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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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BACKGROUND & AIMS: Activation of the transcription factor nuclear factor-κB (NF-κB) has been associated with the development of inflammatory bowel disease (IBD). Copper metabolism MURR1 domain containing 1 (COMMD1), a regulator of various transport pathways, has been shown to limit NF-κB activation. We investigated the roles of COMMD1 in the pathogenesis of colitis in mice and IBD in human beings. **METHODS:** We created mice with a specific disruption of *Commd1* in myeloid cells (Mye-knockout [K/O] mice); we analyzed immune cell populations and functions and expression of genes regulated by NF-κB. 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, and colitis-associated cancer was induced by administration of dextran sodium sulfate and azoxymethane. We measured levels of *COMMD1* messenger RNA in colon biopsy specimens 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 a reduced expression of COMMD1 in colon biopsy specimens 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.

BASIC AND TRANSLATIONAL AT

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 2 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 nuclear factor-κB (NF-κB) plays a master regulatory role.[2] Consequently, this factor also has been linked to the pathogenesis of several chronic inflammatory conditions in human beings,[2] including IBD.[3,4] Canonical NF-κB activity is mediated

Abbreviations used in this paper: **BMDM, bone marrow–derived myeloid cells; CD, Crohn's disease; *cis*-eQTL, *cis*-expression quantitative trait locus; COMMD1, copper metabolism MURR1 domain containing 1; DSS, dextran sodium sulfate; IBD, inflammatory bowel disease; IIBDGC, International IBD Genetic Consortium; Il, interleukin; KEGG, Kyoto encyclopedia of genes and genomes; K/O, knockout; LPS, lipopolysaccharide; mRNA, messenger RNA; Mye-K/O, myeloid-specific Commd1 knockout; NF-κB, nuclear factor-κB; SNP, single-nucleotide polymorphism; Tnf, tumor necrosis factor; UC, ulcerative colitis; WT, wild-type.**

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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 IκB kinase that sits at the cross-roads 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 IκB kinase 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 copper metabolism MURR1 domain containing 1 (COMMD1),[10,12] a prototypical member of the COMMD 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 pleiotropic 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 lipopolysaccharide (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 human beings, highlighting the physiologic importance of this gene in immunity and IBD pathogenesis.

Materials and Methods

Human Studies

All procedures involving human subjects were reviewed and approved by the respective institutional review boards (at the University of Texas Southwestern Medical Center, the Mayo Clinic, and the Tel-Aviv Sourasky Medical Center). Circulating leukocytes and intestinal biopsy specimens were obtained at the time of endoscopy as part of the patients' ongoing medical care.

Genome-Wide Association Studies and Quantitative Trait Locus 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 International IBD Genetic Consortium (IIBDGC).[3] We conducted *cis*-expression quantitative trait locus (cis-eQTL) mapping on whole peripheral blood of 2 cohorts: 1240 samples from a previously published study[21] and 891 samples from the Estonian Biobank, both hybridized to Illumina (San Diego, CA) HT12-v3 oligonucleotide arrays using methodology as described in detail elsewhere.[22]

Statistical Analysis

In all graphs, the mean is presented and the error bars correspond to the SEM. Statistical comparisons between mean values were performed using a 1-tailed, heteroscedastic, Student *t* test. For nonparametric variables, the chi-square test was used. Survival curves were examined using the Kaplan–Meier analysis.

All other materials and methods are described in the Supplementary Materials and Methods section.

Results

Commd1 Represses Proinflammatory Gene Expression in Myeloid Cells

To evaluate the potential role of COMMD1 in inflammation, and given the known embryonic lethality that results from complete *Commd1* deficiency in mice,[23] we generated a tissue-specific mouse model of *Commd1* deficiency.[24] First, *Commd1* was selectively deleted in myeloid cells (Mye-knockout [K/O]), a critical lineage in innate immunity, leading to the expected loss of Commd1 expression in macrophages (Supplementary Figure 1*A*). 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 (Supplementary Figure 1*B* and *C*). 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 (Supplementary Figure 1*B–D*). In line with previous observations,[12,25,26] *Commd1* deficiency did not alter substantially the phosphorylation or turnover of IκB (Figure 1*A*), but had a profound effect on RelA ubiquitination (Figure 1*B*).

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 2 independent series of experiments (Figure 1*C*, and Supplementary Tables 1–3). In addition, the expression of 225 genes was found to be regulated by *Commd1* (Supplementary Tables 1 and 2). Notably, the vast majority of *Commd1*-regulated genes (219 of 225) also was regulated by LPS, and only few *Commd1* target genes (6 of 225) were outside the LPS transcriptional response (Supplementary Tables 2 and 3). 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 1*D*). In most instances, the changes observed consisted of increased gene expression, which often were noted even at basal levels (Figure 1*E*).

To further understand the effect of *Commd1* on gene expression in myeloid cells, we performed a functional