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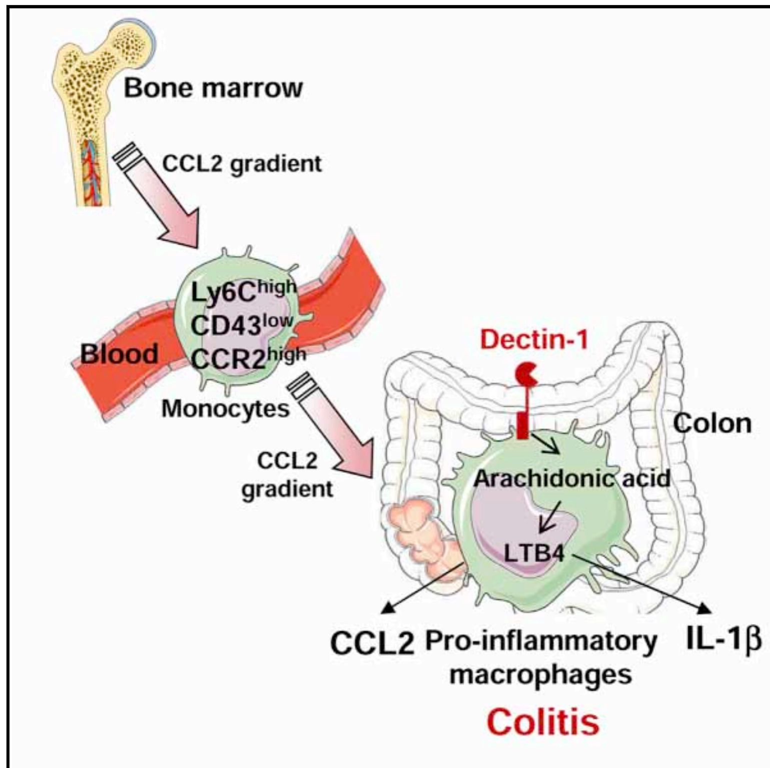
Divergent Roles for Macrophage C-type Lectin Receptors, Dectin-1 and Mannose Receptors, in the Intestinal Inflammatory Response

Graphical Abstract



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In Brief

Rahabi et al. show a critical contribution of dectin-1 on macrophages in intestinal inflammation. Dectin-1 is involved in Ly6C^{high}CCR2^{high} monocyte population enrichment in the blood and their recruitment to inflamed colon, macrophage differentiation toward inflammatory phenotype, and inflammasome-dependent IL-1β secretion through LTB4.

Highlights

- Colitis is attenuated in mice with dectin-1$^{-/-}$ macrophages and severe in mice with MR$^{-/-}$ macrophages
- Dectin-1 on macrophages mediates recruitment of inflammatory classical monocytes in the gut
- Dectin-1 positively controls inflammatory phenotype of intestinal macrophages during colitis
- Gut inflammation in IBD patients is linked to dectin-1 induction and reduced MR

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Divergent Roles for Macrophage C-type Lectin Receptors, Dectin-1 and Mannose Receptors, in the Intestinal Inflammatory Response

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SUMMARY

Colonic macrophages are considered to be major effectors of inflammatory bowel diseases (IBDs) and the control of gut inflammation through C-type lectin receptors is an emerging concept. We show that during colitis, the loss of dectin-1 on myeloid cells prevents intestinal inflammation, while the lack of mannose receptor (MR) exacerbates it. A marked increase in dectin-1 expression in dextran sulfate sodium (DSS)-exposed MR-deficient mice supports the critical contribution of dectin-1 to colitis outcome. Dectin-1 is crucial for Ly6C^{high}CCR2^{high} monocyte population enrichment in the blood and their recruitment to inflamed colon as precursors of inflammatory macrophages. Dectin-1 also promotes inflammasome-dependent interleukin-1β (IL-1β) secretion through leukotriene B4 production. Interestingly, colonic inflammation is associated with a concomitant overexpression of dectin-1/CCL2/LTA4H and downregulation of MR on macrophages from IBD patients. Thus, MR and dectin-1 on macrophages are important mucosal inflammatory regulators that contribute to the intestinal inflammation.

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are chronic inflammatory bowel diseases (IBDs) caused by a combination of intestinal dysbiosis, increased intestinal mucosal permeability to gut bacteria, and inappropriate immunomodulatory response. Genetic or environmental factors also contribute to the pathogenesis of this disease.

Macrophages represent the most abundant mononuclear phagocytic population in the *lamina propria* of the intestine and are essential for local homeostasis and maintaining a balance between the commensal microbiota and the host (Bain and Mowat, 2014). The selective inertia of intestinal macrophages is one of the principal mechanisms preventing inappropriate reactions to commensal microbes. These tissue-resident intestinal macrophages act as noninflammatory scavengers of microbes, and they also assist in the maintenance of regulatory T (Treg) cells and promote epithelial cell renewal (Bain and Mowat, 2014; Kühl et al., 2015).

In IBD, macrophages massively infiltrate the intestinal mucosae, and these newly recruited activated macrophages are thought to be major contributors to the production of inflammatory cytokines, thus playing a central role in the pathogenesis of IBD (Neurath, 2014). Several lines of evidence support the role of inflammatory macrophages in IBD pathogenesis. In addition to the increased presence of inflammatory-polarized macrophages in the *lamina propria*, studies have shown that these macrophages directly contribute to the defective intestinal barrier function in subjects with IBD (Lissner et al., 2015) by secreting pro-inflammatory mediators such as tumor necrosis factor α (TNF-α), interleukin (IL)-1, IL-6, IL-8, IL-12, IL-18, arachidonic acid (AA) metabolites, and reactive oxygen or nitrogen species (Bain and Mowat, 2014; Lissner et al., 2015). Because intestinal macrophages display a rather short half-life, a continuous replenishment by blood monocytes is required. In IBD, inflammatory Ly6C^{high}CCR2^{high} monocytes are recruited from the blood to the *lamina propria* to promote inflammation (Zigmond et al., 2012; Grainger et al., 2017). In tissues, Ly6C^{high}CCR2^{high} and Ly6C^{low}CCR2^{low} monocytes are generally thought to undergo preferential differentiation into inflammatory macrophages and anti-inflammatory macrophages, respectively (Yang et al., 2014).

Pathogen recognition receptors (PPRs) play a crucial role in maintaining the fine balance between immune tolerance to commensal microbes and uncontrolled innate activation leading to inflammation and, subsequently, IBD pathogenesis. Among the PRRs, some C-type lectin receptors (CLRs), such as

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dectin-1, mannose receptor (MR), and dendritic-cell-specific intercellular-adhesion-molecule-3-grabbing non-integrin (DC-SIGN) have been found to be involved in IBD pathogenesis (Heinsbroek et al., 2012; Iliev et al., 2012; Liu et al., 2008; Saunders et al., 2010; Sokol et al., 2016; Tang et al., 2015). Although dectin-1 is the most investigated, its role in the control of gut inflammation remains questionable. On one hand, it has been shown that dectin-1-deficient mice develop more severe dextran sulfate sodium (DSS)-induced colitis. This was accompanied by mycobiota changes and overgrowth of opportunist species, demonstrating that this phenotype was dependent on the inability of dectin-1-deficient mice to control fungal invasion during colitis. This study suggest that host-impaired antifungal immunity is involved in the development of gut inflammation (Iliev and Leonardi, 2017; Iliev et al., 2012; Leonardi et al., 2018). On the other hand, dectin-1-deficient mice were refractory to experimental colitis in the absence of opportunistic fungi due to an increase in *Lactobacillus murinus* in the gut commensal microflora that triggers the expansion of Treg cells (Tang et al., 2015). Thus, although several lines of evidence support the role of dectin-1 in the production of inflammatory mediators (Galès et al., 2010; Lefèvre et al., 2013), the composition of the fungal gut microbiota could be a determining factor in the pro- or anti-inflammatory effect of dectin-1 during colitis (Heinsbroek et al., 2012; Iliev and Leonardi, 2017; Iliev et al., 2012; Tang et al., 2015).

Interestingly, it has been shown that subjects with CD display elevated dectin-1 expression on intestinal macrophages, neutrophils, and other immune cells compared to healthy individuals (de Vries et al., 2009). In contrast to data related to dectin-1, patients with active CD have a reduced number of macrophages expressing MR ($CD68^+CD206^+$) (Hunter et al., 2010). Moreover, a previous study demonstrated that patients who respond to infliximab therapy exhibit a significant induction of these macrophages that contributes to mucosal healing (Vos et al., 2012). This is supported by a report demonstrating the protective effects of a polysaccharide-targeting MR against diarrhea, colonic inflammation, and ulceration in rats with colitis (Liu et al., 2008). In contrast to the protective role of MR, a recent report demonstrated that MR-deficient mice display attenuated intestinal inflammation in experimental colitis. However, this study attributed this effect to a microRNA (miR-511-3p) harbored in intron 5 of the CD206 gene (Heinsbroek et al., 2016).

While the two CLRs, dectin-1 and MR, play a crucial role in pathogen recognition and phagocytosis by macrophages, several studies indicate that they contribute differently to the activation of inflammatory signaling pathways. Indeed, it has been shown that dectin-1, by recognizing carbohydrates on the surface of pathogens, promotes production of reactive oxygen species (Galès et al., 2010; Lefèvre et al., 2013) and IL-1β secretion by macrophages through inflammasome-dependent signaling pathways (Cheng et al., 2011). These findings support the hypothesis that dectin-1 plays an important role in IBD development, as the NLRP3 inflammasome was identified as a critical mechanism by which macrophages are involved in intestinal inflammation (Bauer et al., 2010). Despite its capacity to participate in inflammatory signaling pathways in response to infections (Coste et al., 2003; Galès et al., 2010), MR has also been shown to dampen inflammation and inhibit T helper type 1 (Th1)-polarized immune responses (Chieppa et al., 2003).

In the present study, we identify dectin-1 on macrophages as an important regulator of the mucosal inflammatory response during intestinal inflammation. Dectin-1, through CCL2 production, promotes the infiltration of $Ly6C^{high}CCR2^{high}$ monocyte subset from the blood to the inflamed colon as precursor of inflammatory macrophages. Dectin-1 also mediates inflammasome-dependent IL-1β secretion through the control of LTB4 production. Finally, we demonstrate that intestinal inflammation occurring in subjects with IBD is associated with dectin-1 induction and MR downregulation on macrophages, highlighting the relevant role of these CLRs on macrophages in the IBD pathogenesis in humans.

