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Copper Metabolism Domain-containing 1 Represses Genes that Promote Inflammation and Protects Mice From Colitis and Colitis-associated Cancer

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Abstract

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BACKGROUND & AIMS—Activation of the transcription factor NFκB has been associated with development of inflammatory bowel disease (IBD). COMMD1, a regulator of various transport pathways, has been shown to limit NFκB activation. We investigated the roles of COMMD1 in the pathogenesis of colitis in mice and IBD in humans.

METHODS—We created mice with specific disruption of *Commd1* in myeloid cells (Mye-K/O mice); we analyzed immune cell populations and functions and expression of genes regulated by NFκB. Sepsis was induced in Mye-K/O and wild-type mice by cecal ligation and puncture or intraperitoneal injection of lipopolysaccharide (LPS), colitis was induced by administration of dextran sodium sulfate (DSS), and colitis-associated cancer was induced by administration of DSS and azoxymethane. We measured levels of *COMMD1* mRNA in colon biopsies from 29 patients with IBD and 16 patients without (controls), and validated findings in an independent cohort (17 patients with IBD and 22 controls). We searched for polymorphisms in or near *COMMD1* that were associated with IBD using data from the International IBD Genetics Consortium and performed quantitative trait locus analysis.

RESULTS—In comparing gene expression patterns between myeloid cells from Mye-K/O and wild-type mice, we found that COMMD1 represses expression of genes induced by LPS. Mye-K/O mice had more intense inflammatory responses to LPS and developed more severe sepsis and colitis, with greater mortality. More Mye-K/O mice with colitis developed colon dysplasia and tumors than wild-type mice. We observed reduced expression of COMMD1 in colon biopsies and circulating leukocytes from patients with IBD. We associated single nucleotide variants near *COMMD1* with reduced expression of the gene and linked them with increased risk for ulcerative colitis.

CONCLUSIONS—Expression of COMMD1 by myeloid cells has anti-inflammatory effects. Reduced expression or function of COMMD1 could be involved in the pathogenesis of IBD.

Keywords

mouse model; gene regulation; CD; UC

INTRODUCTION

Persistent inflammation is a common maladaptive component in the pathogenesis of human diseases. A prime example of this paradigm is inflammatory bowel disease (IBD), a chronic inflammatory process of the intestinal tract that clinically presents as two phenotypic entities, ulcerative colitis (UC) and Crohn's disease (CD). This disorder involves an interaction between environmental factors and inherited susceptibility, and is associated with an increased risk for colorectal cancer. [1]

The regulation of the inflammatory cascade is a complex process in which the transcription factor NF-κB plays a master regulatory role. [2] Consequently, this factor has also been linked to the pathogenesis of several chronic inflammatory conditions in humans, [2] including IBD. [3, 4] Canonical NF-κB activity is mediated primarily by NF-κB complexes containing the RelA subunit (also known as p65) or its paralog c-Rel. Under basal conditions, these complexes are kept in the cytosol through interactions with the inhibitory IκB proteins. Their activation requires IκB degradation, an event triggered by a critical kinase complex

known as IKK that sits at the crossroads of numerous signaling pathways. After signaling inputs abate, homeostatic mechanisms that restore basal NF-κB activity are essential for the physiologic function of this pathway. The induction of IκB gene expression, [5] or the expression of IKK inhibitory proteins, such as A20 or CYLD [6, 7] participate in the timely termination of NF-κB activity. The expression of these factors is under the control of NF-κB itself, thus providing negative feedback loops in the pathway. In addition, it has been recognized that ubiquitination and proteasomal degradation of RelA is critical to terminate transcription of a variety of genes. [8–13] One ligase responsible for these effects contains the scaffold protein Cul2 in association with COMMD1, [10, 12] a prototypical member of the COMMD (*Co*pper *m*etabolism *M*URR1 *d*omain containing) protein family. [14]

In addition to its role in NF-κB regulation, [12, 15] COMMD1 has been implicated in a variety of cellular processes, including copper transport, [16] electrolyte balance, [17–19] and hypoxia responses. [20] Given these pleotropic functions, it has remained unclear whether this factor plays a physiologically important role in the control of inflammation *in vivo* and whether it could play a role in chronic inflammatory diseases. Here we report that myeloid-specific deficiency of *Commd1* leads to more intense activation of LPS-inducible genes and is associated with more severe inflammation. In addition, we present genetic evidence linking gene variants associated with reduced *COMMD1* expression to risk for UC in humans, highlighting the physiologic importance of this gene in immunity and IBD pathogenesis.

RESULTS

Commd1 represses pro-inflammatory gene expression in myeloid cells

In order to evaluate the potential role of COMMD1 in inflammation, and given the known embryonic lethality that results from complete *Commd1* deficiency in mice, [21] we generated a tissue-specific mouse model of *Commd1* deficiency. [22] First, *Commd1* was selectively deleted in myeloid cells (Mye-K/O), a critical lineage in innate immunity, leading to the expected loss of Commd1 expression in macrophages (Figure S1A). Mye-K/O mice were healthy and B lymphocyte ($B220^+$) and T lymphocyte populations ($CD3^+$ and CD4/CD8) were not significantly different in the spleen or mesenteric lymph nodes (Figures S1B and S1C). Similarly, myeloid populations, including granulocytes ($Ly6G^+$), monocytes and macrophages ($CD11b^+$, $Ly6C^+$ and F4/80), and dendritic cells ($CD11c^{high}$, and $CD11c^{intermediate}$) were not significantly different in Mye-K/O mice (Figures S1B, S1C, and S1D). In line with previous observations, [12, 23, 24] *Commd1* deficiency did not alter substantially the phosphorylation or turnover of IκB (Figure 1A), but had a profound effect on RelA ubiquitination (Figure 1B).

