CellPress

Immunity
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Macrophage-Restricted Interleukin-10 Receptor Deficiency, but Not IL-10 Deficiency, Causes Severe Spontaneous Colitis

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http://dx.doi.org/10.1016/j.immuni.2014.03.012

SUMMARY

Interleukin-10 (IL-10) is a pleiotropic anti-inflammatory cytokine produced and sensed by most hematopoietic cells. Genome-wide association studies and experimental animal models point at a central role of the IL-10 axis in inflammatory bowel diseases. Here we investigated the importance of intestinal macrophage production of IL-10 and their IL-10 exposure, as well as the existence of an IL-10-based autocrine regulatory loop in the gut. Specifically, we generated mice harboring IL-10 or IL-10 receptor (IL-10Rα) mutations in intestinal lamina propria-resident chemokine receptor CX_3CR1-expressing macrophages. We found macrophage-derived IL-10 dispensable for gut homeostasis and maintenance of colonic T regulatory cells. In contrast, loss of IL-10 receptor expression impaired the critical conditioning of these monocyte-derived macrophages and resulted in spontaneous development of severe colitis. Collectively, our results highlight IL-10 as a critical homeostatic macrophage-conditioning agent in the colon and define intestinal CX_3CR1^{hi} macrophages as a decisive factor that determines gut health or inflammation.

INTRODUCTION

The mammalian intestinal tract represents a unique environment, in which a single epithelial cell layer separates deeper tissues from a dense and potentially harmful microbiota community. Coevolution of the host immune system and the microbiome has generated an environment of mutual benefit. Failure to maintain the exquisite balance of host and microbiota in genetically predisposed individuals results in chronic inflammatory bowel disorders (IBD) including Crohn's disease (CD) and ulcerative colitis (UC).

A critical feature of the gut landscape is ongoing tissue renewal, with a continuously replaced epithelial cell layer (van der Flier and Clevers, 2009) and an equally dynamic immune cell composition (Hapfelmeier et al., 2010; Lathrop et al., 2011). Intestinal macrophages match this dynamic state with a short half-life that is unique among tissue macrophages (Jaensson et al., 2008). Thus, recent studies revealed that most tissue macrophages are established prebirth and subsequently maintain themselves through longevity and limited self-renewal (Ginhoux et al., 2010; Schulz et al., 2012; Yona et al., 2013). Intestinal macrophages rely in contrast on the constant replenishment by blood monocytes (Bogunovic et al., 2009; Varol et al., 2009), potentially attracted by the tonic low-grade inflammatory stimulus present in the gut. Newly arriving $Ly6C^+$ blood monocytes are in the healthy mouse intestine conditioned to acquire a noninflammatory gene-expression profile and differentiate into chemokine receptor CX_3CR1^{hi} macrophages (Bain et al., 2013; Rivollier et al., 2012; Zigmond et al., 2012). Also human lamina propria macrophages display an anergic phenotype under steady-state conditions (Smythies et al., 2005). Under acute inflammatory settings, the conditioning of murine $Ly6C^+$ blood monocytes is impaired and they give rise to proinflammatory cells that promote disease (Zigmond et al., 2012). Local macrophage differentiation and conditioning thus have emerged as a key element for the maintenance of gut homeostasis (Zigmond and Jung, 2013); however, the molecular cues that locally influence this process remain poorly understood.

Interleukin-10 (IL-10) is a pleiotropic cytokine whose activity is aimed at limiting inflammatory responses (Bogdan et al., 1991; Moore et al., 2001). In the gut, IL-10 is produced by T cells, B cells, and macrophages, as well as certain nonhematopoietic cells, usually after activating stimuli (Saraiva and O'Garra, 2010). Most hematopoietic cells also sense IL-10 via expression of a dedicated IL-10 receptor, composed of an IL-10-binding chain (IL-10Rα) and an accessory molecule shared with other receptors of IL-10 superfamily members (IL-10Rβ) (Moore et al., 2001). Genome-wide association studies (GWASs) revealed a central role of the IL-10 axis in IBD pathogenesis. Sequence variants in the IL-10 locus predispose to UC development, without however affecting the CD risk (Franke et al., 2008). Homozygous

IL-10RA and *IL-10RB* loss-of-function mutations that impair ligand-triggered signaling cause severe early onset colitis (Glocker et al., 2009). Highlighting conservation, IL-10-deficient mice develop spontaneous enterocolitis (Kühn et al., 1993). Moreover, also animals that harbor a STAT3 transcription factor deficiency in myeloid cells, including neutrophils and intestinal macrophages, succumb to colitis (Takeda et al., 1999). Activity of STAT3 is however not restricted to the IL-10 receptor but also serves the receptors of IL-5, IL-6, epidermal growth factor (EGF) and others.

Here we report on the specific roles of IL-10 production and IL-10 sensing by intestinal CX_3CR1^{hi} macrophages in gut homeostasis. Specifically, we took advantage of Cx_3cr1^{cre} animals (Yona et al., 2013) to generate mice with macrophage-restricted IL-10 or IL-10Rα deficiencies. We have shown that IL-10 production by macrophages was dispensable for gut homeostasis and T regulatory (Treg) cell maintenance in the mouse. In contrast, animals harboring intestinal macrophages that fail to sense IL-10 due to the IL-10Rα deficiency developed a spontaneous severe UC-like disorder. This defines IL-10 as critical homeostatic macrophage conditioning agent in the colon and highlights CX_3CR1^{hi} macrophages as decisive factor for gut health or inflammation.