RESULTS

Dectin-1 and MR Have Distinct Contributions to the Pathogenesis of Colitis

To explore whether dectin-1 and MR on myeloid compartment participate in the development of DSS-induced colitis, we evaluated intestinal inflammatory responses and mucosal injury in dectin-1- or MR-conditionally deficient (LysM-Cre/dectin-1 L2/L2 mice [dectin-$1^{-/-}$] or LysM-Cre/MR L2/L2 mice [$MR^{-/-}$]) mice, in which the *Clec7a* or *Mrc-1* gene were selectively disrupted in myeloid-derived cells, and their corresponding wild-type (WT) littermates (STAR Methods; Figure S1). Compared to WT mice exposed to DSS, weight loss was significantly lower in the DSS-exposed myeloid-specific dectin-$1^{-/-}$ mice (Figure 1A). Macro- and microscopic scores, and colonic myeloperoxidase (MPO) activity (Figure 1B), a marker of neutrophil and macrophage infiltrations, were consistently and significantly reduced in the colons of DSS-exposed dectin-$1^{-/-}$ mice compared to those in the colons of their DSS-exposed WT littermates. The reduced inflammation in the colons of dectin-$1^{-/-}$ mice was characterized by a decrease in glandular ulceration, fewer inflammatory infiltrates, and the absence of necrotic regions (Figure 1J), highlighting dectin-1 as a susceptibility factor for colitis. By contrast, weight loss increased significantly in DSS-exposed $MR^{-/-}$ mice compared to their DSS-exposed WT littermates (Figure 1D). Consistent with this finding, DSS-exposed $MR^{-/-}$ mice displayed a higher degree of colonic inflammation, as reflected by elevated macro- and microscopic scores, higher MPO activity (Figure 1E), extensive inflammatory infiltrates associated with glandular ulceration and the loss of crypts, and hypersecreting appearance of the adjacent glands (Figure 1J), suggesting a protective role of MR against colitis. While the mild colonic inflammation in the DSS-exposed dectin-$1^{-/-}$ mice was associated with low levels of the pro-inflammatory cytokines IL-6 and IL-1β in the colon (Figure 1C), the exacerbated colonic inflammation observed in DSS-exposed $MR^{-/-}$ mice was related to the excessive secretion of these cytokines (TNF-α, IL-6, and IL-1β) (Figure 1F). The severity of colitis resulted in a significantly higher survival rate of dectin-$1^{-/-}$ mice and a lower survival rate of $MR^{-/-}$ mice compared with their WT littermates, supporting a role for dectin-1 and MR in colitis outcome (Figure 1K). The role of dectin-1 and MR in the development of colitis was further supported in another TNBS (2,4,6-trinitrobenzenesulfonic acid solution)-induced colitis murine experimental model (Figure S2).

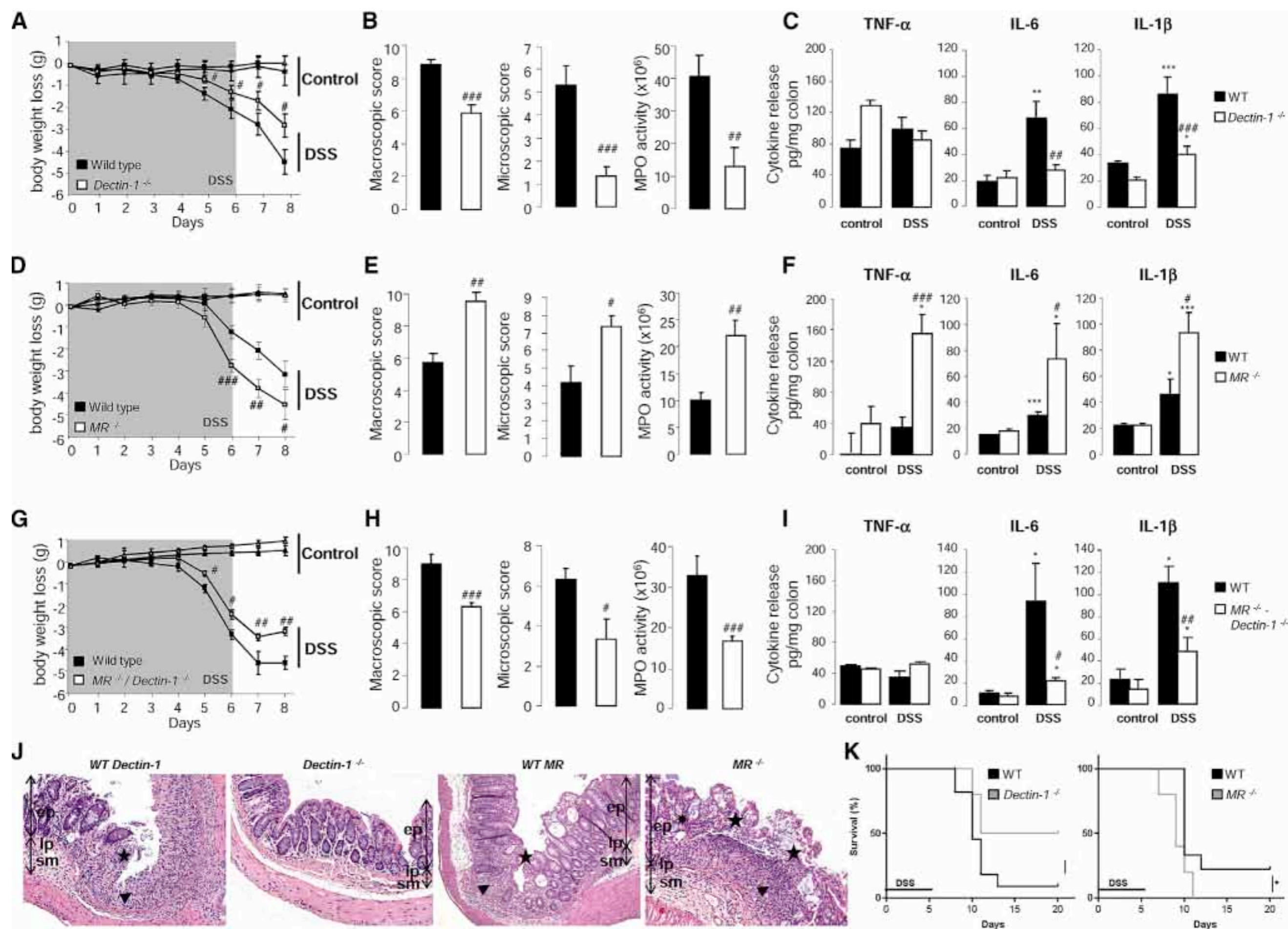


Figure 1. Different Contributions of Dectin-1 and MR on Macrophages in the Development of Gut Inflammation

(A–I) Body weight (A, D, and G); macro- and microscopic scores, MPO activity (B, E, and H); and TNF-α, IL-6, and IL-1β levels (C, F, and I) were determined at 8 days in the colons of control or DSS-exposed dectin-1$^{-/-}$, MR$^{-/-}$, and dectin-1$^{-/-}$-MR$^{-/-}$ mice and their WT littermates (n = 12 per group). $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, and $^{\#\#\#}p \leq 0.005$ compared to DSS-exposed corresponding WT littermates. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.005$ compared to respective unexposed mice (control).

(J) H&E staining of representative cross-sections of distal colon from DSS-exposed WT, dectin-1$^{-/-}$, and MR$^{-/-}$ mice. Glandular ulceration and loss of colonic epithelium (ep) are marked with a star; hypersecretory appearance of adjacent glands is marked with an asterisk; inflammatory infiltrate in the *lamina propria* (lp) and submucosa (sm) is marked by black triangles. Data are representative of three independent experiments.

(K) Survival curve of dectin-1$^{-/-}$, MR$^{-/-}$ mice, and their WT littermates (n = 10 per group) treated with 2% DSS for 6 days. Mice were then followed until day 20. $^{*}p \leq 0.05$.

See also Figure S2.

Remarkably, the use of a mouse model deficient in both dectin-1 and MR in myeloid-derived cells demonstrated that these DSS-exposed mice exhibited less severe inflammation than WT, as demonstrated by reduced weight loss (Figure 1G) and decreased macro- and microscopic scores, MPO activity (Figure 1H), and TNF-α, IL-6, and IL-1β levels in the colons (Figure 1I). These data are similar to that observed in DSS-exposed dectin-1$^{-/-}$ mice and highlight a greater role of dectin-1 compared to MR in the development of colitis.

We then thoroughly investigated the mucosae-associated fungal and bacterial colonization of unexposed or DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$ mice and their WT littermates (Figures S3A and S3B). Although no significant difference was observed in the fungal and bacterial content between dectin-1$^{-/-}$ and MR$^{-/-}$ mice and their WT littermates, as expected, DSS treatment increases pro-inflammatory fungal burden from *Candida sp.* and pro-inflammatory bacterial burden from *Bacteroïdetes* and *Enterobacteria* phylum (Ohkusa and Koido, 2015). In dectin-1$^{-/-}$ mice, reduced severity of colitis was associated with a decrease in the content of pro-inflammatory bacteria (*Bacteroïdetes* and *Enterobacteria*) and fungi (*Candida sp.*) and with an increase in protective bacteria (*Firmicutes* and *F. prausnitzii*) (Figure S3A). Inversely, the severity of colitis in MR$^{-/-}$ mice correlated with increased *Bacteroïdetes*, *Enterobacteria*, and *Candida sp.* (Figure S3B).

To evaluate that *Candida* burden may influence the inflammatory phenotype of DSS-treated mice, we evaluated the macroscopic score in DSS-exposed dectin-1$^{-/-}$ mice and their WT

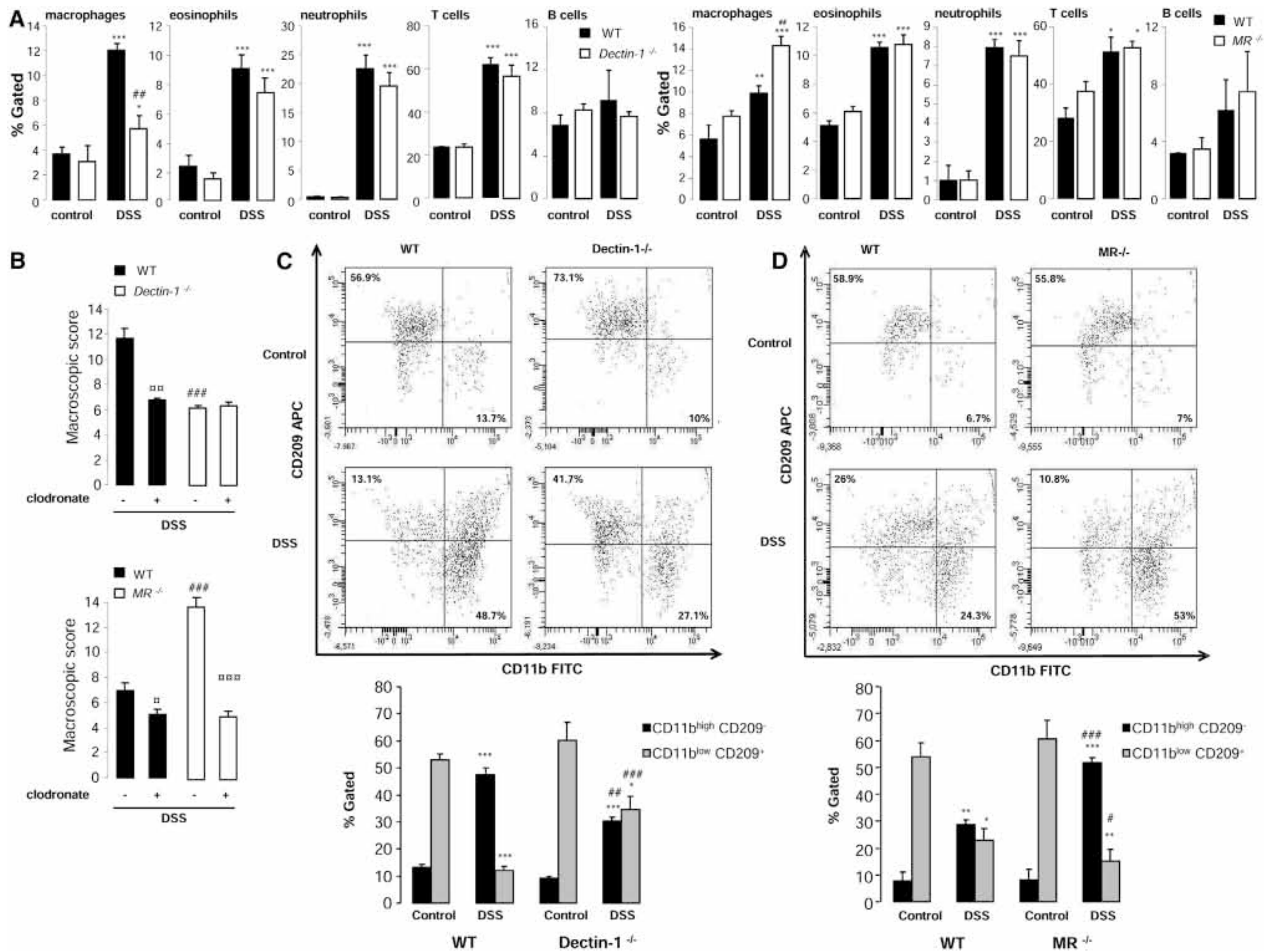


Figure 2. Dectin-1 Is Involved in the Phenotypic Differentiation of Colonic Macrophages toward a Pro-inflammatory Phenotype during Colitis

(A) Percentages of macrophages, eosinophils, neutrophils, T cells, and B cells in the colons of unexposed (control), DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$ mice and their WT littermates. Among viable cells, macrophages were identified as CD45$^+$, CD11b$^+$, F4/80$^+$, SiglecF$^-$, Ly6G$^-$, eosinophils (CD45$^+$, F4/80$^+$, CD11b$^+$, SiglecF$^+$, Ly6G$^-$), neutrophils (CD45$^+$, F4/80$^-$, CD11b$^+$, Ly6G$^+$, SiglecF$^-$), and T and B cells (CD45$^+$, CD3$^+$ and CD45$^+$, CD19$^+$) by flow cytometry. *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.005$ compared to respective unexposed mice (control). ##$p \leq 0.01$ compared to DSS-exposed corresponding WT littermates. Data are representative of three independent experiments.