Next, using bone marrow derived myeloid cells (BMDMs) from the Mye-K/O mice, we assessed the impact of *Commd1* on the LPS transcriptional response at a genome-wide level. High density microarray experiments indicated that 1008 genes were regulated by LPS at least 3-fold in two independent series of experiments (Figure 1C, and Tables S1–S3). In addition, the expression of 225 genes was found to be regulated by *Commd1* (Tables S1 and S2). Notably, the vast majority of *Commd1*-regulated genes (219 of 225) was also regulated by LPS, and only few *Commd1* target genes (6 out of 225) were outside the LPS transcriptional response (Tables S2 and S3). Hierarchical clustering of these 225 genes was

used to visualize the pattern of deregulated expression in *Commd1*-deficient myeloid cells. Both early and late LPS-inducible genes were affected by *Commd1* deficiency (Figure 1D). In most instances, the changes observed consisted of increased gene expression, which were often noted even at basal levels (Figure 1E).

In order to further understand the effect of *Commd1* on gene expression in myeloid cells, we performed a functional analysis using the KEGG database. This revealed that only 51 genes (23%) participate in immune regulation (as defined by any of a number of KEGG entries, Table S4), indicating that a large number of *Commd1* gene targets in myeloid cells are not known inflammatory regulators (Figure 1F). Next, we assessed the contribution of NF-κB regulation to the effects noted in *Commd1*-deficient myeloid cells. To that end, NF-κB regulation of genes of interest was ascertained through two overlapping approaches: first, using a curated database of published NF-κB gene targets (publicly available at www.NF-κB.org), and second, using ChIP seq data for RelA in LPS-stimulated BMDMs. [25] This indicated that only 66 genes regulated by *Commd1* (29%, Table S4) are known NF-κB or RelA targets, suggesting that a substantial proportion of the effects of Commd1 are either secondary or mediated through other transcriptional regulators.

Commd1 exerts gene selective effects among NF-κB regulated targets

Altogether, these findings suggest a substantial repressive role for *Commd1* on the LPS response in myeloid cells and confirmed a negative regulatory role for *Commd1* on selected NF-κB regulated genes. Indeed, qRT-PCR analysis corroborated that certain NF-κB target genes were selectively regulated (for example, *Mx1*, *Cd86* and *Sell*, Figure 2A), while others were relatively insensitive to *Commd1* deficiency (such as *Il6*, *Tnfaip3* and *Mmp13*, Figure S2A). These findings are in line with previous reports of gene specific effects for COMMD1 in cancer cell lines. [12] Enhanced expression of selected genes that are not directly regulated by NF-κB was confirmed as well (Figure 2B). Interestingly, in contrast to these effects, several hypoxia-induced genes were not affected by *Commd1* deficiency in BMDMs (Figure S2B), despite its reported role in hypoxia-dependent gene expression. [20, 21] In aggregate the data indicate that *Commd1* primarily exerts a gene selective repressive function on the LPS response.

Myeloid deficiency of Commd1 leads to exaggerated inflammatory responses in vivo

In view of the effects of *Commd1* on LPS-induced gene expression, we utilized two models to contrast the inflammatory response of Mye-K/O mice and wild-type (WT) littermate controls. First, we used the cecal ligation and puncture (CLP) model of polymicrobial sepsis. Animal survival was compromised in Mye-K/O mice compared to WT controls (Figure 3A). In contrast, cultures of peritoneal fluid demonstrated lower bacterial counts in the Mye-K/O mice (Figure 3B), indicating that their increased mortality was not due to greater bacterial load. Rather, after accounting for bacterial invasion (by examining animals with negative bacterial cultures), we found that Mye-K/O mice had increased cytokine expression (Figure 3C), suggesting that exaggerated pro-inflammatory gene expression was responsible for their increased susceptibility to sepsis. Next, animals were directly challenged with intraperitoneal LPS injection. Once more, Mye-K/O mice demonstrated greater mortality (Figure 3D) and increased morbidity (Figure S3A). Tissue injury, manifested by LDH

release in plasma, was also more pronounced in Mye-K/O mice (Figure S3B), which also displayed greater plasma elevations of Il-6 and Tnf (Figure 3E). In contrast, plasma levels of the anti-inflammatory cytokine Il-10 were comparable to WT. Tissue expression of pro-inflammatory genes was more pronounced in Mye-K/O mice, as exemplified by *Tnf*, as well as other pro-inflammatory genes (Figure 3F and S4). Altogether, these data indicate that *Commd1* deficiency in the myeloid lineage leads to more profound acute inflammatory responses.

Reduced expression of COMMD1 is a common feature of IBD

Given the findings made in the Mye-K/O mouse model, we decided to explore whether *COMMD1* could play a role in the pathophysiology of inflammatory disorders in humans, choosing IBD as a disease model. To this end, we evaluated the expression of *COMMD1* in IBD patients. We found that *COMMD1* mRNA levels in colonic endoscopic biopsies were reduced in an Israeli cohort of patients with colitis (n=29, 18 with Crohn's disease and 11 with ulcerative colitis) compared to unaffected controls (n=16) (p=0.04, Figure 4A, left). This finding was replicated in an independent patient cohort in the USA (p= 0.04, 22 normal controls and 17 patients with IBD, 9 of which had CD and 8 with UC, Figure S5). Similarly, we found that *COMMD1* mRNA was reduced in circulating leukocytes from IBD patients with active disease (n=12, 9 with CD and 3 with UC) compared to normal individuals (n=14) (p=0.001, Figure 4A, right). Moreover, in a murine model of colitis induced by Dextran sodium sulfate (DSS) administration, we also observed decreased mRNA and protein expression of *Commd1* and other *Commd* genes in the colon (Figures 4B and 4C). In aggregate, the data indicate that mucosal inflammation is associated with decreased *COMMD1* gene expression in clinical and experimental situations.

Genetic variants associated with decreased COMMD1 expression are associated with UC risk

In view of the reduced expression of *COMMD1* in IBD patients, we investigated whether genetic polymorphisms of this gene might affect IBD risk in humans. Interestingly, genome-wide association studies (GWAS) have linked an intergenic region near *COMMD1* to the risk for various inflammatory conditions including IBD (Figure 4D) [3, 26–30], but the precise gene responsible for these effects remains unknown.