RESULTS

Resident Macrophages Accumulate and Gain Proinflammatory and Migratory Traits in an IL-10-Deficient Environment

IL-10-deficient animals develop severe spontaneous colitis (Kühn et al., 1993) driven by commensal microbiota (Rakoff-Nahoum et al., 2006). To evaluate CX_3CR1^{hi} macrophages in this chronic IBD model, we backcrossed *IL-10*$^{-/-}$ mice (Kühn et al., 1993) to *Cx3cr1*^{gfp} mice (Jung et al., 2000). Reporter gene introduction facilitates the identification of these cells (Varol et al., 2009; Zigmond et al., 2012). In our animal facility, *IL-10*$^{-/-}$ *Cx3cr1*$^{gfp/+}$ mice develop spontaneous intestinal inflammation mainly restricted to the large bowel. Flow cytometric and immunohistochemical analysis of colons of these mice revealed the accumulation of CX_3CR1^{hi} macrophages (Figures 1A and 1B). Although the majority of gut macrophages exhibited high expression of the chemokine receptor, akin to resident cells, *Il-10*$^{-/-}$ *Cx3cr1*$^{gfp/+}$ mice notably also displayed a CX_3CR1^{int} cell infiltrate, recently reported to be associated with acute colitis (Bain et al., 2013; Zigmond et al., 2012) (Figure 1A; see Figure S1A available online). To study molecular aspects of resident CX_3CR1^{hi} macrophages under chronic inflammation, we performed a microarray analysis on cells purified from the colonic lamina propria of 6-week-old *IL-10*$^{-/-}$ *Cx3cr1*$^{gfp/+}$ mice, i.e., a time before overt histological disease signs, and compared them to $CX_3CR1\text{-}GFP^{hi}$ macrophages of *Cx3cr1*$^{gfp/+}$ animals (Figure S1A). Comparative transcriptome analysis revealed a marked proinflammatory signature of macrophages isolated from *IL-10*$^{-/-}$ mice with high expression of Trem1, Nos2, IL-23a, Ccl5, and Serum amyloid A3 (Saa3) mRNA. *Il10*$^{-/-}$, but not WT macrophages, also upregulated mRNA of the C-type lectin Clec9A, a steady state marker of $CD8\alpha^+$ dendritic cells (DCs) (Poulin et al., 2012), as well as mRNA of CCR7, a chemokine receptor granting migration potential toward mesenteric lymph nodes (mLN) (Jang et al., 2006). Notably, *Il10*$^{-/-}$ *Cx3cr1*$^{gfp/+}$ macrophages also displayed reduced mRNA expression of the metalloproteinase subunit meprin A, reported to confer ulcerative colitis susceptibility (Banerjee et al., 2009) and of the DC-specific ICAM-3 grabbing nonintegrin (DC-SIGN) isoform A (CD209a) (Figure 1C). Quantitative PCR (qPCR) analysis confirmed the differential expression of these genes (Figure 1D). Under steady-state conditions, CX_3CR1^{hi} macrophages are nonmigratory (Schulz et al., 2009), as indicated by their lack of CCR7 expression. Accordingly, CX_3CR1^{hi} cells are absent from the mLN, a feature that also extends to acute inflammation (Schulz et al., 2009; Figure S1B). In accordance with the observed CCR7 expression in our array analysis, mLN of *Il10*$^{-/-}$ *Cx3cr1*$^{gfp/+}$ mice harbored though a sizable population of CX_3CR1^{hi} cells (Figure 1E). Moreover, the CX_3CR1^{hi} cells in the inflamed mLN expressed CD11c, MHC II, and the Fc receptor CD64, akin to lamina propria-resident colonic macrophages (Figure 1F). These data are consistent with the recent notion that CX_3CR1^{hi} macrophages might under chronic inflammation migrate from the tissue to the LN (Diehl et al., 2013).

Collectively, this demonstrates that resident CX_3CR1^{hi} macrophages fail in absence of IL-10 to adopt a noninflammatory signature but gain proinflammatory and migratory capacity. Furthermore, these data suggest that macrophages might be key initiators of the pathology in this system.

Cultured Macrophages Are Subject to an IL-10-Based Autocrine Regulatory Loop Curbing Activation and Proinflammatory Activity

CX_3CR1^{hi} macrophages are established major producers of intestinal IL-10 (Hadis et al., 2011; Murai et al., 2009), and also sense this cytokine by virtue of their IL-10R expression (Pils et al., 2010a). Indeed, qPCR analysis demonstrated unique high expression of IL-10 and IL-10Rα mRNA by macrophages among immune cells sorted from the colonic lamina propria (Figure 2A). To investigate the physiological importance of macrophage-derived IL-10 and the consequences of macrophages exposure to IL-10 in the gut, we generated mice with macrophage-restricted IL-10 or IL-10Rα mutations. Specifically, we crossed *Cx*$_3$*cr1*^{cre} animals (Yona et al., 2013) to mice harboring conditional mutant *IL10* or *IL10ra* loci (Pils et al., 2011b; Roers et al., 2004).