(B) Macroscopic score of DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$ mice and WT littermates treated or not with clodronate liposomes. ###$p \leq 0.005$ compared to corresponding WT littermates. ¤$p \leq 0.05$, ¤¤$p \leq 0.01$, and ¤¤¤$p \leq 0.005$ compared to clodronate-exposed mice. See also Figure S4.

(C and D) Representative dot-plot of CD209$^+$ and CD11b$^+$ macrophages from the colons of control, DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$ mice. Macrophages were identified as before. Histograms representing the mean values of the percentage of CD11b^{high} CD209$^-$ macrophages and CD11b^{low} CD209$^+$ macrophages in the colons of control, DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$ mice. Data are representative of three independent experiments. *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.005$ compared to respective unexposed mice (control). #$p \leq 0.05$, ##$p \leq 0.01$, and ###$p \leq 0.005$ compared to DSS-exposed corresponding WT littermates.

littermates after a gastrointestinal infection with *C. albicans*. *C. albicans* supplementation of dectin-1$^{-/-}$ mice aggravated colitis in response to DSS (Figure S3C), supporting that the absence of dectin-1 can also promote DSS-induced colitis because of the expansion of pathogenic commensal fungi in the gut (Iliev et al., 2012; Leonardi et al., 2018).

Dectin-1 Promotes Pro-inflammatory Polarization of Colonic Macrophages during Colitis

To establish whether dectin-1 and MR on myeloid cells influence colonic inflammation, we evaluated the mucosal infiltration of macrophages (CD45$^+$, CD11b$^+$, F4/80$^+$, SiglecF$^-$, Ly6G$^-$), eosinophils (CD45$^+$, F4/80$^+$, CD11b+, SiglecF$^+$, Ly6G$^-$), neutrophils (CD45$^+$, F4/80$^-$, CD11b+, Ly6G$^+$, SiglecF$^-$), T and B cells (CD45$^+$, CD3$^+$ and CD45$^+$, CD19$^+$) in colonic tissues from DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$ mice (Figures S4 and 2A). As expected, DSS induced significant macrophages, eosinophil, neutrophil, and T cell infiltration in the colonic mucosae of WT mice (Figure 2A). Interestingly, although mucosal macrophage infiltration was reduced in DSS-exposed dectin-1$^{-/-}$ mice, the DSS-exposed MR$^{-/-}$ mice displayed increased macrophage proportion in the colonic mucosae. Moreover, the frequency of eosinophils, neutrophils, T cells, and B cells in colonic mucosa was not altered both in DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$

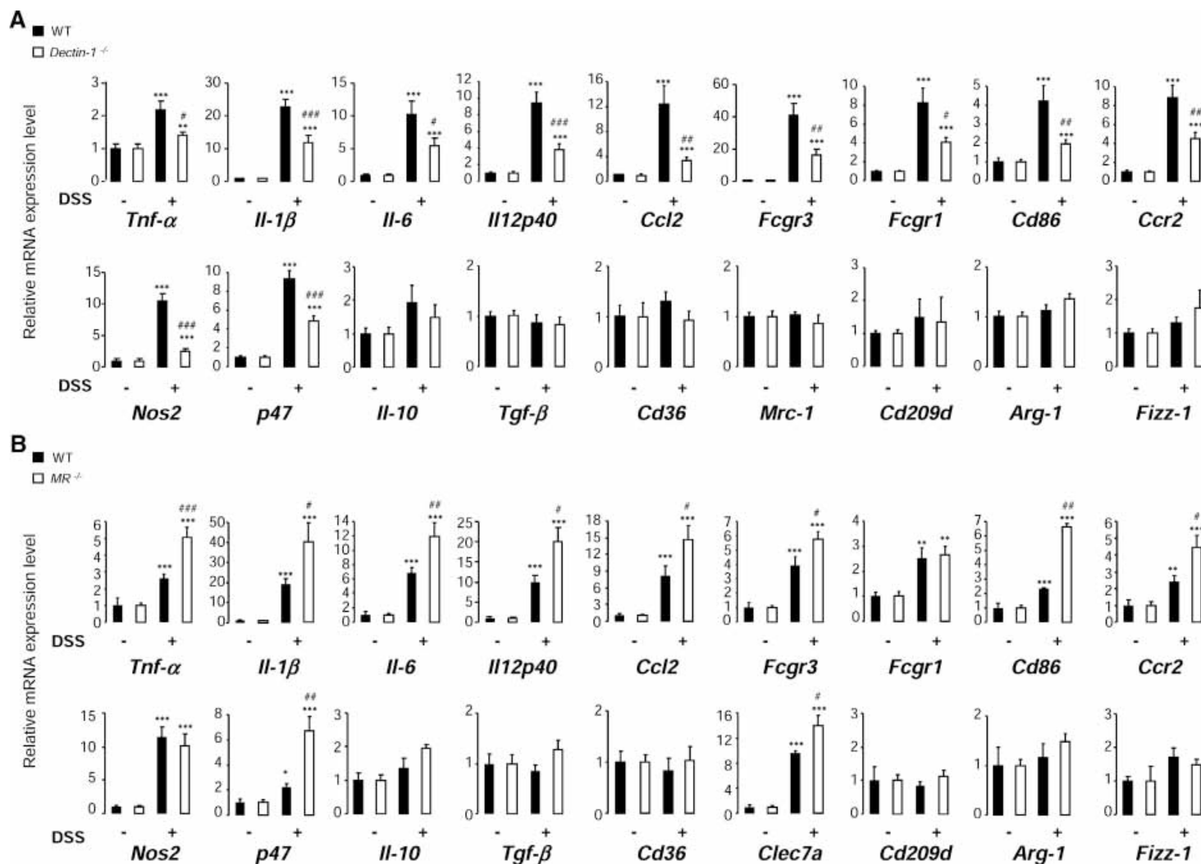


Figure 3. Dectin-1 Controls Inflammatory-Specific Gene Expression of Colonic Macrophages during Colitis

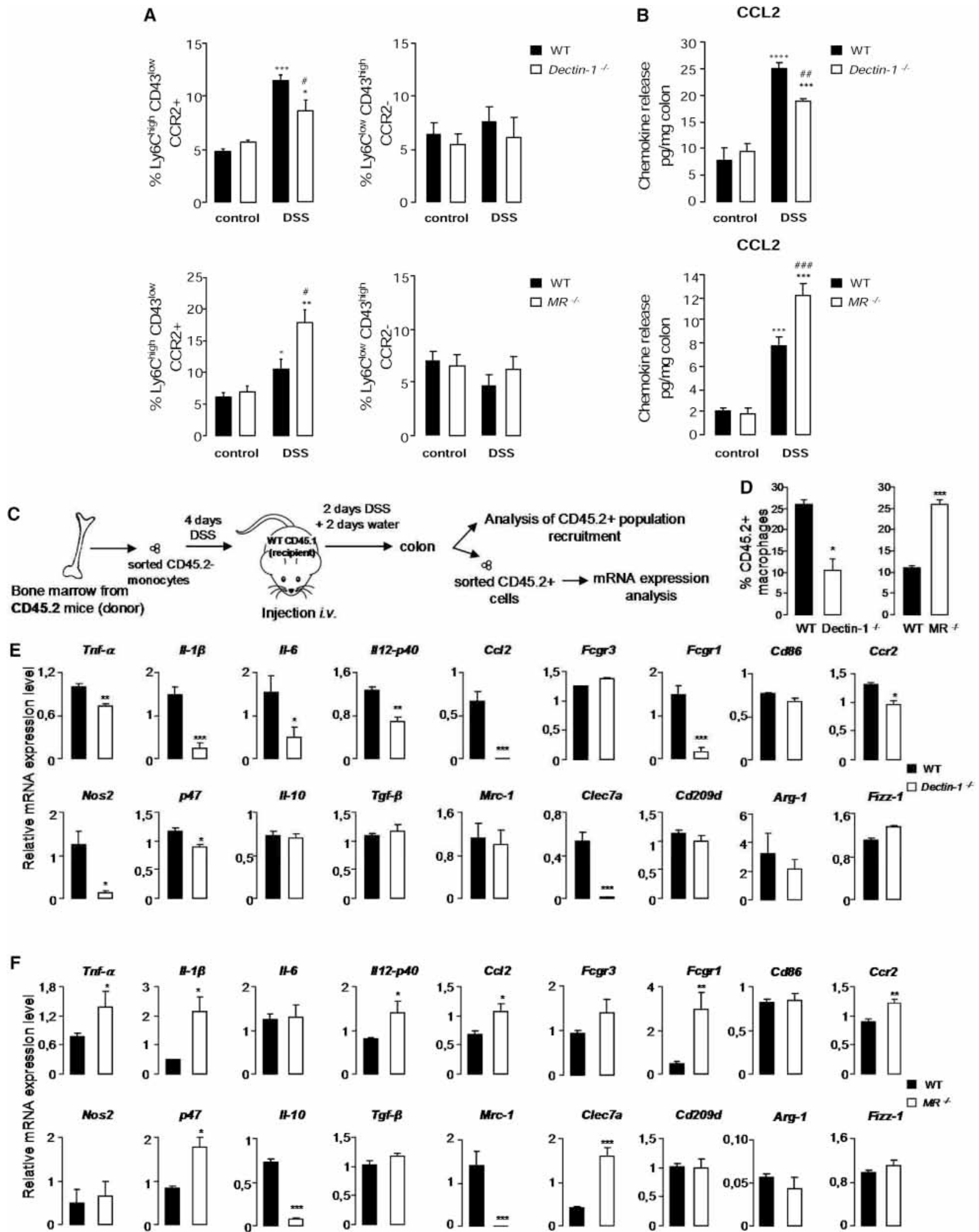
Gene expression analysis of inflammatory and anti-inflammatory polarization markers in colonic macrophages from control or DSS-exposed WT and dectin-1$^{-/-}$ (A) or MR$^{-/-}$ (B) mice. Data are representative of three independent experiments. *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.005$ compared to respective unexposed mice. $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, and $^{\#\#\#}p \leq 0.005$ compared to DSS-exposed corresponding WT littermates.

mice. These data suggest that only macrophage recruitment in colon during colitis is dependent on the expression of dectin-1 or MR.