We began by interrogating the most recent meta-analysis of GWAS for CD and UC [3] by the International IBD Genetics Consortium (IIBDGC), which is currently available at the Ricopili server (www.broadinstitute.org/mpg/ricopili/). These data indicated a suggestive association signal with UC for SNPs located just downstream of *COMMD1* including rs3943540 ($p=7.89 \times 10^{-5}$) and rs921319 ($p=7.47 \times 10^{-5}$) (Figure 4E). Interestingly, both SNPs are in linkage disequilibrium (LD) with each other ($r^2=1$, $D'=1$). Additional analysis of the 1000 Genomes imputed IIBDGC UC and CD meta-analyses also revealed a suggestive association with UC for a SNP located in the same region, rs6545924 ($p=2.27 \times 10^{-5}$, OR=1.104, 95% confidence interval of the OR between 1.06–1.15).

Epigenetic analysis in myeloid cells performed by the ENCODE project, [31] demonstrated that this genomic locus displays features of a gene regulatory region (Figure 4D and 4E),

such as Histone 3 lysine 4 monomethylation (H3K4me1) and lysine 27 acetylation (H3K27Ac), both associated with active enhancers. [32] In agreement with this notion, the SCAN database [33] indicates that there is a significant eQTL effect of rs921319 on *COMMD1* gene expression. To further examine the possibility that these variants have an effect on *COMMD1* expression, we conducted *cis*-eQTL mapping on whole peripheral blood of two cohorts (1,240 samples previously studied [34] and 891 samples from the Estonian Biobank). Since the SNPs in question were not present in our eQTL dataset, we tested rs921320, located in very close proximity to rs921319 and a perfect proxy SNP for rs6545924 (r^2=0.9, D′=1). We observed a significant eQTL effect of rs921320 on *COMMD1* expression, with the same allelic direction for the Dutch and Estonian cohorts (the p values for individual cohorts were p= 0.057 and p=0.0099, respectively; weighted Z-method meta-analysis P = 0.0033). The A–allele of rs921320, which is linked to the risk allele for rs6545924, was associated with reduced expression of *COMMD1* (Figure 4F). On the other hand, we did not observe any eQTL effects of rs921320 on the two other neighboring genes, *B3GNT2* or *TMEM17*. Altogether, these findings suggest that genetic variation in the 3′ region of *COMMD1*, which has regulatory effects on gene expression, is linked to risk for UC.

Myeloid cell deficiency of Commd1 leads to more severe colitis

In order to further examine the possible involvement of *COMMD1* in IBD pathogenesis, we begun by assessing the *in situ* expression of this gene in the colon. Immunofluorescence staining of mouse colon tissue demonstrated staining in the epithelium as well as in mononuclear cells in the lamina propria (Figure S6). Furthermore, confocal microscopy images confirmed that resident lamina propria macrophages ($F4/80^+$) are also COMMD1-positive, in agreement with western blot analysis of primary myeloid cells (Figure 1A). With this in mind and in view of the known contributions of both epithelial and myeloid cells in the pathogenesis of human and murine colitis, [35, 36] we evaluated the role of this gene in this disease process using both epithelial and myeloid cell specific knockout mice.

First, we used the DSS model, which triggers colonocyte injury and an innate immune response to luminal bacteria that result in acute colitis. Mye-K/O mice demonstrated more severe disease that WT animals, with excess mortality, more profound weight loss, and worse disease activity (Figure 5A). In agreement with this, colon shortening (a macroscopic marker of tissue injury) and histologic evaluation of the colonic mucosa both indicated more damage in Mye-K/O mice (Figures 5B and 5C). In contrast to these results, *Commd1* inactivation in the intestinal epithelium did not result in any appreciable alteration of DSS-induced colitis (Figure S7). The more severe inflammatory response in Mye-K/O mice was correlated with greater numbers of $CD68^+$ myeloid cells in the colon as seen by immunohistochemistry (Figures 5D, left panel and 5E), and a consistent trend was observed at the mRNA level for the macrophage-specific gene *Emr1* (*F4/80*, Figure 5D, right panel). In addition, Mye-K/O mice displayed increased expression of pro-inflammatory genes, such as *Il6* and *Il1b* (Figure 5F). However, myeloid and lymphoid populations in the MLN (Figure S8) and the spleen (not shown), were not significantly different between the groups.

Myeloid cell deficiency of Commd1 promotes colitis-dysplasia progression

Further analysis indicated that recurrent DSS administration, which mimics the chronic nature of IBD, also resulted in more severe disease in Mye-K/O mice, manifested as greater weight loss (Figure 6A) and more severe anemia (Figure 6B). Colonic tissue from these animals also produced greater amounts of several pro-inflammatory factors such as Tnf (Figure 6C and S9). In addition, azoxymethane (AOM) followed by recurrent DSS treatments was utilized to examine progression to dysplasia. In this model, Mye-K/O mice also displayed more severe disease, evidenced by more severe colonic shortening, splenomegaly, and histologic distortion of the mucosal architecture (Figure 6D). Moreover, after 7 weeks of treatment, Mye-K/O mice had a greater propensity to develop dysplasia, high-grade dysplasia (HGD) or multifocal dysplasia (MFD) as assessed histologically (Figure 6D). By 10 weeks, small adenomas could be visualized, but the total number of lesions was not increased in Mye-K/O mice (Figure 6E). Nevertheless, only Mye-K/O mice developed proximal colonic tumors and there was a tendency for the tumor burden to be greater in these animals as a result of greater adenoma volume (Figure 6E). In colonic tissue, Mye-K/O animals displayed greater NF-κB activation as evidenced by the ratio of the transcriptionally active form of RelA (phosphorylated at Ser536) to total RelA. However, STAT3 activation, another pathway known to promote cancer in the context of chronic tissue inflammation, [37] was not altered in these mice (Figure 6F). Altogether, these data highlight a role for myeloid cell expression of Commd1 in chronic colonic inflammation and progression to dysplasia.