FACS analysis of the colonic lamina propria of *Cx*$_3$*cr1*^{cre} *rosa26-rfp*$^{fl/fl}$ reporter mice revealed that almost all intestinal macrophages were affected by this transgenic system, whereas only a third of the $CD11c^+$ $CD11b^-$ DCs, which represent the main colonic DC population, displayed reporter gene activation. Rearrangements were also detected in two thirds of $CD103^+$ $CD11b^+$ DCs and $CD103^-$ $CD11b^+$ DCs (Figure 2B) (data not shown). T cells reported to express CX_3CR1 at low percentages are largely spared by this system with less than 6% percent of $CD4^+$ $CD25^-$, $CD4^+CD25^+$ and $CD8^+$ T cells being affected, a frequency that did not change upon inflammation (Figures S2A and S2B). qPCR analysis of colonic tissue of *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ mice revealed reduced IL-10 mRNA expression as compared to *Il10*$^{fl/fl}$ littermates, supporting the notion of macrophages as significant colonic IL-10 source (Figure 2C).

To probe for the existence of an IL-10-based autocrine regulatory loop, we established monocyte-derived macrophage

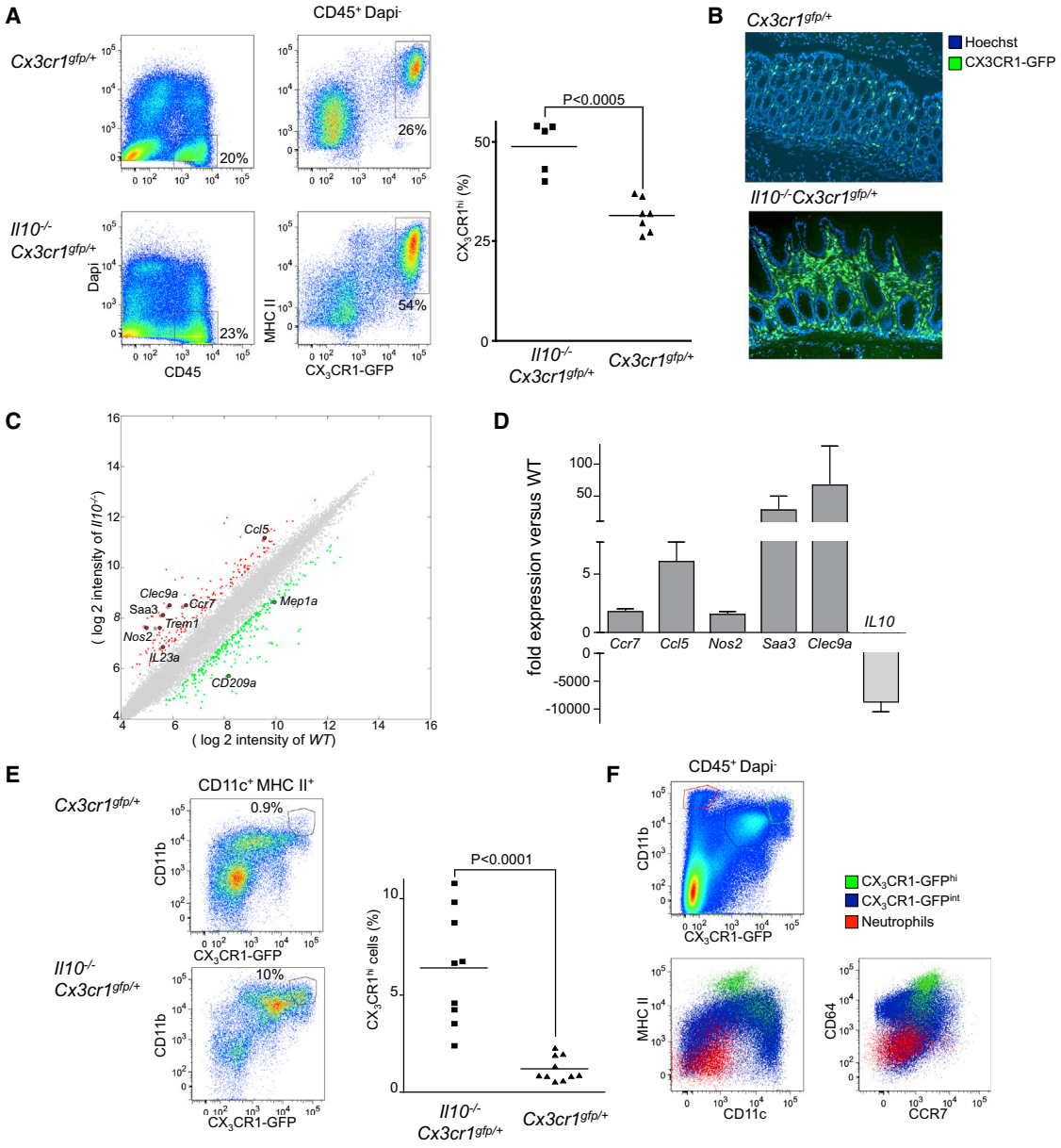

Figure 1. Resident Macrophages Accumulate and Gain Proinflammatory and Migratory Traits in IL-10-Deficient Environment

(A and B) Flow cytometry analysis (A) and histology (B) of colons of 6-month-old *Il10*$^{-/-}$*Cx3cr1*$^{gfp/+}$ and *Cx3cr1*$^{gfp/+}$ mice. Percentages of macrophages are out of CD45$^+$ living cells. Data are pooled from two independent experiments (n = 3–4) and depict the percentage of CX_3CR1^{hi} cells out of CD45$^+$ living cells. Statistical comparisons were performed with the Student's t test.