Interestingly, intraperitoneal (i.p.) administration of clodronate-containing liposomes to mice, leading to a selective depletion of macrophages (Figure S5), decreased the macroscopic score in both dectin-1$^{-/-}$, MR$^{-/-}$ mice and their WT littermates, supporting the critical involvement of macrophages in the development of colitis (Figure 2B).

Inflammatory activity in colitis is closely dependent on the polarization state of macrophages. To further explore whether dectin-1 and MR influence the phenotype of recruited macrophages, we analyzed macrophage populations present in colonic tissues from dectin-1$^{-/-}$ and MR$^{-/-}$ mice following DSS treatment. As depicted in Figures 2C and 2D, a large number of the colonic-resident macrophages in the non-DSS-exposed WT, dectin-1$^{-/-}$, and MR$^{-/-}$ mice exhibited a CD11b^{low} and CD209^{+} anti-inflammatory phenotype. In the colons of DSS-exposed WT mice, the proportion of CD11b^{high} and CD209^{-} pro-inflammatory macrophages increased significantly. Interestingly, whereas the induction of the proportion of CD11b^{high} and CD209^{-} macrophages in DSS-exposed WT mice was greatly reduced in dectin-1$^{-/-}$ mice, it increased substantially in MR$^{-/-}$ mice, demonstrating that the severity of colitis is related to the distinct pro-inflammatory macrophage population.

To assess the role of dectin-1 and MR in macrophage activation during the development of colonic inflammation, we evaluated the gene expression of pro- and anti-inflammatory markers and specific classical and alternative activation markers in colonic macrophages from DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$ mice (Figure 3). Overall, macrophages from DSS-treated WT mice displayed an upregulation of pro-inflammatory cytokines, such as *Tnfα*, *Il1β*, *Il6*, and *Il12p40*. In line, chemokine ligand 2 (*Ccl2*), Fcγ receptor *Fcgr3* (Cd16), *Fcgr1* (Cd64), co-stimulatory protein *Cd86*, chemokine receptor 2 (*Ccr2*), inducible nitric oxide synthase (*Nos2*), and *p47^{phox}* (a cytosolic subunit of the NADPH oxidase complex) pro-inflammatory factors were also upregulated (Figures 3A and 3B). Consistent with the reduced susceptibility of dectin-1$^{-/-}$ mice to colitis, the transcriptional induction of pro-inflammatory markers upon DSS treatment was reduced in the macrophages from dectin-1$^{-/-}$ mice (Figure 3A). In contrast, with the more severe colonic inflammation in MR$^{-/-}$ mice, colonic macrophages displayed a robust expression of inflammatory markers (Figure 3B). Thus, more pronounced colonic inflammation was associated with the presence of colonic macrophages that expressed high levels



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of pro-inflammatory markers, highlighting that the polarization state of macrophages is critical for colitis development. Next, we evaluated the expression of anti-inflammatory and specific alternative markers. DSS treatment did not alter the expression of *Il10*, *Tgf-β*, *Cd36*, *Mrc-1* (MR), *Cd209*, *Arginase-1*, and *Fizz-1* (Figures 3A and 3B). Only *Clec7a* (dectin-1) increased significantly in DSS-exposed WT mice, and its expression was amplified in MR$^{-/-}$ mice (Figure 3B), further supporting the significant contribution of dectin-1 to the development of colonic inflammation.

Dectin-1 Mediates the Infiltration of the Ly6C^{high}CCR2^{high} Monocyte Subset from the Blood to the Inflamed Colon as Inflammatory Macrophage Precursors

The inflammatory Ly6C^{high}CCR2^{high} monocytes in the blood selectively traffic to sites of injury to promote inflammation, and once within the tissues, they differentiate into inflammatory macrophages (Yang et al., 2014; Zigmond et al., 2012). To elucidate whether the dectin-1-mediated inflammatory phenotype in colonic macrophages was accompanied by a specific phenotypic profile of blood monocytes, the characterization of monocyte subsets was performed in DSS-exposed dectin-1$^{-/-}$ or MR$^{-/-}$ mice (Figure 4A). As expected, DSS induced a significant increase in the inflammatory Ly6C^{high}CD43^{low}CCR2^{high} monocyte subset in the blood of WT mice. Interestingly, this augmentation was reduced in DSS-exposed dectin-1$^{-/-}$ mice, whereas it was amplified in DSS-exposed MR$^{-/-}$ mice. The proportion of the anti-inflammatory Ly6C^{low}CD43^{high}CCR2^{low} monocyte subset was not altered in DSS-exposed WT or deficient mice (Figure 4A).

Consistent with the increase in the Ly6C^{high}CCR2^{high} monocyte subset in DSS-exposed mice, we observed an elevated amount of CCL2 (the major chemokine driving Ly6C^{high}CCR2^{high} monocyte recruitment from the bone marrow to the bloodstream and sites of inflammation) in the colon (Figure 4B). Moreover, the amount of CCL2 decreased in DSS-exposed dectin-1$^{-/-}$ mice and increased in DSS-exposed MR$^{-/-}$ mice relative to that in DSS-exposed WT mice, suggesting that dectin-1-mediated CCL2 production in the colon during colitis is involved in the enrichment of the Ly6C^{high}CCR2^{high} population in the blood and the recruitment of these inflammatory macrophage precursors to inflamed intestinal tissues.

To further confirm the involvement of dectin-1 or MR in the recruitment of macrophages into colonic mucosae from the blood monocytes during colitis, CD45.1 recipient mice were transferred with sorted monocytes isolated from CD45.2 donor dectin-1$^{-/-}$ or MR$^{-/-}$ mice and their WT littermates (Figure 4C). Interestingly, the percentage of CD45.2 macrophages in the colon of CD45.1 recipient mice was strongly decreased when the transferred monocytes were derived from dectin-1$^{-/-}$ mice (Figure 4D). Conversely, when the transferred monocytes were derived from CD45.2 MR$^{-/-}$ mice, the percentage of CD45.2 macrophages in colonic mucosae of CD45.1 recipient mice was robustly increased, supporting the involvement of dectin-1 and MR in the recruitment of colonic macrophages derived from blood monocytes during colitis.

To determine the capacity of activation of macrophages derived from monocytes recruited in colon during colitis, gene expression of pro- and anti-inflammatory and specific classical and alternative activation markers was evaluated in colonic CD45.2 cells isolated from CD45.1 recipient mice (Figure 4C). Remarkably, mRNA levels of *Tnfα*, *Il1β*, *Il6*, *Il12p40*, *Ccl2*, *Fcgr1*, *Ccr2*, *Nos2*, and *p47^{phox}* pro-inflammatory markers were decreased in CD45.2^{+} cells from CD45.1 recipient mice transferred with monocytes from dectin-1$^{-/-}$ mice (Figure 4E). Inversely, CD45.2^{+} cells from CD45.1 recipient mice transferred with monocytes from MR$^{-/-}$ mice displayed an upregulation of pro-inflammatory markers (Figure 4F). Interestingly, *Clec7a* expression was strongly increased in CD45.2^{+} cells from CD45.1 recipient mice transferred with monocytes from MR$^{-/-}$ mice (Figure 4F), further supporting the critical contribution of dectin-1 in the activation of colonic macrophages toward an inflammatory phenotype.

Therefore, altogether, these experiments confirm that dectin-1 contributes to Ly6C^{high}CCR2^{high} monocyte population enrichment in the blood, their recruitment to inflamed colon as precursors of colonic macrophages, and the activation of these macrophages toward an inflammatory profile.

Dectin-1 Is Critical for 5-Lipoxygenase/LTA4H-Mediated Leukotriene B4 (LTB4) Production during Colitis

As one of the most important mediators of IBD, AA metabolites (including prostaglandins and leukotrienes) are essential to the development of colonic inflammation. Because the major pathways for AA metabolism that are relevant for IBD include COX-1/COX-2 cyclooxygenases, 5-LOX, and 12/15-LOX, their expression was evaluated in colonic macrophages from DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$ mice (Figure 5A). The COX-2

Figure 4. Dectin-1 Enriches the Ly6C^{high}CCR2^{high} Blood Monocyte Subset through CCL2 Production and Enhances Its Recruitment to Inflamed Colons during Colitis

(A) Histograms representing the proportion of CD11b^{+}, Ly6C^{high}, CD43^{low}, and CCR2^{+} monocytes and CD11b^{+}, Ly6C^{low}, CD43^{high}, and CCR2^{-} monocytes in the blood of control, DSS-exposed WT, dectin-1$^{-/-}$ and MR$^{-/-}$ mice.

(B) CCL2 chemokine production in colons from control, DSS-exposed WT, dectin-1$^{-/-}$ and MR$^{-/-}$ mice.

Data are representative of three independent experiments. *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.005$ compared to respective unexposed mice (control). $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, and $^{\#\#\#}p \leq 0.005$ compared to DSS-exposed corresponding WT littermates.

(C) Experimental procedure. CD45.1 mice received DSS for 4 days. CD45.1 recipient mice were injected into the ophthalmic venous sinus with sorted monocytes isolated from CD45.2 donor mice. After 2 days of DSS treatment followed by 2 days of drinking water, mice underwent colon resection, and CD45.2^{+} cells isolated by enzymatic digestion of colons were analyzed by flow cytometry (D) and sorted from the colon to evaluate mRNA expression (E and F).

(D) Analysis of CD45.2^{+} macrophages of DSS-exposed CD45.1 mice injected with monocytes from CD45.2 WT, dectin-1$^{-/-}$ or MR$^{-/-}$ mice.

(E and F) Gene expression analysis of inflammatory and anti-inflammatory markers of macrophages in CD45.2 sorted-cells isolated from the colon of CD45.1 mice injected with monocytes from CD45.2 WT, dectin-1$^{-/-}$ (E) or MR$^{-/-}$ (F) mice. *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.005$ compared to WT.

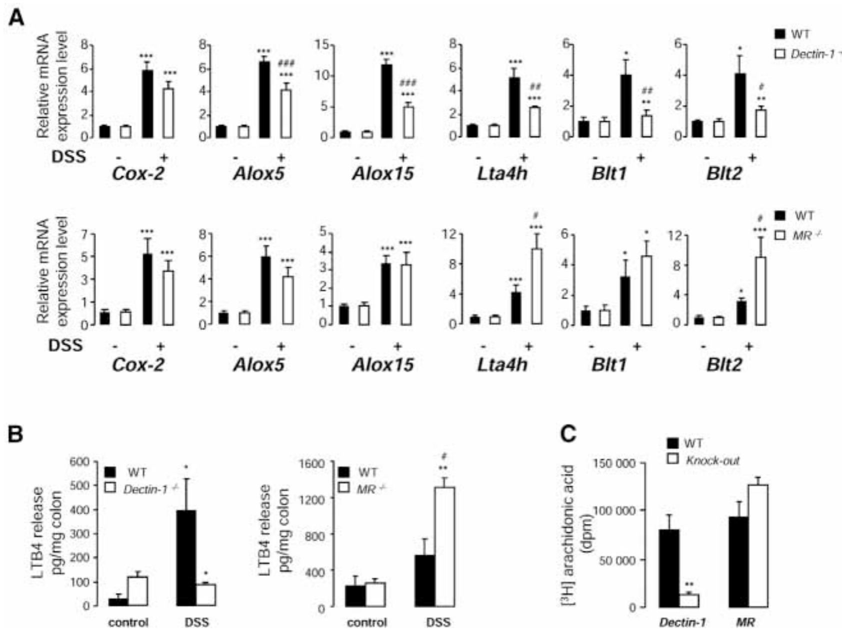

Figure 5. Dectin-1 Induces LTB4 Secretion through the Control of AA/5-LOX/LTA4H Signaling during Colitis

(A) Gene expression analysis of major enzymes involved in AA metabolism and LTB4 production in colonic macrophages from control, DSS-exposed WT, dectin-1$^{-/-}$ and MR$^{-/-}$ mice.

