CD11c, such as macrophages (16, 64, 65). We carefully excluded the macrophages by using certain relatively macrophage-specific markers, such as F4/80 and CD64, for our in vitro DC and DC/T cell analysis. Although our in vitro coculture result showed that p38α in intestinal macrophages was not required for Tr1 differentiation, additional studies are needed to explore the role of p38α signaling in DCs in regulating the pathogenesis of colitis: for example, by using the various DC-Cre system (e.g., zDC-Cre), Batf3-deficient mice to make chimeras or conditional deletion of p38α in macrophages.

In conclusion, our study uncovered a previously underappreciated role of DC p38α-dependent IL-27 in the pathogenesis of colitis and CACRC. Considering that p38 has been shown as a promising candidate for targeted inhibition in acute and chronic inflammation and that most specific p38 inhibitors have been withdrawn from clinical trials due to severe side effects and safety concerns (66, 67), selectively targeting p38α signaling in DCs may offer an attractive strategy for treatment of inflammatory intestinal diseases.

Materials and Methods

Mapk14$^{fl/fl}$, *Il27p28*$^{fl/fl}$, and CD11c-Cre mice have been described previously (34, 68–70). All mice were bred and maintained in specific pathogen-free conditions in the Animal Resource Center at Shanghai Jiao Tong University School of Medicine. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Shanghai Jiao Tong University School of Medicine. Acute colitis was induced by administration of 3% (wt/vol) DSS (molecular weight: 36,000–50,000; MP Biomedicals) in sterile drinking water for 7 d followed with normal drinking water until the end of the experiment. For induction of CACRC, mice were intraperitoneally injected with 10 mg/kg AOM (Sigma-Aldrich). After 5 d, mice were supplied with 2% DSS solution for 5 d followed with normal drinking water for 15 d. The DSS cycle was repeated twice, and mice were killed 30 d after the last DSS cycle. Detailed materials and methods, including isolation of LPLs and IECs, intestinal permeability analysis, flow cytometry, cell purification and coculture, histopathological analysis, BMDC culture, Western blot analysis, ELISA, real-time qPCR, ChIP assay, luciferase reporter assay, and statistics, are available in *SI Appendix, SI Materials and Methods*.

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