DISCUSSION

COMMD1 plays an essential role to restrain the activity of NF-κB, a master regulator of inflammation. Specifically, COMMD1 acts to terminate NF-κB transcriptional activation through the ubiquitin-mediated degradation of NF-κB subunits. A detailed molecular mechanism by which COMMD1 restrains NF-κB has been previously examined and reported. [8, 10, 12, 13] The studies presented here confirm the negative regulatory role of this factor on the NF-κB pathway, [10, 12, 13, 15] and also underscore the gene selective nature of these effects. Moreover, the studies in this model corroborate that key aspects of the reported mechanism are evident in the knockout mice, namely normal IκB phosphorylation and turnover, but diminished ability to terminate NF-κB transcriptional activity (ie, reduced RelA/p65 ubiquitination). Importantly, this study investigates for the first time the physiological relevance of these events in disease pathogenesis. The data indicate that COMMD1 expression in the myeloid compartment plays an important role in the regulation of inflammatory responses *in vivo*. Given that diverse functions have been reported for this gene, [17, 38–40] this report fills a critical void by underscoring that COMMD1 participates in immune regulation. In addition, the results also suggest that COMMD1 has broader effects on pro-inflammatory gene expression programs that might extend beyond the NF-κB pathway. Whether these effects are the result of secondary perturbations or are the result of direct regulation of transcription factor(s) other than NF-κB, should be further examined in the future.

In the context of intestinal inflammation and progression to cancer, previous studies have underscored the importance of epithelial and myeloid cells in these processes. [14, 36, 37] In particular, epithelial intrinsic NF-κB activation has been shown to be a protective response that promotes cell survival and barrier function,[41] while myeloid cell NF-κB activation is important in sustaining the inflammatory response.[35] The increased propensity to inflammation and colitis observed as a result of myeloid cell deficiency of *Commd1* is in agreement with this paradigm and suggests that the immune regulatory roles of this gene are physiologically. In addition, the increased progression to dysplasia in Mye-K/O mice is in agreement with the role of myeloid cells in promoting tumor growth through various pro-inflammatory cytokines. [42] In this regard, our model displays increased production of Tnf and greater tissue activation of NF-κB, both of which have been shown to promote cancer progression in the setting of colitis. [35, 43] In addition, we also found no appreciable phenotype of enterocyte-specific deficiency of *Commd1*, which stands in contrast to other models of disrupted NF-κB activity in the epithelium.[35, 41] This may reflect the fact that unlike other models, cell survival genes are not regulated by COMMD1 in the transcriptome analysis presented here. Furthermore, it suggests that other reported roles of *Commd1*, such as the regulation of various transport mechanisms, may be more relevant in these cells.

These observations in the animal model, coupled with the finding that genetic variants linked to lower *COMMD1* expression are also associated with increased risk for UC, suggest that this gene participates in the pathogenesis of this disorder in humans. In this context, it is important to note a recent report of a risk association for *COMMD7*, another member of the *COMMD* gene family. [44] Moreover, COMMD1 acts in concert with Cul2 to promote RelA ubiquitination, [10, 12, 13] and the *CUL2* gene has been linked to IBD susceptibility. [45] Additional studies, including ongoing resequencing efforts, will be required to validate the genetic association seen here with greater statistical confidence. However, the aggregate of our data and published reports suggest the possibility that homeostatic mechanisms that restore NF-κB to its basal state, when decreased, may enhance the risk for IBD.

Our studies also identified that inflammation can lead to suppressed expression of *COMMD1*, as seen in patients with colitis as well as in animal models. Our data suggest that this is likely the consequence rather than the cause of inflammation in human disease, because this suppression can be induced by inflammation itself in animal studies. The decrease in *COMMD1* expression probably plays a physiologic role in the initiation of an appropriate inflammatory response, but in view of the more severe inflammatory responses seen when this gene is deleted in myeloid cells, persistent *COMMD1* suppression is probably maladaptive during chronic inflammation. Therefore, this work highlights the need to gain a refined understanding of the molecular events that control *COMMD1* gene expression in the setting of inflammation. With this knowledge, we may begin to envision potential interventions that might restore gene expression, which could be tested for their therapeutic potential in chronic inflammatory disorders.

MATERIALS AND METHODS

Human studies

All procedures involving human subjects were reviewed and approved by the respective Institutional Review Boards (at UT Southwestern Medical Center, The Mayo Clinic and Tel-Aviv Sourasky Medical Center). Circulating leukocytes and intestinal biopsies were obtained at the time of endoscopy as part of the patients' ongoing medical care.

GWAS and QTL analysis

Genetic association data were obtained from the 1000 genomes imputed meta-analysis from 15 genome-wide association studies of CD and/or UC conducted by the IIBDGC. [3] We conducted cis-eQTL mapping on whole peripheral blood of two cohorts; 1,240 samples from a previously published study [34] and 891 samples from the Estonian Biobank, both hybridized to Illumina HT12-v3 oligonucleotide arrays using methodology as described in detail elsewhere. [46]

Statistical analysis

In all graphs, the mean is presented and the error bars correspond to the standard error of the mean (SEM). Statistical comparisons between mean values were performed using one-tailed, heteroscedastic, Student's *t* test. For non-parametric variables, the Chi-square test was employed. Survival curves were examined using the Kaplan-Meier analysis.