(B) LTB4 secretion in colons from DSS-exposed WT, dectin-1$^{-/-}$ and MR$^{-/-}$ mice.

(C) (^{3}H) AA levels in the culture media of LPS-primed WT, dectin-1$^{-/-}$ and MR$^{-/-}$ macrophages exposed to DSS. Data are representative of three independent experiments. $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, and $^{***}p \leq 0.005$ compared to respective unexposed mice (control). $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$, and $^{\#\#\#}p \leq 0.005$ compared to DSS-exposed corresponding WT littermates.

mRNA level was strongly induced in macrophages from DSS-exposed WT mice, while it remained unaffected in macrophages from DSS-exposed dectin-1$^{-/-}$ and MR$^{-/-}$ mice (Figure 5A). Although the mRNA levels of 5-LOX (*Alox5*) and 12/15-LOX (*Alox15*) were increased substantially in macrophages from DSS-exposed WT mice, their expression was significantly decreased in macrophages from DSS-exposed dectin-1$^{-/-}$ mice and unaltered in the macrophages from DSS-exposed MR$^{-/-}$ mice (Figure 5A). In line with reduced 5-LOX and 12/15-LOX gene expression in macrophages from DSS-exposed dectin-1$^{-/-}$ mice, mRNA expression of LTA4 hydrolase (*Lta4h*), which controls LTB4 production, decreased substantially. Accordingly, LTB4 production was completely abrogated in the colons of DSS-exposed dectin-1$^{-/-}$ mice (Figure 5B). Conversely, the mRNA level of LTA4H was substantially increased in macrophages from DSS-exposed MR$^{-/-}$ mice (Figure 5A), correlating with augmented LTB4 levels (Figure 5B). Consistent with the altered LTB4 production in macrophages from DSS-exposed dectin-1$^{-/-}$ mice, the LTB4 receptors BLT1 and BLT2 remained poorly expressed (Figure 5A). Inversely, mRNA levels of both BLT1 and BLT2 increased in macrophages from DSS-exposed MR$^{-/-}$ mice (Figure 5A). Furthermore, DSS treatment resulted in the induction of AA secretion by WT and MR$^{-/-}$ macrophages, but not dectin-1$^{-/-}$ macrophages (Figure 5C). Altogether, these data indicate that dectin-1 is critical for the mobilization of AA and for the regulation of 5-LOX and LTA4H, supporting the importance of dectin-1 in the control of LTB4 production during colitis.

Dectin-1 Mediates Inflammasome-Dependent IL-1β Secretion through the Control of LTB4 Production

Considering the involvement of dectin-1 and MR in the regulation of LTB4 production in macrophages during colitis and the importance of LTB4 in inflammasome-dependent IL-1β release (Amaral et al., 2012), we next evaluated the role of LTB4 in the control of IL-1β production in dectin-1$^{-/-}$ and MR$^{-/-}$ macrophages (Figure 6). These macrophages were primed with lipopolysaccharide (LPS), mannan, and (1, 3)-β-glucans (zymosan) *in vitro* to trigger transcription of pro-IL-1β and then stimulated with DSS, a potassium ionophore activating caspase-1, which is involved in the proteolytic processing of pro-IL-1β (Bauer et al., 2010). As depicted in Figure 6A, LTB4 production was strongly increased in LPS-primed WT macrophages exposed to DSS compared to resident macrophages. This induction was totally abolished in LPS-primed dectin-1$^{-/-}$ macrophages exposed to DSS, while it was improved in LPS-primed MR$^{-/-}$ macrophages exposed to DSS. These results support the major role of dectin-1 in the control of LTB4 production by macrophages. Although IL-1β production was not affected in LPS-primed *Alox15*$^{-/-}$ macrophages exposed to DSS, it was strongly decreased in the *Alox5*$^{-/-}$ macrophages (Figure 6B). Interestingly, the addition of LTB4 restored IL-1β secretion in *Alox15*$^{-/-}$ macrophages, supporting the importance of 5-LOX-mediated LTB4 production in IL-1β generation during colonic inflammation. While the addition of exogenous LTB4 efficiently restored IL-1β production in LPS-primed dectin-1$^{-/-}$ macrophages exposed to DSS, it did not affect IL-1β secretion by MR$^{-/-}$ macrophages (Figure 6C). These data clearly demonstrate the critical role for dectin-1 and MR in LTB4-mediated IL-1β production.

The use of a specific caspase-1 inhibitor Z-YVAD revealed that the secretion of IL-1β by LPS-primed macrophages exposed to DSS was dependent on caspase-1 activation, as demonstrated by an inability of WT, dectin-1$^{-/-}$, and MR$^{-/-}$ macrophages to produce IL-1β (Figure 6C). Moreover, the processing of pro-caspase-1 into its p10 subunit, which is a hallmark of caspase-1 activation, was also determined in LPS-primed dectin-1$^{-/-}$ or MR$^{-/-}$ macrophages exposed to DSS (Figure 6D). The level of p10 subunit was significantly increased in WT macrophages. Interestingly, while the level of p10 subunit was diminished in dectin-1$^{-/-}$ macrophages, it was still elevated in MR$^{-/-}$ macrophages, suggesting that dectin-1 induces caspase-1 activation to secrete IL-1β. Therefore, dectin-1 mediates

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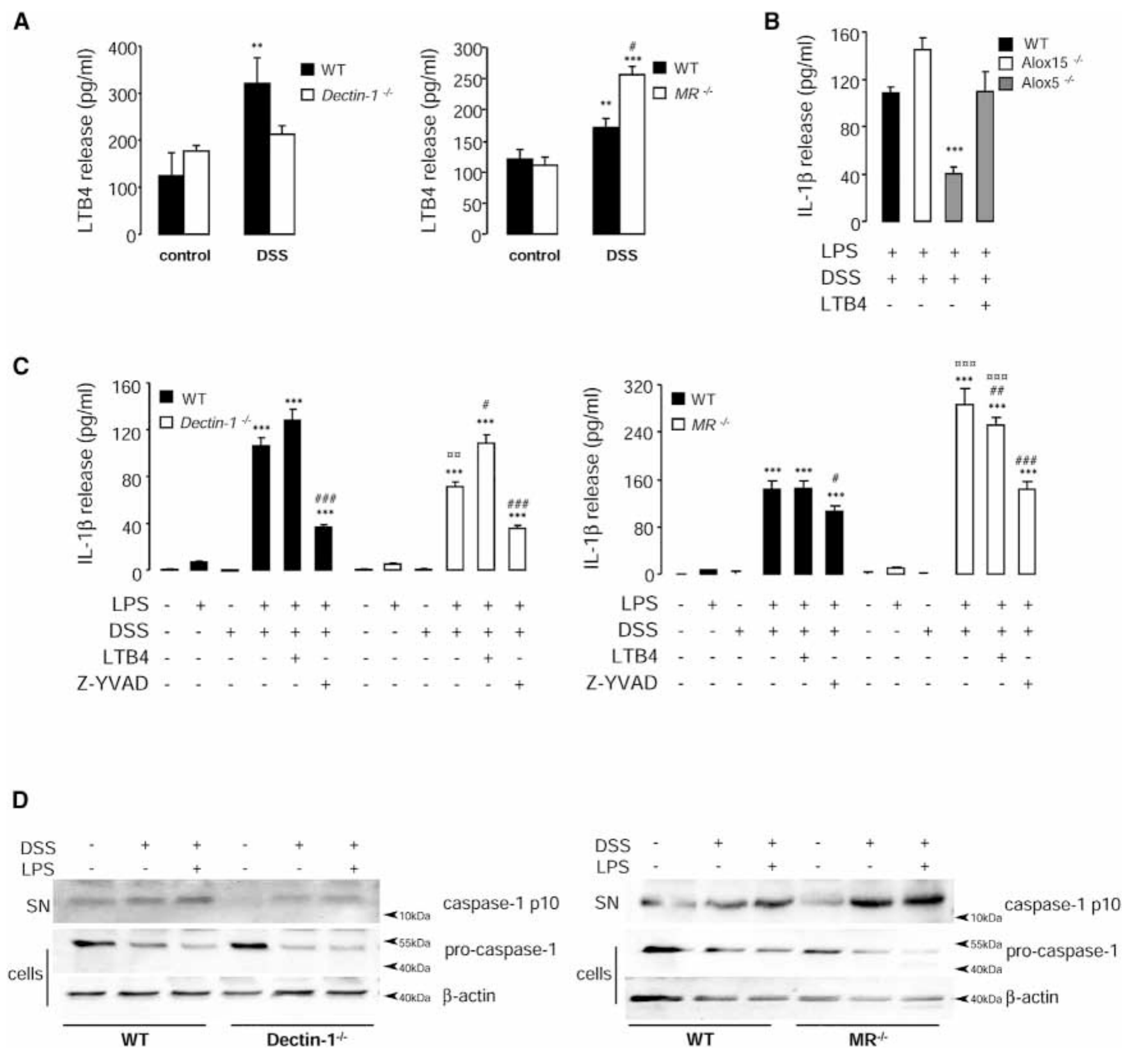

Figure 6. Dectin-1 Promotes LTB4-Dependent Inflammasome Activation and IL-1β Secretion

(A) LTB4 release by LPS-primed WT, dectin-1$^{-/-}$, and MR$^{-/-}$ macrophages exposed to DSS or not.

(B) IL-1β release by LPS-primed WT, 12/15-LOX (*Alox15*$^{-/-}$), or 5-LOX (*Alox5*$^{-/-}$) macrophages stimulated or not with DSS. Data are representative of three independent experiments. **$p \leq 0.01$ and ***$p \leq 0.005$ compared to respective DSS non-exposed macrophages (control). $^{\#}p \leq 0.05$ compared to DSS-exposed corresponding WT macrophages.

(C) IL-1β release by LPS-primed WT, dectin-1$^{-/-}$ and MR$^{-/-}$ macrophages exposed or not to DSS in the presence of LTB4 or Z-YVAD. ***$p \leq 0.005$ compared to respective DSS non-exposed macrophages. Data are representative of three independent experiments. $^{\#\#}p \leq 0.01$ and $^{\#\#\#}p \leq 0.005$ compared to respective DSS-exposed macrophages. ¤¤$p < 0.01$ and ¤¤¤$p < 0.005$ compared to DSS-exposed corresponding WT untreated or LTB4 or Z-YVAD-treated macrophages.

(D) Immunoblot analysis of caspase-1 p10 mature form in the supernatant (SN) and p45 pro-form in the lysate (cells) of LPS-primed WT, dectin-1$^{-/-}$ and MR$^{-/-}$ macrophages exposed or not to DSS.

inflammasome-dependent IL1β secretion by controlling LTB4 production during colitis.