(C) Scatterplot presenting the differentially expressed genes in 6-week-old *Il10*$^{-/-}$ versus WT resident colonic macrophages. Genes are plotted based on their expression level (log2 intensity). Genes downregulated or upregulated above 2-fold are colored in green and red, respectively (based on microarray data). Specific genes that participate in selected biological pathways are indicated

(D) Graphical summary of qPCR analysis showing the mRNA ratio of indicated molecules between *Il10*$^{-/-}$ and WT resident macrophages sorted from the colonic lamina propria of 6-week-old mice. Data represent mean ± SEM of three independent experiments with sorted cells from a pool of six mice per experiment.

(E) Flow cytometry analysis of mesenteric lymph nodes of 6-month-old *Il10*$^{-/-}$*Cx3cr1*$^{gfp/+}$ and *Cx3cr1*$^{gfp/+}$ mice and graphical summary of the prevalence of CX_3CR1-GFP^{hi} macrophages out of CD11c$^+$ MHC II$^+$ cells. Data are pooled from three independent experiments (n = 3–4) and depict the number of CX_3CR1^{hi} macrophages out of CD11c$^+$ MHC II$^+$ cells. Statistical comparisons were performed with the Student's t test.

(F) Flow cytometry analysis of mesenteric lymph nodes of 6-month-old *Il10*$^{-/-}$*Cx3cr1*$^{gfp/+}$ mice. CD45$^+$ Dapi$^-$ cells were gated for CD11b^{hi} CX_3CR1^{neg} neutrophils (red), CX_3CR1^{hi} cells (green), and CX_3CR1^{int} cells (blue). Data are representative of two independent experiments (n = 3).

cultures by exposing CD115$^+$ bone marrow (BM) fractions of *Cx3cr1*$^{gfp/+}$, *Cx3cr1*^{cre}*Il10*$^{fl/fl}$, and *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice to Csf-1 (M-CSF). This protocol yielded a homogeneous population of F4/80$^+$MHC II$^+$ macrophages displaying uniform high CX_3CR1-GFP expression (Figure 2D). Following LPS stimulation, WT and *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$, but not *Cx3cr1*^{cre}*Il10*$^{fl/fl}$