Supplementary materials and methods

All other materials and methods are described in the supplementary section.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Abbreviations used in this paper

AOM	azoxymethane
BMDM	bone marrow derived myeloid cells

Cfu	colony forming units
ChIP	chromatin immunoprecipitation
***cis*-eQTL**	*cis*-expression quantitative trait locus
CLP	cecal ligation and puncture
COMMD1	Copper metabolism MURR1 domain containing 1
DAI	disease activity index
DSS	dextran sodium sulfate
GWAS	genome wide association studies
H3K4m1	histone 3 lysine 4 monomethylation
H3K4m3	histone 3 lysine 4 trimethylation
H3K27Ac	histone 3 lysine 27 acetylation
HGD	high-grade dysplasia
IIBDGC	International IBD genetic consortium
IKK	IκB kinase
IQR	inter-quartile range
KEGG	Kyoto encyclopedia of genes and genomes
K/O	knockout
LD	linkage disequilibrium
LDH	lactate dehydrogenase
LGD	low-grade dysplasia
LPS	lipopolysaccharide
MFD	multifocal dysplasia
MLN	mesenteric lymph node
Mye-K/O	myeloid-specific Commd1 knockout
NF-κB	nuclear factor-κB
qRT-PCR	quantitative reverse transcription polymerase chain reaction
SEM	standard error of the mean
SNP	single nucleotide polymorphism
WT	wild-type

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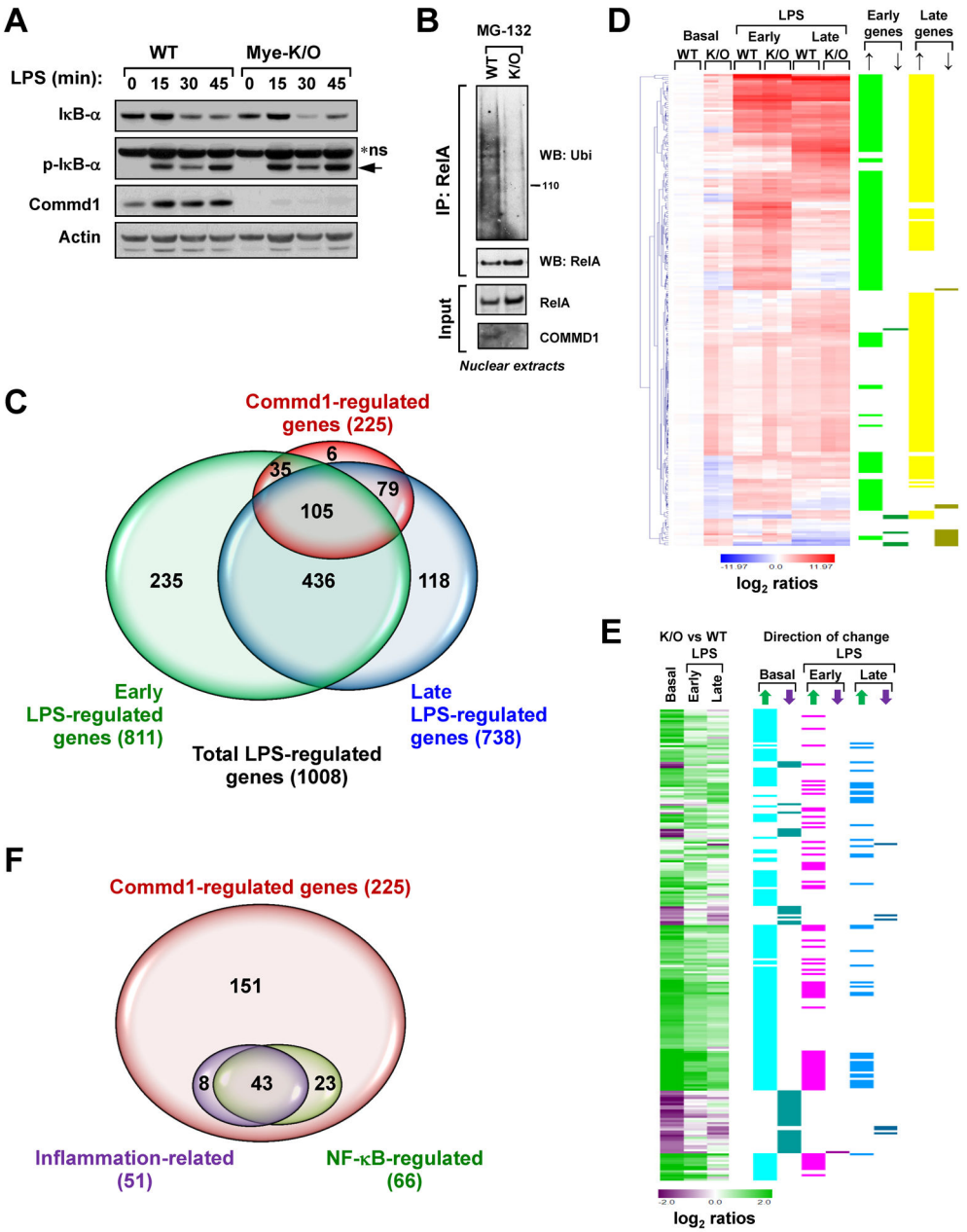


Figure 1. *Commd1* regulates the LPS transcriptional program in myeloid cells

(A) Commd1 does not affect IκB turnover in BMDMs. IκB-α phosphorylation (p-IκB-α) and degradation after LPS treatment were examined by western blotting. * ns, non-specific band (arrow points to pIκB-α) **(B)***Commd1* deficiency reduces RelA ubiquitination. *Commd1* deficient mouse embryo fibroblasts (K/O) or control cells (WT) were used to detect ubiquitinated RelA by ubiquitin immunoblotting of immunoprecipitated RelA. **(C)** Gene expression differences resulting from *Commd1* deficiency substantially overlap with the LPS response. BMDMs from WT and Mye-K/O mice were utilized in duplicate microarray analysis to ascertain genes regulated by *Commd1* or by LPS (100ng/mL) at early (3h) or late (24h) time points. The degree of overlapping genes is presented in Venn diagram

form. **(D)** Hierarchical cluster analysis of *Commd1*-regulated genes during the LPS response. Expression levels normalized to basal expression in WT samples is displayed as color-coded $\log_2$-transformed ratios. The gene induction pattern, either early (>3- fold induction at 3h) or late LPS response (>3-fold induction at 24h), is also indicated. The specific effect of *Commd1* deficiency is noted by up or down arrows. **(E)** The genes analyzed in (D) are reanalyzed by calculating ratios between K/O and WT BMDMs, displayed as color-coded $\log_2$-transformed manner. The direction of change is indicated in adjacent columns by up or down arrows. **(F)** Venn diagram of *Commd1*-regulated genes, indicating those involved in inflammatory responses or regulated by NF-κB.