Dectin-1, CCL2, and LTA4H Overexpression and MR Downregulation Are Related to Colonic Inflammation Grade in IBD Subjects

Based on the observation that dectin-1$^{-/-}$ mice are less susceptible and MR$^{-/-}$ mice are more susceptible to the development of colitis, we next analyzed the mRNA expression of dectin-1 (*Clec7a*), MR (*Mrc-1*), and LTA4H (*Lta4h*) in colonic biopsy specimens from healthy subjects and biopsy specimens taken from healthy and inflamed mucosae of CD and UC subjects (Figure 7A). As expected, the inflamed intestines of IBD pathologies showed an increase in the pro-inflammatory markers TNF-α (*Tnfα*) and IL-1β (*Il1β*) relative to the macroscopically and histologically healthy intestine (Figure S6). Interestingly, dectin-1 mRNA levels increased considerably in inflamed colonic biopsy specimens from both CD and UC subjects compared with those in biopsy specimens from healthy subjects and healthy-looking mucosae. This was accompanied by a reduction in MR levels (Figure 7A). Accordingly, the percentage of dectin-1^{+} macrophages (CD64^{+} dectin-1^{+}) increased substantially and

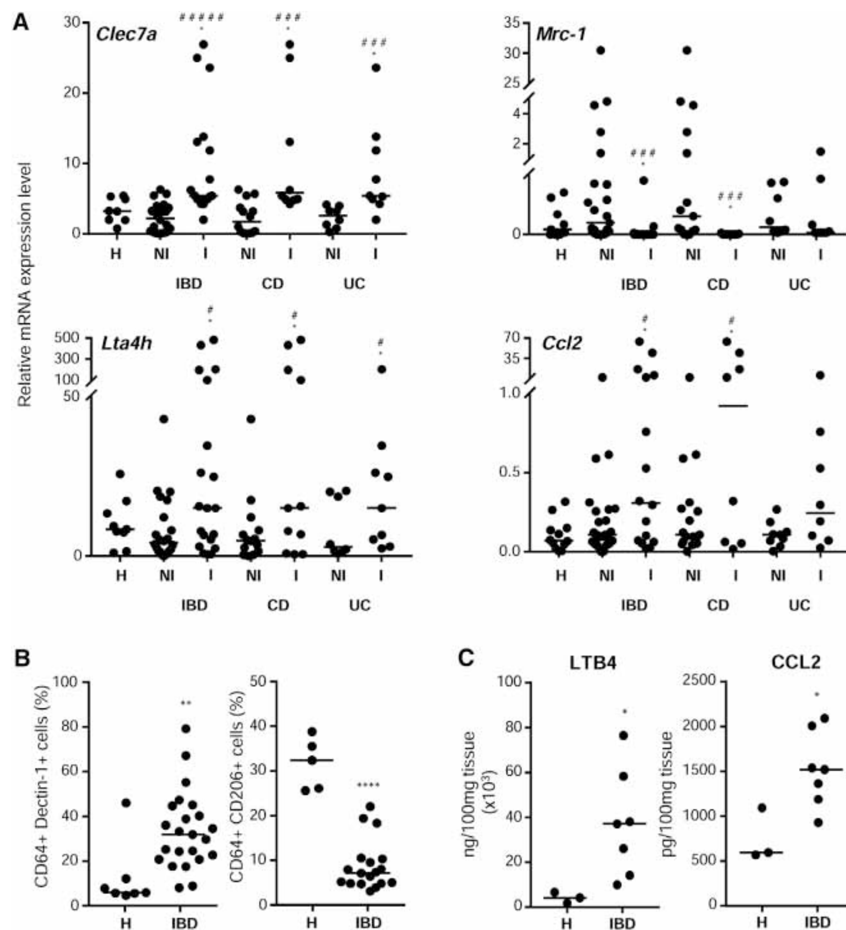

Figure 7. Severity of Colonic Inflammation in Subjects with IBD Is Correlated with Dectin-1, CCL2, and LTB4 Upregulation and MR Downregulation

(A) Gene expression analysis of *Clec7a*, *Mrc-1*, *Lta4h*, and *Ccl2* in colonic biopsy specimens from healthy individuals (H) (n = 9) and healthy (NI) (n = 20) or inflamed (I) (n = 20) mucosae of subjects with IBD, CD, or UC. Differences were determined by Mann-Whitney test. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to NI mucosae. $^{\#}p < 0.05$, $^{\#\#\#}p < 0.005$, $^{\#\#\#\#}p < 0.001$, and $^{\#\#\#\#\#}p < 0.0001$ compared to healthy mucosae.
(B) Flow cytometry analysis of CD64^{+} and dectin-1^{+} or CD206^{+} cells in the colonic mucosae of healthy individuals (H) (n = 7) and subjects with IBD (n = 20).
(C) CCL2 and LTB4 levels in the colonic mucosae of healthy individuals (H) (n = 4) and subjects with IBD (n = 12). Differences were determined by Mann-Whitney test. $^{*}p < 0.05$, $^{***}p < 0.005$, $^{****}p < 0.001$, and $^{*****}p < 0.0001$ compared to healthy mucosae.
See also Figure S5.

the percentage of MR^{+} macrophages (CD64^{+} CD206^{+}) decreased in the colonic mucosae of IBD subjects compared to healthy subjects (Figure 7B). Consistent with the elevated dectin-1 and reduced MR expression in inflamed colonic biopsy specimens from subjects with IBD, the mRNA expression of CCL2 and LTA4h strongly increased (Figure 7A). Additionally, the protein level of CCL2 and the LTB4 release robustly increased in the colonic mucosae of IBD subjects (Figure 7C). Therefore, the intestinal inflammation occurring in IBD in humans is associated with a specific induction of dectin-1, CCL2, and LTB4 and a reduction in MR expression, suggesting that this mechanism may contribute to the pathogenesis of IBD in humans.

DISCUSSION

The regulation of intestinal immunity through the activation of CLRs on macrophages is an emerging concept in our understanding of IBD pathogenesis. Here, we report that mice deficient in dectin-1 in myeloid-derived cells have a decreased susceptibility to colitis, with a significant reduction in colonic inflammatory parameters in two independent models of colitis, highlighting the pro-inflammatory function of dectin-1 in macrophages. This finding is in accordance with reports showing that dectin-1 can be involved in the aggravation of intestinal inflammation in the absence of opportunistic fungi in the gut (Tang et al., 2015) and that orally administered β-glucans aggravate DSS-induced intestinal inflammation (Heinsbroek et al., 2015). Moreover, in line with previous data showing that the absence of dectin-1 can also promote DSS-induced colitis because of the expansion of pathogenic commensal fungi in the gut (Iliev et al., 2012; Leonardi et al., 2018), our study demonstrated that *C. albicans* supplementation aggravates colitis in dectin-1$^{-/-}$ mice (Figure S3C), demonstrating that dectin-1 deficiency can affect host antifungal immunity and hence stimulates inflammatory responses in the gut. Thus, dectin-1 can influence the development of colitis not only by directly triggering inflammatory signaling pathways but also by contributing to the control of fungal and bacterial microbiota.

Interestingly, the selective deletion of dectin-1 in myeloid cells and the decrease of colonic inflammation in macrophage-depleted mice by clodronate excluded the contribution of other dectin-1-expressing cells, such as intestinal epithelial cells and other dectin-1-expressing immune cells. Altogether, these results establish a direct link between the expression of dectin-1 on macrophages and colonic inflammatory status.

In contrast to dectin-1$^{-/-}$ mice, MR$^{-/-}$ mice developed more severe colonic inflammation, highlighting the divergent roles of these CLRs in the inflammatory response during colitis. Consistent with the protective effect for MR against colonic inflammation, the treatment of TNBS-exposed rats with a polysaccharide-targeting MR significantly reduced diarrhea, colonic inflammation, and ulceration (Liu et al., 2008). Moreover, in conjunction with more severe colonic inflammation in DSS-exposed dectin-1$^{-/-}$ mice, we showed a marked increase in dectin-1 expression in macrophages, suggesting that the greater extent of intestinal inflammation in MR$^{-/-}$ mice could be

mediated by dectin-1. In support of the critical role of dectin-1 in the development of colonic inflammation in DSS-exposed $MR^{-/-}$ mice, a similar decrease in inflammation was observed in mice with dectin-1 or both dectin-1 and MR deficiencies in myeloid-derived cells. Consistent with these results, our findings in clinical samples showed that intestinal inflammation in IBD subjects is associated with a specific increase in dectin-1 expression and a reduction in MR expression on macrophages. This correlates with reports demonstrating elevated dectin-1 expression in macrophages of actively inflamed colonic tissues relative to non-inflamed tissues (de Vries et al., 2009). This is also supported by a reduced number of macrophages expressing MR in subjects with active CD (Hunter et al., 2010). Thus, these data reinforce our findings, thereby highlighting dectin-1 in macrophages as a crucial susceptibility factor in the development of intestinal inflammation and demonstrating the preponderant contribution of dectin-1 relative to MR.

In this report, in accordance with the well-established role of inflammatory macrophages in the pathogenesis of IBD (Hunter et al., 2010; Lin et al., 2014; Lissner et al., 2015), we showed that dectin-1 could contribute to colitis pathogenesis by promoting the inflammatory polarization of colonic macrophages. The involvement of dectin-1 as an inducer of inflammatory macrophage differentiation in the colon was supported by a previous report showing that the absence of dectin-1 during pulmonary paracoccidioidomycosis impairs M1 differentiation while promoting M2-like phenotype (Loures et al., 2014). Interestingly, we also demonstrated that the increase in dectin-1-dependent inflammatory macrophage recruitment in the colon was associated with both the augmentation of the $Ly6C^{high}CCR2^{high}$ monocyte subset in the blood and CCL2 production in the gut. Accordingly, CCL2 was described as the major chemokine driving $Ly6C^{high}CCR2^{high}$ monocyte recruitment from the bone marrow to the circulatory system and inflammation sites (Yang et al., 2014; Zigmond et al., 2012). Moreover, this monocyte subset is known to preferentially differentiate into inflammatory macrophages in tissues (Yang et al., 2014). These data provide evidence that dectin-1 plays a critical role in CCL2 production by macrophages, which is involved in the recruitment of $Ly6C^{high}CCR2^{high}$ monocytes as inflammatory macrophage precursors during colitis. Thus, we have revealed a mechanism whereby dectin-1 regulates intestinal inflammation by promoting inflammatory monocyte recruitment and macrophages differentiation.

Because dectin-1 is involved in macrophage differentiation toward inflammatory phenotype, and because caspase-1-dependent IL-1β secretion is strongly upregulated in inflammatory macrophages (Pelegrin and Surprenant, 2009), the loss of dectin-1 during colitis compromises the ability of the macrophages to produce IL-1β due to impaired caspase-1 activation. This is in agreement with reports showing that dectin-1 can act as an extracellular sensor for pathogens to induce IL-1β release through inflammasome-dependent signaling pathways (Cheng et al., 2011; Lefèvre et al., 2013; van de Veerdonk et al., 2009). Thus, in line with the fact that the inflammasome has been identified as a critical mechanism mediating intestinal inflammation in the DSS-induced colitis model (Bauer et al., 2010) and that IL-1β levels correlate with the severity of IBD (Mao et al., 2018; McAlindon et al., 1998), we showed that dectin-1 participates in IBD development by positively controlling inflammasome-dependent IL-1β secretion in colonic macrophages. Although many studies have shown that IL-1β contributes to the development of inflammation in colitis (Mao et al., 2018), depending on genetic and environmental factors, IL-1β can also participate in tissue repair and thus contribute to the healing of intestinal lesions (Bersudsky et al., 2014; Dupaul-Chicoine et al., 2010). Thus, IL-1β can play a dual role depending on intestinal state. This cytokine first participates in the triggering of inflammation, and then, in conditions of active inflammation, it is required for tissue repair.