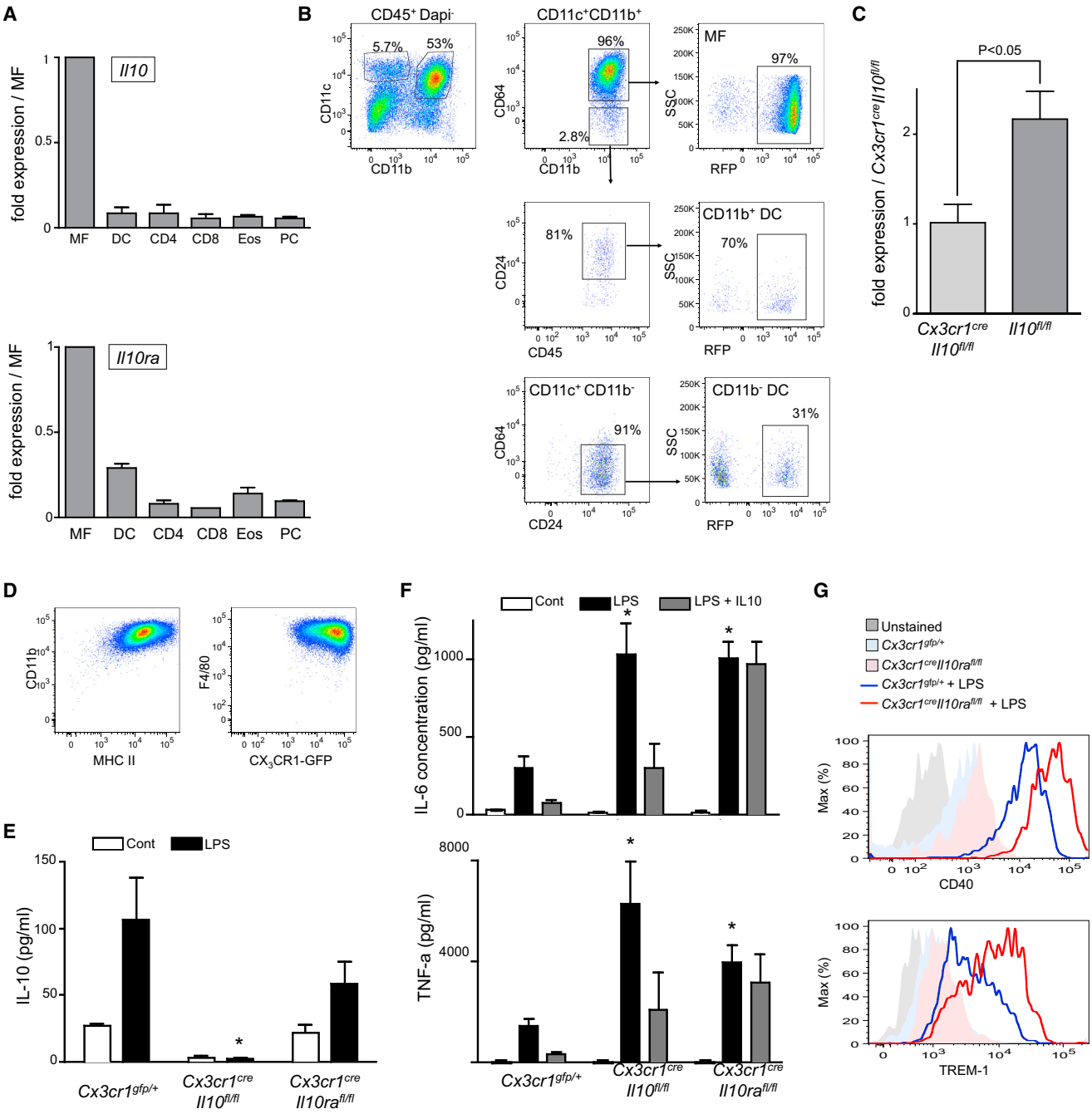

Figure 2. IL-10-Based Autocrine Regulatory Loop

(A) qPCR analysis for IL-10 (upper) and IL-10Ra (lower) mRNA performed on RNA extracted from cells sorted from the colonic lamina propria of *Cx3cr1*$^{gfp/+}$ mice. MF, macrophages; DC, CD103^{+}CD11b^{-} dendritic cells; CD4, CD4^{+} T cells; CD8, CD8^{+} T cells; Eos, Eosinophils; PC, Plasma cells. Data are pooled from two independent experiments (n = 10). Results are shown as mean ± SEM.

(B) Flow cytometry analysis of mononuclear phagocytes in the colonic lamina propria of *Cx3cr1*^{cre}*rosa26-rfp*$^{fl/fl}$ mice. Data are representative of three independent experiments.

(C) qPCR for IL-10 mRNA performed on RNA extracted from the colons of *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ and *Il10*$^{fl/fl}$ mice. Data are pooled from two independent experiments (n = 3). Results are shown as mean ± SEM.

(D) Flow cytometry analysis of macrophages derived from BM of *Cx3cr1*$^{gfp/+}$ mice at day 6 of culture. Data are representative of three independent experiments.

(E) BM-derived macrophages from *Cx3cr1*$^{gfp/+}$, *Cx3cr1*^{cre}*Il10*$^{fl/fl}$, and *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice were stimulated with LPS. Supernatants were analyzed by ELISA for IL-10. Data are pooled from two independent experiments. Results are shown as mean ± SEM.

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macrophages, secreted IL-10 (Figure 2E), establishing efficient rearrangement of the "floxed" *Il10* loci in the latter cells. Macrophages stimulated with LPS responded by secretion of TNF-α and IL-6 (Figure 2F), and upregulated CD40 and TREM-1 (Figure 2G). Proinflammatory cytokine secretion by IL-10- and IL-10Rα-deficient macrophages was significantly higher than that of WT cells. Simultaneous addition of recombinant IL-10 and LPS reduced IL-6 and TNF-α production by *Cx$_3$cr1*^{cre}*Il10*$^{fl/fl}$ macrophages to WT amounts, whereas *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ macrophages remained unaffected (Figure 2F). Collectively, these data corroborate the previously reported autocrine regulatory loop (Siewe et al., 2006; Pils et al., 2010a).

Macrophage-Restricted IL-10 Deficiency Does Not Result in Spontaneous Enterocolitis or Impaired T Regulatory Cell Compartment

To probe the importance of macrophage-derived IL-10 and the above described IL-10-based autoregulatory loop in the control of physiological macrophage hyperactivation, we monitored *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ mice and control littermates weekly for body mass gain and health. Mice harboring IL-10-deficient resident CX$_3$CR1^{hi} macrophages developed normally and did not display any signs of colitis assessed by endoscopic and histological examination up to the age of six months (Figures 3A–3E). Notably, macrophage-derived IL-10 was proposed to be critical for gut homeostasis through its impact on Treg cells (Murai et al., 2009; Hadis et al., 2011; Liu et al., 2011). Comparison of the intestinal Treg cell pool of *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ mice to that of their *Il10*$^{fl/fl}$ littermates revealed, however, no difference in the prevalence of these cells (Figures 3F and 3G). This was in contrast to colitic *Il10*$^{-/-}$ mice that displayed lower percentages of Treg cells (out of total CD4$^+$ T cells), likely due to inflammation-associated CD4$^+$ non-Treg cell infiltrates (Figures 3F and 3G). Although unchallenged *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ mice did not develop spontaneous gut pathology, they could be more susceptible to challenge. To test this issue, we subjected *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ mice and their *Il10*$^{fl/fl}$ littermates to the acute dextran sodium sulfate (DSS) colitis model (Okayasu et al., 1990). Colitis severity and recovery were, however, similar in both groups of animals as evaluated by body mass change and colonoscopy (Figures 3H–3J). Collectively, these data establish that CX$_3$CR1^{hi} macrophage-derived IL-10 is dispensable for the maintenance of gut homeostasis, potentially due to redundant IL-10 sources.