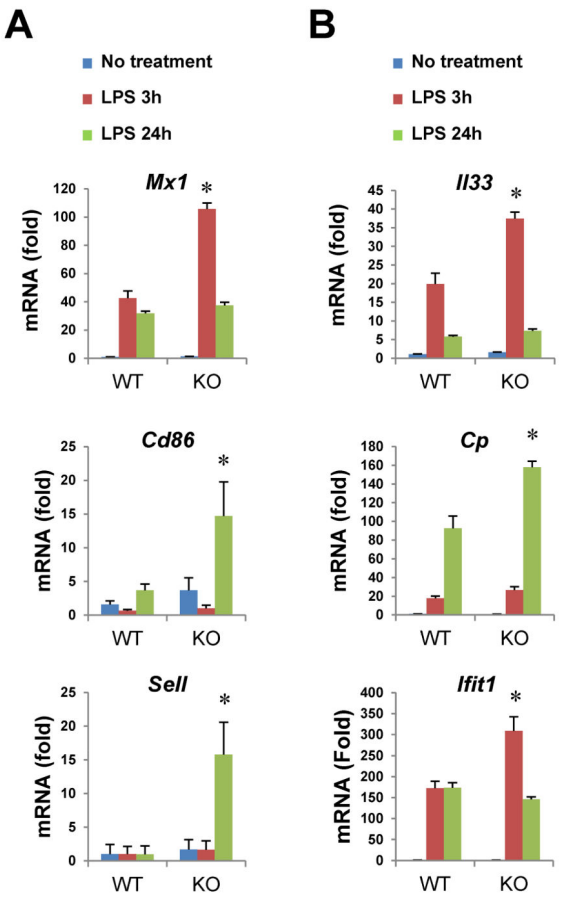


Figure 2. Gene selective effects of *Commd1* on NF-κB regulated targets

BMDMs (2 mice per group, with triplicate cultures per mouse) were stimulated with LPS and gene expression was determined by qRT-PCR. Selected NF-κB target genes are shown **(A),** while other LPS-inducible genes that are not NF-κB targets are also shown **(B)**.

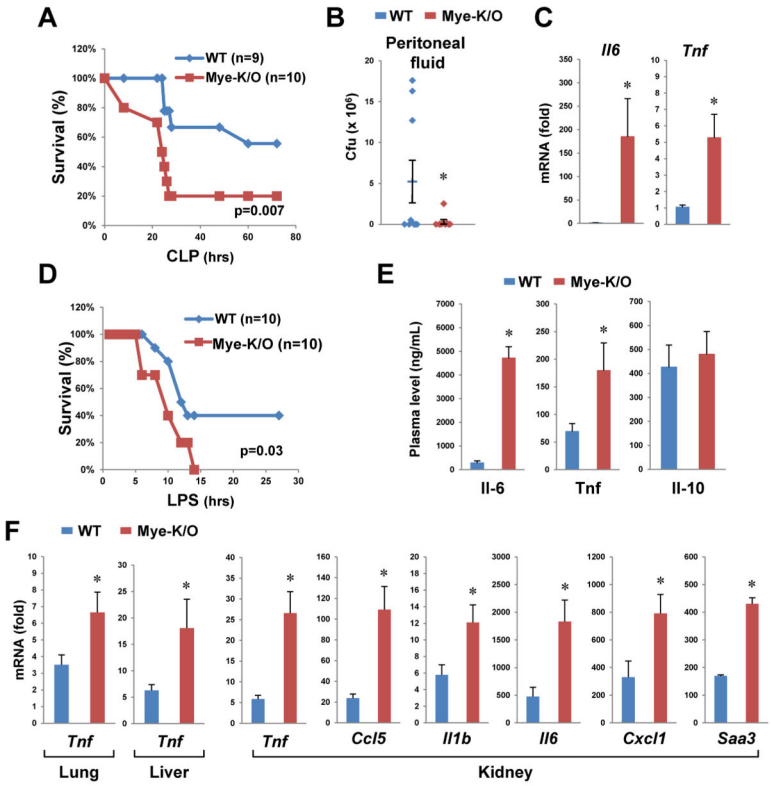


Figure 3. Mye-K/O mice have a more severe sepsis response

(A) CLP leads to higher mortality rate in Mye-K/O mice. Mye-K/O animals (*Commd1* $^{F/F}$, LysM-Cre) were compared to wild-type (WT) littermate controls (*Commd1* $^{F/F}$). Survival without need for euthanasia was plotted and the Kaplan-Meier estimator was calculated. **(B)** Peritoneal cultures yield lower bacterial counts in Mye-K/O mice (9 mice in each group). Colony forming units (Cfu) in blood agar were determined by progressive dilution. **(C)** Induction of pro-inflammatory gene expression is greater in Mye-K/O mice. Tissue RNA was analyzed by qRT-PCR (in kidney). To normalize for bacterial load, animals with no detectable bacterial counts were used for this analysis (WT=4, Mye-K/O=6 mice). **(D)** Mye-K/O mice have greater mortality after LPS administration. Survival was analyzed as in (A). **(E)** Plasma levels of Il-6, Tnf and Il-10 were determined in Mye-K/O mice (n=9) and WT controls (n=10) at the time of euthanasia in the LPS model. **(F)** LPS induction of pro-inflammatory genes was determined by qRT-PCR in tissues of Mye-K/O mice (n=10) and WT controls (n=10). * $p < 0.05$.