In addition to the key role of dectin-1 on macrophages in caspase-1-dependent IL-1β secretion during colitis, this study also provides mechanistic insights into the link between this CLR and inflammasome activation. Our findings showing both impaired AA mobilization and LTB4 production in macrophages from DSS-exposed dectin-$1^{-/-}$ mice substantiate the importance of dectin-1 in the control of LTB4 production during colitis. This observation is consistent not only with our results showing that gene expression of 5-LOX and LTA4 hydrolase (whose enzymatic activity is critical for LTB4 synthesis) is downregulated in macrophages of DSS-exposed dectin-$1^{-/-}$ mice but also with the known capacity of dectin-1 to activate AA secretion in response to the β-glucan component of yeasts (Suram et al., 2006). Furthermore, the restoration of IL-1β production in DSS-exposed dectin-$1^{-/-}$ macrophages by exogenous LTB4 highlights the critical role of dectin-1 in LTB4-mediated IL-1β production, which is best supported by the concomitant overexpression of pro-IL-1β, dectin-1, and Lta4h, and the secretion of LTB4 in clinical biopsy specimens from the mucosae of IBD subjects. Our data are consistent with the results of previous studies demonstrating that subjects with active IBD display significantly higher LTB4 synthesis and 5-LOX and LTA4H expression compared to healthy controls (Jupp et al., 2007). Thus, the identification of the AA/LTB4/dectin-1 axis in activating IL-1β secretion in macrophages during gut inflammation represents a major breakthrough in the understanding of intestinal innate immune responses during colitis.

In conclusion, we have shown that dectin-1 gene deletion in macrophages prevents intestinal inflammation, whereas the loss of MR results its exacerbation. Interestingly, we demonstrated that the severity of intestinal inflammation in $MR^{-/-}$ mice is mediated by dectin-1 overexpression, further supporting the major contribution of dectin-1 to colitis development. Dectin-1 mediates inflammatory macrophage recruitment in the colon through CCL2 production, which is responsible for the infiltration of the $Ly6C^{high}CCR2^{high}$ monocyte subset. Our findings also revealed a dectin-1-coupled signaling pathway in colonic macrophages that controls intestinal inflammation by positively controlling inflammasome-dependent IL-1β secretion via the regulation of LTB4 production. All of these results are reinforced by increased $CD64^{+}$ dectin-1^{+} macrophages in colonic mucosae from IBD subjects associated with a higher intestinal CCL2 level and the concomitant upregulation of IL-1β, dectin-1, LTA4H expression, and LTB4 secretion. Finally, this study has relevance and provides important insights into the pathogenesis of inflammatory diseases of the bowel and the role of dectin-1 and MR in macrophages during intestinal inflammation and mucosal

immunity. Our study could set the stage for further development of therapeutic strategies to control the imbalanced inflammatory response in the gut.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

H.A., L.A., and A.C. were involved in the study concept and design and in drafting the manuscript. M.R., G.J., H.A., and A.C. performed, acquired, and analyzed experiments. M.D. and D.B. acquired and analyzed histological data. We thank M.P., E.M., M.A., B.B., L.L., K.B., A.A., and P.B. for technical support and J.A., S.K., and L.A. for material support.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-CD45	Miltenyi Biotec	Cat # 130-110-661, RRID: AB_2658229
Mouse anti-CD45.1	Miltenyi Biotec	Cat # 130-121-204, RRID: AB_2784205
Mouse anti-CD45.2	Miltenyi Biotec	Cat # 130-096-242, RRID: AB_10829797
Mouse anti-CD45.2	Miltenyi Biotec	Cat # 130-102-458, RRID: AB_2660717
Mouse anti-F4/80	Miltenyi Biotec	Cat # 130-118-327, RRID: AB_2751483
Mouse anti-SiglecF	Miltenyi Biotec	Cat # 130-112-332, RRID: AB_2653439
Mouse anti-Dectin-1	Miltenyi Biotec	Cat # 130-102-225, RRID: AB_2651540
Mouse anti-Cd209b (SIGN-R1)	Miltenyi Biotec	Cat # 130-117-499, RRID: AB_2727966
Mouse anti-Ly6G	Miltenyi Biotec	Cat # 130-120-803, RRID: AB_2752201
Mouse and human anti-CD11b	Miltenyi Biotec	Cat # 130-081-201, RRID: AB_244270
Mouse anti-Ly6C	Miltenyi Biotec	Cat # 130-111-921, RRID: AB_2652806
Mouse anti-CD43	Miltenyi Biotec	Cat # 130-102-594, RRID: AB_2661309
Mouse anti-CCR2	Miltenyi Biotec	Cat # 130-119-658, RRID: AB_2751785
Mouse anti-CD326 (EpCAM)	Miltenyi Biotec	Cat # 130-117-871, RRID: AB_2728059
Mouse anti-MR (CD206)	Ozyme	BLE141721
Mouse anti-CD3	Miltenyi Biotec	Cat # 130-120-076, RRID: AB_2751991
Mouse anti-CD19	Miltenyi Biotec	Cat # 130-102-310, RRID: AB_2661120
Human anti-CD64	Serotec	Cat # MCA756C, RRID: AB_322877
Human anti-CD206	BD PharMingen	Cat # 550956, RRID: AB_398480
Human anti-Dectin-1	Miltenyi Biotec	Cat # 130-107-693, RRID: AB_2651550
anti-FITC microbeads	Miltenyi Biotec	Cat # 130-048-701, RRID: AB_244371
caspase-1 p10 antibody	Santa Cruz Biotechnology	Cat # sc-514, RRID: AB_2068895
anti-β-actin (C11)	Santa Cruz Biotechnology	Cat # sc-1615, RRID: AB_630835
Yeast Strains		
Candida albicans	ATCC	sc-5314
Chemicals, Peptides, and Recombinant Proteins		
Dextran sulfate sodium	MP biomedicals	MW 36.000-50.000
2,4,6-Trinitrobenzenesulfonic acid solution, TNBS	Sigma-Aldrich	92822
clodronate encapsulated liposomes/control liposome	Clinisciences	16001003
lipopolysaccharides	Sigma-Aldrich	L4391
mannan (from S. cerevisiae)	Sigma-Aldrich	M7504
zymosan	Sigma-Aldrich	Z4250
caspase-1 inhibitor (Z-YVAD)	Calbiochem	218746
LTB4	Cayman Cheminal	71160-24-2
collagenase D	Roche	11088866001
dispase-II	Roche	04942078001
Dnase-I	Roche	11284932001
Percoll®	Sigma-Aldrich	P4937
Ficoll 1.077 ± 0.001 g/ml	GE Healthcare	17-1441-02
5-amino-2,3-dihydro-1,4-phthalazinedione	Sigma-Aldrich	A8511
hydrogen peroxide	Sigma-Aldrich	H1009
Formaldéhyde 4%	VWR Chemicals	9713.901
Hematoxylin Solution, Mayer's	Sigma-Aldrich	MHS16

(*Continued on next page*)

Continued		
REAGENT or RESOURCE	SOURCE	IDENTIFIER
Eosin Y solution, alcoholic	Sigma-Aldrich	HT110116
RIPA Buffer	Sigma-Aldrich	R0278
trichloroacetic acid	Sigma-Aldrich	T4885
Critical Commercial Assays		
Monocytes isolation kit	Miltenyi Biotec	130-100-629
Total RNA Minipreps super kit	BioBasic	BS784
Verso cDNA kit	Thermo Fisher Scientific	AB1453B
LightCycler SYBR Green I Master	Roche Diagnostics	4887352001
High Pure PCR Template preparation kits	Roche	11796828001
Live/Dead® Fixable Violet dead cell stain kits	Molecular Probes	L34955
ELISA Duoset CCL2/MCP-1	R&D Systems	DY479-05
ELISA kits for IL-1β	BD Biosciences	Cat # MA1-22725, RRID: AB_559603
ELISA kits for TNF-α	BD Biosciences	BD 555268
ELISA kits for IL-6	BD Biosciences	BD 555240
Leukotriene B4 EIA kit	Oxford	EA35
Experimental Models: Organisms/Strains		
myeloid-specific Dectin-1$^{-/-}$ mice and WT littermates	This paper	N/A
myeloid-specific MR$^{-/-}$ mice and WT littermates	This paper	N/A
myeloid-specific Dectin-1$^{-/-}$ / MR$^{-/-}$ mice and WT littermates	This paper	N/A
Alox5$^{-/-}$ mice: B6.129S2-Alox5^{tm1Fun}/J	The Jackson laboratory	Cat # JAX: 004155, RRID: IMSR_JAX: 004155
Alox15$^{-/-}$ mice: B6.129S2-Alox15^{tm1Fun}/J	The Jackson laboratory	Cat # JAX: 002778, RRID: IMSR_JAX: 002778
WT mice: C57BL/6J	The Jackson laboratory	Cat # JAX: 000664, RRID: IMSR_JAX: 000664
Mouse: B6.SJL-*Ptprc^{a}Pepc^{b}*/BoyCrl congenic	Charles River	Cat # CRL: 494, RRID: IMSR_CRL: 494
Software and Algorithms		
FACS Diva software	BD Biosciences	N/A

LEAD CONTACT AND MATERIALS AVAIBILITY

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Agnès Coste (agnes.coste@univ-tlse3.fr). This study did not produce new unique reagents.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Mice

All mouse experiments were performed according to protocols approved by the institutional ethics committee (CEEA122) with permit number 5412-2016051917498658 in accordance with European legal and institutional guidelines (2010/63/UE) for the care and use of laboratory animals. All mice were bred in the same facility, and age- and sex-matched WT mice were first co-housed with genetically modified mice just after weaning at 3 weeks old for at least 4 weeks before use. At the start of the experiments, animals weighed (mean ± SD) 24 ± 0.36 g. Animals were housed in 425x266x185 mm cages (Tecniplast, 1291H Type III H, France) and given access to maintenance food (Global Diet, Harlan, France) and water *ad libitum*. Environmental enrichment included bedding and one hut. The experimenters were blinded to the mice genotype for monitoring animals.

The myeloid-specific Dectin-1$^{-/-}$ or MR$^{-/-}$ mice have been described earlier (Galès et al., 2010; Lefèvre et al., 2013). The corresponding floxed littermates were used as control in all experiments. To generate MR floxed (MR L2/L2) mice or Dectin-1 floxed (Dectin-1 L2/L2) mice, genomic DNA covering the MR locus or Dectin-1 locus from the 129Sv strain was amplified using high fidelity PCR. The resulting DNA fragments were assembled into the targeting vector that after linearization by NotI was electroporated into 129Sv ES cells. G418-resistant colonies were selected and analyzed for homologous recombination using PCR and Southern blot hybridization. Positive clones were verified using Southern blot hybridization. The genomic DNA was prepared from ES cells, digested with XbaI or SacI, electrophoresed and transferred to a positively charged nylon transfer membrane (Amersham Biosciences, Saclay, France). A 0.5 kb DNA fragment (NotI–NheI) located between exons 12 and 13, and a 0.5 kb DNA fragment (NotI SacII) placed between exons 19 and 20, were used as probes for MR. A 0.5 kb DNA fragment (NotI–NheI) located between exons 6 and 7 (39 probe) and a 0.5 kb DNA fragment (NotI–SacII) placed between exons 2 and 3 (59 probe) were used as probes for Dectin-1. The karyotype

was verified and several correctly targeted ES cell clones were injected into blastocysts from C57BL/6J mice. These blastocysts were transferred into pseudo-pregnant females, resulting in chimeric offspring that were eventually mated with female C57BL/6J mice, which express the Flp recombinase under the control of the ubiquitous CMV promoter. The offspring that transmitted the mutated allele (in which the selection marker was excised) and that had lost the Flp transgene (MR+/L2 mice) were then selected and used for systematic backcrossing with C57BL/6J mice to generate congenic MR floxed mouse lines or Dectin-1 floxed mice. A PCR genotyping strategy was subsequently used to identify MR+/+, +/L2, and L2/L2 mice. To generate myeloid-specific mutant (LysM-MR L2/L2) mice, MR L2/L2 mice were mated with LysM-Cre C57BL/6J mice in which the Cre recombinase was expressed under the control of the phagocyte-selective lysozyme promoter (Clausen et al., 1999) LysM-Cre/MR L2/+ mice, heterozygous for the floxed *Mrc1* allele, were selected and subsequently inter-crossed to generate pre mutant LysM-Cre/MR L2/L2 mice (MR$^{-/-}$). At least two more rounds of breeding were required to generate age- and sex-matched mice for the experimental cohorts. The same strategy was used to generate LysM-Cre/Dectin-1 L2/L2 mice (Dectin-1$^{-/-}$).

mRNA and protein expression of Dectin-1 and MR revealed that these CLR are expressed in myeloid lineage (macrophages, eosinophils, neutrophils) and epithelial cells but not in B and T immune cells (Figures S1A–S1D). The disruption of Dectin-1 and MR is only detected in myeloid lineage but not in other CLR-expressing cells, such as colonic epithelial cells (Figures S1A–S1D).