Macrophage-Restricted IL-10 Receptor Deficiency Results in Severe Enterocolitis

To evaluate the significance of IL-10 receptor expression by resident macrophages, we evaluated *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice and control littermates weekly for well-being. *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ males exhibited growth retardation compared to their *Il10ra*$^{fl/fl}$ littermates, with reduced body mass and about 20 percent reduction in average weight at the age of 6 months, similar to IL-10-deficient animals (Figure 4A). Moreover, all male *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice, but none of their *Il10ra*$^{fl/fl}$ littermates, developed progressive rectal prolapse (Figure 4B). Female *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice were less affected, with a milder reduction in body mass and about 60 percent incidence of rectal prolapse (Figure S3). *Il10ra*$^{fl/fl}$ animals were cohoused up to an age of 12 months with their sick *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ littermates. However, they did not display any sign of pathology, arguing against an emergence of dominant colitogenic microbiota in this experimental system. Colonoscopy assessment revealed features compatible with chronic colitis (Figures 4C and 4D). Histological assessment revealed chronic inflammation characteristics only in *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice with normal appearance of their *Il10ra*$^{fl/fl}$ littermates (Figures 4E and 4F). Histopathological alterations in *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice included multifocal infiltrates composed of mononuclear cells, neutrophils, and lymphocytes in the lamina propria, submucosa, and rarely transmural, accompanied by epithelial hyperplasia, erosions, and ulcers (Figures 4E and 4F). Changes were most prominent in the cecum and distal colon, whereas only few mice displayed mild inflammatory infiltrates in their terminal ileum. Multiplex cytokine analysis of sera and supernatants of colon explant cultures disclosed a significant increase in IL-6, IL-17, and IL-10 in *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice (Figure 4G). Histological staining revealed prominent accumulation of T cells and neutrophils in the lamina propria of *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice (Figure 4H). Collectively, this established that CX$_3$CR1^{hi} macrophages have to sense IL-10 to maintain gut homeostasis. Together with the above data obtained from CX$_3$CR1^{hi} macrophages isolated from *Il10*$^{-/-}$ mice, these findings highlight IL-10 as a critical conditioning factor of the monocyte-derived cells.

IL-10 Receptor-Deficient Macrophages Exhibit a Proinflammatory Expression Signature

Next we performed a microarray analysis on resident CX$_3$CR1^{hi} macrophages freshly isolated from the colonic lamina propria of 6-week-old *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice (pre-colitis onset), and compared them to cells isolated from *Cx3cr1*$^{gfp/+}$ animals (Figure S4). Hierarchical sample clustering demonstrated high resemblance of replicate data sets (Figure 5A). Microarray analysis revealed 156 upregulated and 176 downregulated genes that had significantly changed at least 2-fold in IL-10Rα-deficient versus WT macrophages (Figure 5B; Tables S1A and S1B). The genes upregulated in *Il10ra*$^{-/-}$ macrophages revealed a marked proinflammatory signature similar to the profile of macrophages isolated from *Il10*$^{-/-}$ mice (Figure 1C), including elevation of Trem-1, Nos2, IL-23a, Ccl5, Clec9A, Ccr7, and Saa3 mRNA. qPCR analysis confirmed the differential expression of these genes (Figure 5C). Because IL-10R-deficient intestinal macrophages initiated the inflammatory process in the *Cx3cr1*^{cre} *Il10ra*$^{fl/fl}$ model, their gene signature might offer insights into pathogenesis and potential therapeutic IBD targets. Accordingly, comparison of genes upregulated in colonic *Cx3cr1*^{cre} *Il10ra*$^{fl/fl}$ macrophages with IBD susceptibility genes retrieved

(F) ELISA for IL-6 and TNF-α performed on culture supernatants of BM-derived macrophages treated with LPS or LPS and IL-10 isolated from *Cx3cr1*$^{gfp/+}$, *Cx3cr1*^{cre}*Il10*$^{fl/fl}$, and *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice. Data are pooled from three independent experiments. Results for (E) and (F) are shown as mean ± SEM. Statistical comparisons were performed with one-way ANOVA followed by Bonferroni (*$p < 0.05$).
(G) BM-derived macrophages from *Cx3cr1*$^{gfp/+}$ and *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ were analyzed by flow cytometry for specific surface markers. Data are representative of two independent experiments.