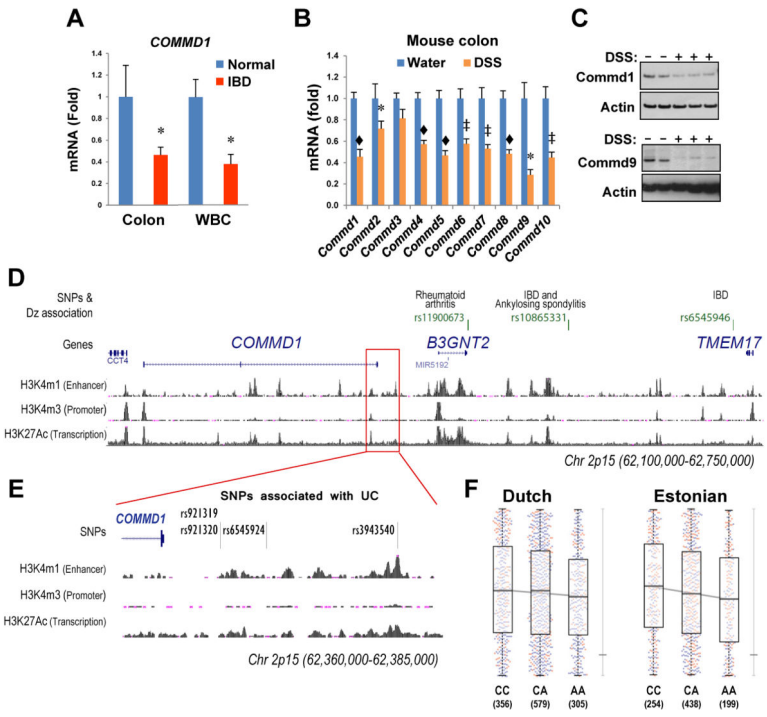


Figure 4. Lower *COMMD1*expression is linked to IBD

(A) *COMMD1* gene expression is suppressed in IBD patients. *COMMD1* mRNA expression was determined by qRT-PCR in endoscopic colonic biopsies from IBD patients with colitis (n=29, 18 with CD and 11 with UC) and normal controls (n=17). Similarly, *COMMD1* mRNA levels in white blood cells (WBC) were determined in IBD patients (n=12, 9 with CD and 3 with UC) and unaffected controls (n=14). **(B)** and **(C)** *Commd* gene expression at the mRNA level (B) was similarly decreased in colonic tissue after DSS-induced acute colitis (7 mice in the DSS group compared to 8 mice in the water group), and this was demonstrated at the protein level for Commd1 and Commd9 (C). * $p < 0.05$, ‡ $p < 0.001$, ◆ $p < 0.0001$. **(D)** Polymorphisms near *COMMD1* are associated with UC risk. A map of the 2p15 locus, including SNPs previously implicated in inflammatory diseases, is shown. The region located 3′ of the *COMMD1* gene, where SNPs associated to UC were found, is marked by a red box. **(E)** A closer view of the location of the SNPs of interest downstream of *COMMD1* is shown, including chromatin modifications such as histone 3 K4 monomethylation (H3K4m1), trymethylation (H3K4m3) and histone 3 K27 acetylation (H3K27Ac). **(F)** *cis*-eQTL analysis for rs921320 in two independent cohorts. *COMMD1* gene expression (in the Y axis) is shown in a box plot for all three genotypes for this SNP (CC, CA or AA). The horizontal bar in the box is the median, the top and bottom of the box show the Inter-quartile range (IQR; Q3 – Q1); the whiskers show the quartile + 1.5x the IQR. The number of individuals in each group is indicated (under the genotype); males and females are denoted by blue and red circles, respectively.