Dectin-1$^{-/-}$, MR$^{-/-}$ and Dectin-1$^{-/-}$ MR$^{-/-}$ mice and their WT littermates are recombinant inbreeding populations. The random fixation and selection during the inbreeding process can explain the variability of some parameters observed between mouse models (Ashbrook et al., 2019).

Alox5$^{-/-}$, *Alox15*$^{-/-}$ mice and C57BL/6 mice (corresponding controls) were purchased from The Jackson Laboratory (USA).

Dextran Sulfate Sodium (DSS) and 2,4,6-trinitrobenzenesulfonic acid (TNBS) models

From day 1 to day 6, mice were given 1.5% DSS (MW 36.000-50.000, MP Biomedicals, France) through drinking water to induce intestinal inflammation. The health status of animals was monitored once daily during 8 days. After treatment, the mice were euthanized by CO_2, and their colons were collected for further analyses.

For induction of TNBS colitis, anesthetized mice were given an intrarectal injection of a 150 mg/kg TNBS solution (Sigma, France) dissolved in 0.9% NaCl and mixed with an equal volume of ethanol. Control mice received 50% ethanol intrarectally. Animals were killed by CO_2 asphyxiation 24h after TNBS administration. Body weight and rectal bleeding were monitored during the experiment. Mice were killed, colons were measured and weighed, and macroscopic features were scored. All mice treated with DSS or TNBS develop colitis.

In vivo macrophage depletion protocol

Mice received intraperitoneal injections of 5μg/kg of clodronate encapsulated liposomes or liposome for control (Clinisciences, France) one day before DSS treatment and 3 days after.

In vivo tracking of macrophages origin

Ly5.1 mice (CD45.1 expressing B6.SJL-*Ptprc*^{a}*Pepc*^{b}/BoyCrl congenic) from Charles River received DSS for 4 days before injection into the ophthalmic venous sinus with sorted monocytes isolated from CD45.2 donor mice. Monocytes were isolated from bone marrow of Dectin-1$^{-/-}$ or MR$^{-/-}$ mice and their WT littermates according to Monocytes isolation kit recommendation's (Miltenyi, France). After 2 days of DSS treatment following by 2 days with drinking water, Ly5.1 mice were sacrificed. Cells were isolated by enzymatic digestion of colons (see below). Viable cells were analyzed by flow cytometry and the percent of macrophages (CD45.2^{+}, CD11b^{+}, F4/80^{+}) were determined. CD45.2 cells from colons were sorted using CD45.2-FITC antibody and anti-FITC microbeads according to Miltenyi's protocol and the mRNA expression profile of pro and anti-inflammatory markers were evaluated using qRT-PCR.

Human subjects

The part of the study involving human subjects was sponsored by the University Hospital of Toulouse for regulated and ethical submission (Clinical trials.gov NCT01990716). Colonic tissue samples were obtained from well-characterized CD and UC subjects undergoing colonoscopy or colonic resection procedures at the Toulouse Hospital Centre (France). Freshly isolated colonic biopsies from 9 healthy patients (5 females and 4 males; mean age 59, range 21-87 years), from 11 CD patients (5 females and 6 males; mean age 39, range 20-58 years) and from 9 UC patients (3 females and 6 males; mean age 49, range 34-67 years) were quick-frozen at −80°C until use for RNA extraction and gene expression analysis. Finally, 20 IBD patients (8 females and 12 males; mean age 42, range 20-67 years) were included in Figures 7A and S6A. For cytokines (IL1β, TNFα), chemokines (CCL2) and LTB4 quantification by ELISA and EIA (Figures 7C and S6B), colonic biopsies or resection from 4 healthy patients (3 females and 1 males; mean age 68, range 68-69 years) and from 12 IBD patients (6 females and 6 males; mean age 45, range 16-73 years). Colonic tissue samples from 7 healthy patients (5 females and 2 males; mean age 62, range 43-69 years) and from 20 IBD patients (9 females and 12 males; mean age 40, range 16-73 years) were digested to isolate the cells and flow cytometry analysis.

Isolation of mice and human colonic macrophages

This protocol was developed and modified as described previously (Weigmann et al., 2007). Excised mouse colons or colonic mucosae from healthy or IBD subjects undergoing colectomy sections from surgery were washed with cold PBS 1% BSA and cut longitudinally into approximatively 1cm lengths. The pieces were incubated twice in RPMI 5% FCS, 0.2 mM EDTA for 20 min at 37°C with slow, regular shaking. The pieces were passed through a 100 μm nylon membrane after each incubation. The flow-through contains colonic content with epithelial cells and intra-epithelial lymphocytes. The pieces were then digested 3 times for 20 min at 37°C with collagenase D (0.2 mg/ml, Sigma, France), dispase-II (3 mg/ml, Roche, Mannheim, Germany), Dnase-I (0.2 mg/ml, Roche) in RPMI 10% FCS with slow, regular shaking. Cells were collected by centrifugation and subjected to a gradient of Percoll® (gradient 30%/40%/70%; Sigma, France).

Isolation of blood monocytes

Monocytes were isolated from blood using a Ficoll gradient (Abcys).

Macrophage culture

Resident murine peritoneal cells were harvested from Dectin-1$^{-/-}$ and MR$^{-/-}$ mice and their respective wild-type (WT) littermates. The cells were primed with lipopolysaccharide (LPS, 10 ng/ml; Sigma, France) overnight before treatment with mannan (from *S. cerevisiae*, 1.25 mg/ml; Sigma, France), zymosan (10 μg/ml, Sigma, France) and with or without 5% DSS, caspase-1 inhibitor (Z-YVAD, 50 μM; Calbiochem) or LTB4 (10^{-7} M; Cayman) for 7 h. Culture supernatants were used for enzyme-linked immunosorbent assay (ELISA), Enzyme Immunoassay (EIA) or immunoblotting. The cells were used for immunoblotting.

METHOD DETAILS

Myeloperoxidase activity (MPO)

MPO activity in colon tissue was measured via chemiluminescence in the presence of 5-amino-2,3-dihydro-1,4-phthalazinedione (100 μM) and hydrogen peroxide (0.1 M) using a luminometer (EnVision, PerkinElmer). Statistical analysis was performed using the area under the curve expressed in counts × second.

Macroscopic scoring and histological analysis

Macroscopic scores of colons were determined according to the criteria defined earlier (Kimball et al., 2004). One part of the distal colon was fixed overnight in 4% paraformaldehyde and embedded in paraffin; sections of mouse colon were stained with hematoxylin and eosin (H&E) and scored according to the Ameho criteria (Ameho et al., 1997).

Reverse transcription and qPCR

mRNA from colonic macrophages or from human biopsies were prepared and cDNA were synthetized according to the manufacturer's recommendations (Total RNA Minipreps super kit, BioBasic; Verso cDNA kit, Thermo Fisher Scientific). qRT-PCR was performed on a LightCycler 480 system with LightCycler SYBR Green I Master (Roche Diagnostics, France). Serially diluted samples of pooled cDNA were used as external standards in each run for the quantification and results were expressed in fold induction relative to the respective control. The primers are listed in Tables S1 and S2. GAPDH, β-actin or 18S mRNA were used as housekeeping genes.

Flow Cytometry

To distinguish viable cells, cells were incubated with Live/Dead® Fixable Violet dead cell stain kits (Lifetechnology, Oregon, USA) before antibodies. The murine cells were labeled with the following antibodies: anti-CD45, anti-CD45.1, anti-CD45.2, anti-F4/80, anti-SiglecF, anti-Ly6G, anti-Dectin-1, anti-Cd209b (SIGN-R1), anti-CD11b, anti-Ly6C, anti-CD43, anti-CCR2, anti-CD326 (EpCAM) (Miltenyi, France), and anti-MR (CD206) (Ozyme, France). Epithelial cells were identified as EpCAM^{+} cells. Macrophages were identified as CD45^{+}, CD11b^{+}, F4/80^{+}, SiglecF^{-}, Ly6G^{-} cells. Eosinophils were identified as CD45^{+}, F4/80^{+}, CD11b+, SiglecF^{+}, Ly6G^{-} cells. Neutrophils were identified as CD45^{+}, F4/80^{-}, CD11b+, Ly6G^{+}, SiglecF^{-} cells. Monocytes were identified as CD11b^{+}, CD45^{+}, Ly6C^{+}, CD43^{+}, CCR2$^{-/+}$ cells.

The human cells were labeled with the following antibodies: anti-CD64 (Serotec, France), anti-CD206 (BD PharMingen), anti-Dectin-1 (Miltenyi, France).

Flow cytometry was performed using a BD LSR Fortessa (BD Biosciences). Flow cytometry data were analyzed with FACS Diva software (BD Biosciences).

Immunoblot analysis

Total protein was extracted from cell lysates using an RIPA buffer. Proteins from the culture supernatant were concentrated using trichloroacetic acid precipitation. Rabbit polyclonal anti-caspase-1 p10 antibody or goat anti-β-actin (C11) antibody (Santa Cruz Biotechnology, USA) was used.

ELISA, enzyme immunoassay lipid quantification (EIA) and AA mobilization assay

Cytokines and chemokine were quantified with ELISA kits for IL1β, TNFα, IL6 and CCL2 (BD Biosciences, R&D Systems). LTB4 levels were evaluated with an EIA kit (Oxford). The kits were used according to the manufacturer's instructions. The AA mobilization assay was performed as described previously (Lefèvre et al., 2013).

DNA isolation and analysis of fungal and bacteria colonization

DNA was purified using High Pure PCR Template preparation kits (Roche, Mannheim, Germany). For evaluation of mucosae-associated fungal or bacteria colonization, semiquantitative PCR was performed on DNA isolated from intestinal mucosae using primers listed in Table S3. Relative quantity was calculated and normalized to the amount of β-actin. For amplicon detection, the LightCycler FastStart DNA SYBR Green I kit was used as described by the manufacturer (Roche).

QUANTIFICATION AND STATISTICAL ANALYSIS

Data are expressed as mean ± SEM. For each experiment, the data was subjected to one-way analysis of variance followed by multiple comparisons of means using the Bonferroni-Dunnett method. P value < 0.05 was defined as the level of statistical significance.

DATA AND CODE AVAILABILITY

This study did not generate/analyze datasets/code.