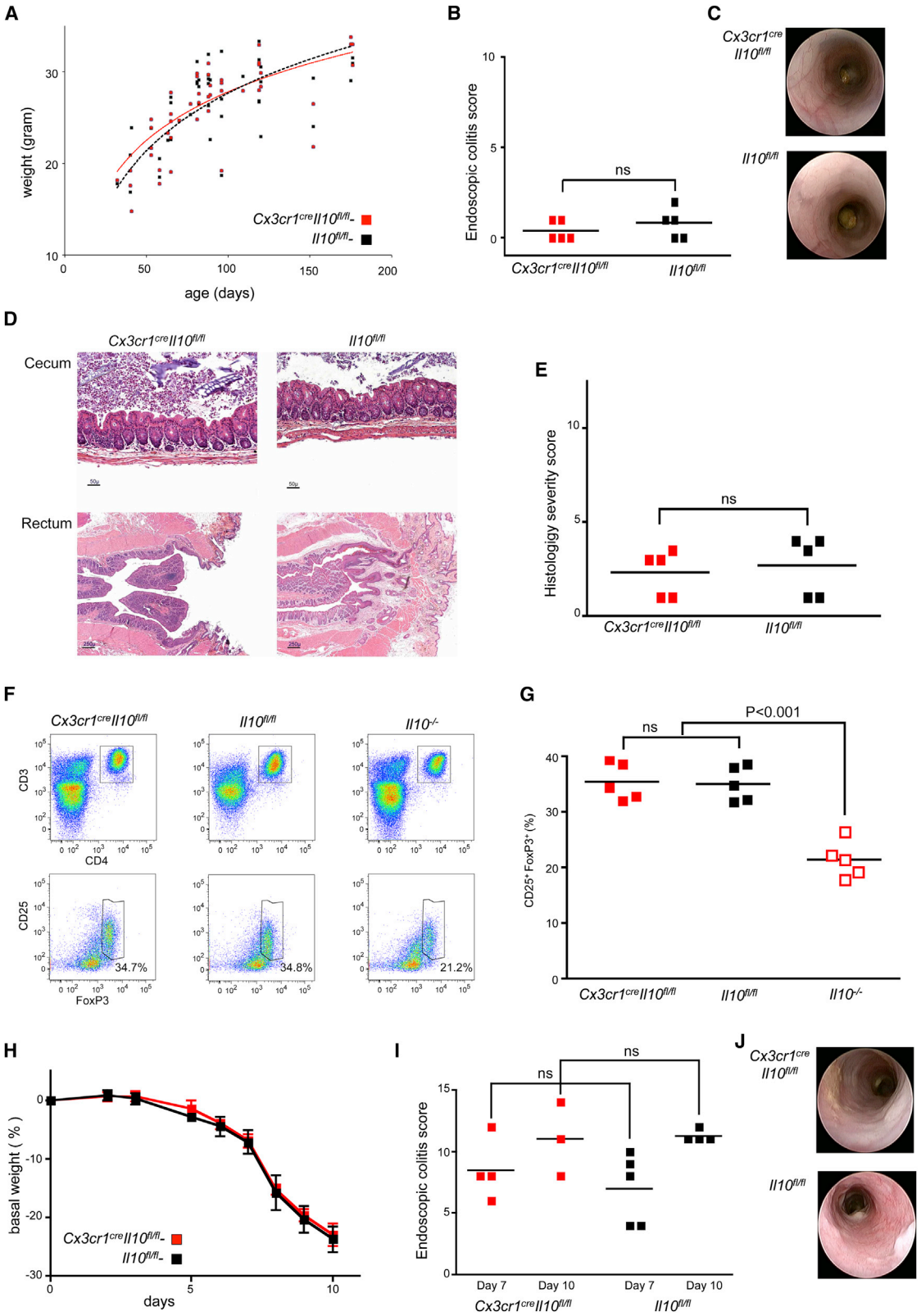

Figure 3. Mice Harboring Macrophage-Restricted IL-10 Mutations Display neither Developmental Abnormalities nor Intestinal Pathology

(A) Graphical summary of body mass follow up of male *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ and *Il10*$^{fl/fl}$ mice. ns, nonsignificant.

(B) Graphical summary of endoscopic colitis grades for 6-month-old *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ and their *Il10*$^{fl/fl}$ littermates.

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from GWASs (Jostins et al., 2012) revealed considerable overlap (Figure 5D). Interestingly, this included a signature of altered lipid metabolism with a shift in eicosanoid synthesis from leukotriene to prostaglandin, suggesting a potential rise of prostaglandin E_2 (PGE_2). Local concentrations of this highly bioactive fatty-acid-derived lipid compound, which has been implicated in the IBD pathophysiology and correlates with UC disease severity (Lauritsen et al., 1988), are determined by the balance of PGE_2 synthesis by cyclooxygenases (COX1 and COX2) and PGE_2 degradation by 15-hydroxyprostaglandin dehydrogenase (*Hpgd*) (Figure 5E). *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ macrophages displayed reduced expression of Hpgd mRNA, a finding confirmed by qPCR (Figure 5F). The cells furthermore exhibited a reduction of arachidonate 5-lipoxygenase (*Alox5*) and leukotriene C4 synthase (*Ltc4s*) expression, i.e., two enzymes required for leukotriene synthesis (Figure 5E). Moreover, culture supernatants of *Cx3cr1*^{cre}*Il10Ra*$^{fl/fl}$ colon explants displayed significantly elevated PGE_2, as compared to littermate controls (Figure 5G). Finally, and supporting the notion of an increased PGE_2 concentration due to reduced degradation in immediate vicinity of the mutant macrophages, we detected a number of genes reported to be induced by PGE_2 in the gene-expression signature of the *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ macrophages, most notably *Il23a* and *Ccr7* (Muthuswamy et al., 2010; Sheibanie et al., 2007). Collectively, the molecular profiling defined the impact of the inability of the CX_3CR1^{hi} macrophages to sense IL-10 and thus the impaired conditioning of their monocytic progenitors.