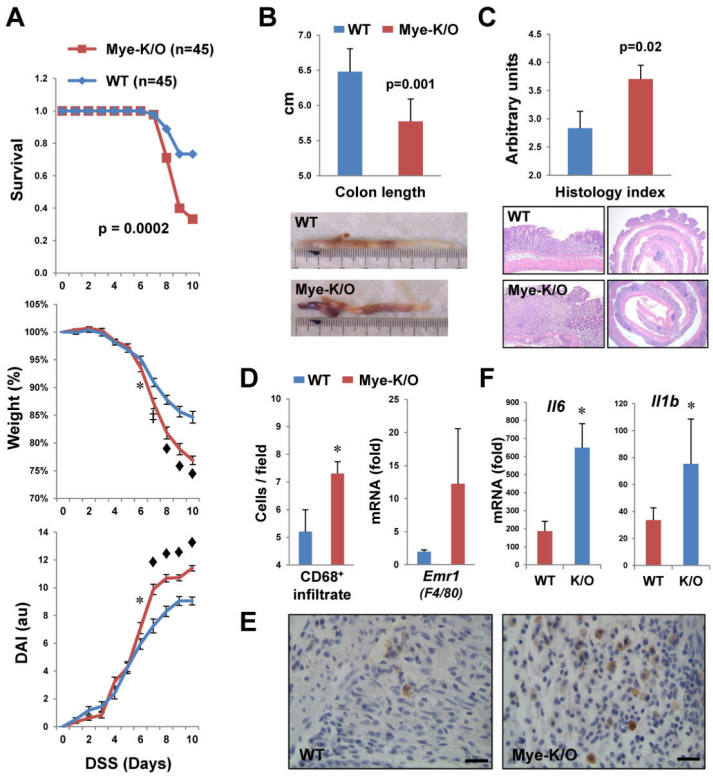
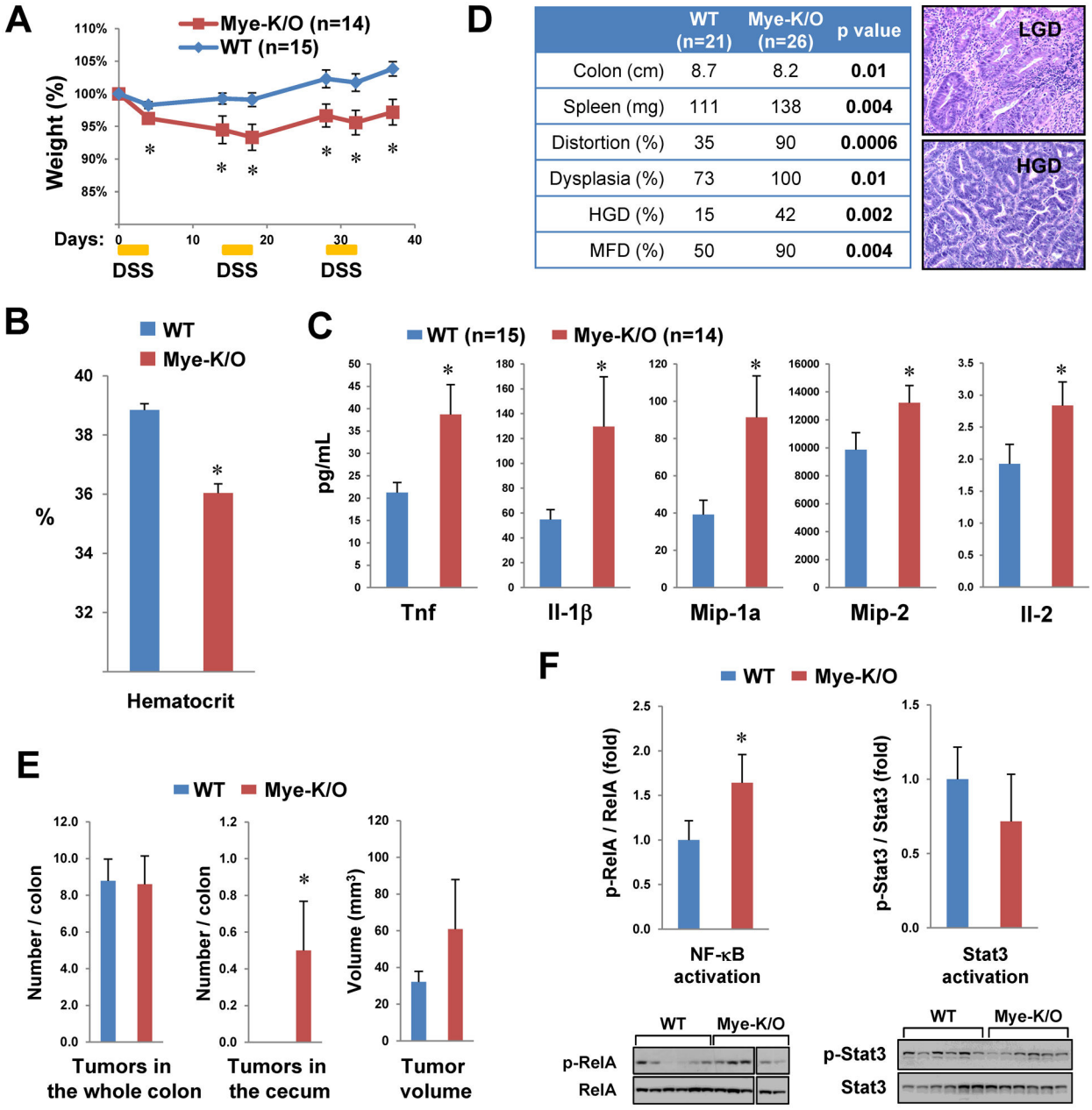


Figure 5. Myeloid deficiency of *Commd1* predisposes to more severe colitis

(A) Worse survival, weight loss and disease activity index (DAI) were noted in Mye-K/O mice compared to wild-type littermate controls during acute DSS-induced colitis. * $p < 0.05$, ‡ $p < 0.001$, ◆ $p < 0.0001$. **(B)** Colon length was significantly shorter in Mye-K/O mice (n=26) after acute colitis compared to WT controls (n=20). Representative images are shown. **(C)** Worse tissue injury was also evident histologically. Using a severity score, colonic sections were examined (11 Mye-K/O and 9 WT mice). Representative histologic sections are shown. **(D)** and **(E)** Expansion of macrophage populations in the colonic mucosa of Mye-K/O mice. In the left panel, the number of $CD68^+$ cells present in the mucosa was assessed by immunohistochemistry in a tissue microarray of DSS colitis samples (Mye-K/O=13, WT=12 samples). In the right panel, the colonic expression of the macrophage marker gene *Emr1* (*F4/80*) was determined by qRT-PCR (Mye-K/O=18, WT=23 samples). Representative images of the CD68 immunohistochemistry are shown in (E), magnification bar = 20μm. **(F)** RNA extracted from colonic tissue was utilized for qRT-PCR analysis for the genes indicated (Mye-K/O=18, WT=23 samples).



	WT (n=21)	Mye-K/O (n=26)	p value
Colon (cm)	8.7	8.2	**0.01**
Spleen (mg)	111	138	**0.004**
Distortion (%)	35	90	**0.0006**
Dysplasia (%)	73	100	**0.01**
HGD (%)	15	42	**0.002**
MFD (%)	50	90	**0.004**

Figure 6. *Commd1* deficiency leads to worse chronic colitis and transition to dysplasia

(A) Recurrent administration of DSS caused more weight loss in Mye-K/O mice. **(B)** Chronic colitis led to more severe anemia in Mye-K/O mice (5 mice per group). **(C)** Pro-inflammatory cytokine production is elevated in colonic tissue of Mye-K/O mice. Colonic full-thickness tissue harvested from colitic mice was cultured overnight and the secretion of cytokines into the growth media was determined. **(D)** Progression to dysplasia was worse in Mye-K/O animals. AOM was administered prior to chronic colitis induction and dysplasia was assessed histologically at 7 weeks. Representative images are displayed. **(E)** Tumor number and volume in mice at 10 wks of colitis/dysplasia induction is depicted. * $p < 0.05$. **(F)** Greater NF-κB activation in inflamed colonic tissue of Mye-K/O mice. Activation of

NF-κB/RelA and Stat3 was assessed in inflamed colonic tissue of animals treated with AOM/DSS. Western blot analysis was performed with tissue lysates and the active phosphorylated form was quantitated and normalized to expression of the respective target.