DISCUSSION

Here we investigated the role of the IL-10 axis in the establishment of the noninflammatory gene-expression signature of intestinal CX_3CR1^{hi} macrophages. We have shown that macrophage production of IL-10 is dispensable for gut homeostasis. In contrast, macrophages unable to sense IL-10 fail to acquire a mandatory noninflammatory gene signature resulting in overt spontaneous colitis.

IBD are considered polygenic diseases that develop in genetically susceptible individuals under certain environmental conditions (Cho, 2008). GWASs revealed strong association of the allelic variants of the *IL10* and *IL10R* genes with IBD, and in particular UC. This suggested impaired IL-10 signaling as key afflicted pathway in the development of human intestinal inflammation (Engelhardt et al., 2013; Franke et al., 2008). Indeed, severe early onset colitis in children can be a monogenic disease caused by mutations in IL-10 or its receptor (Glocker et al., 2009). However, the cell type that requires silencing by IL-10 to maintain gut homeostasis has remained elusive. Our finding that mice harboring IL-10R-deficient colonic lamina propria macrophages develop spontaneous colitis that is in its severity comparable to *Il10*$^{-/-}$ mice highlights CX_3CR1^{hi} macrophages as critical initiators of the inflammatory process. Of note, the transgenic *Cx3cr1*^{cre} system also partially affected intestinal DCs. Impaired IL-10R signaling in these cells, potentially resulting in enhanced effector T cell responses, could therefore contribute to the development of gut inflammation in our model. This should be evaluated in the future as experimental models that allow the specific conditional targeting of intestinal DC subsets become available.

Homeostatic resident CX_3CR1^{hi} macrophages are derived from $Ly6C^+$ monocytes (Bogunovic et al., 2009; Varol et al., 2009) and in the healthy colon locally conditioned to be anergic and noninflammatory (Zigmond and Jung, 2013). Resident CX_3CR1^{hi} macrophages are nonmigratory, as indicated by their lack of expression of the chemokine receptor CCR7 (Schulz et al., 2009; Zigmond et al., 2012) that is required for tissue exit (Jang et al., 2006). Accordingly, in steady-state conditions, but also during chemical-induced acute colitis, mLN lack CX_3CR1^{hi} cells. Recently, it was suggested that dysbiosis caused by antibiotica exposure and *Salmonella* infection, triggers expression of CCR7 by CX_3CR1^{hi} cells and their migration toward mLN (Diehl et al., 2013). Here we corroborate this finding by showing that CX_3CR1^{hi} macrophages of both *Il10*$^{-/-}$ and *cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice display CCR7 expression. Moreover, CCR7 was also found to be expressed by monocyte culture-derived macrophages of patients harboring IL-10R loss-of-function mutations (Shouval et al., 2014). Interestingly, analysis of *Il10*$^{-/-}$*cx3cr1*$^{gfp/+}$ mice revealed the presence of CX_3CR1^{hi} cells in their mLN. Although we cannot exclude that these cells arose locally from blood monocytes that entered the inflamed LN (Tamoutounour et al., 2012), their CD64, CD11c, MHC II, and CCR7 expression would be consistent with their lamina propria origin. Collectively, these data suggest that the absence of migratory capacity of intestinal CX_3CR1^{hi} macrophages requires active enforcement by environmental cues, rather than being an inherent epigenetically preprogrammed feature of these monocyte-derived cells.

The spontaneous colitis of IL-10-deficient animals (Kühn et al., 1993) and most likely also that observed in *cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ model are driven by the commensal gut microbiota (Rakoff-Nahoum et al., 2006). Thus, a Myd88 deficiency in macrophages can restore homeostasis in *Il10*$^{-/-}$ mice (Hoshi et al., 2012). As opposed to other models of gut inflammation (Elinav et al., 2011), we however found no evidence for a selection of a colitogenic microbiota. Interestingly, *Helicobacter hepaticus*-bearing WT mice, but not animals lacking these bacteria, respond to antibody-mediated IL10R neutralization by colitis development

(C) Representative colonoscopy images of indicated mice.
(D) Representative histological images of cecum and rectum of 6-month-old *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ and *Il10*$^{fl/fl}$ mice.
(E) Graphical summary of histological severity score of indicated mice.
(F) Flow cytometry analysis of $CD4^+$ $CD25^+FoxP3^+$ regulatory T cells in the lamina propria of *Cx3cr1*^{cre}*Il10*$^{fl/fl}$, *Il10*$^{fl/fl}$, and *Il10*$^{-/-}$ mice. Graph depicts the percentage of $CD25^+$ $FoxP3^+$ T cells out of $CD4^+$ T cells.
(G) Graphical summary of regulatory T cell prevalence in the lamina propria of indicated mice out of $CD4^+$ T cells.
(H) Graphical summary of body mass changes following DSS challenge of *Cx3cr1*^{cre}*Il10*$^{fl/fl}$ and *Il10*$^{fl/fl}$ mice.
(I) Graphical summary of endoscopic colitis grades in days 7 and 10 following DSS initiation in indicated mice.
(J) Representative colonoscopy images of indicated mice in day 10 following DSS initiation.
Data are representative of at least three independent experiments (n = 5), results are shown as mean ± SEM. Statistical comparisons were performed using the Student's t test (B, E, I), or one-way ANOVA followed by Bonferroni (G).

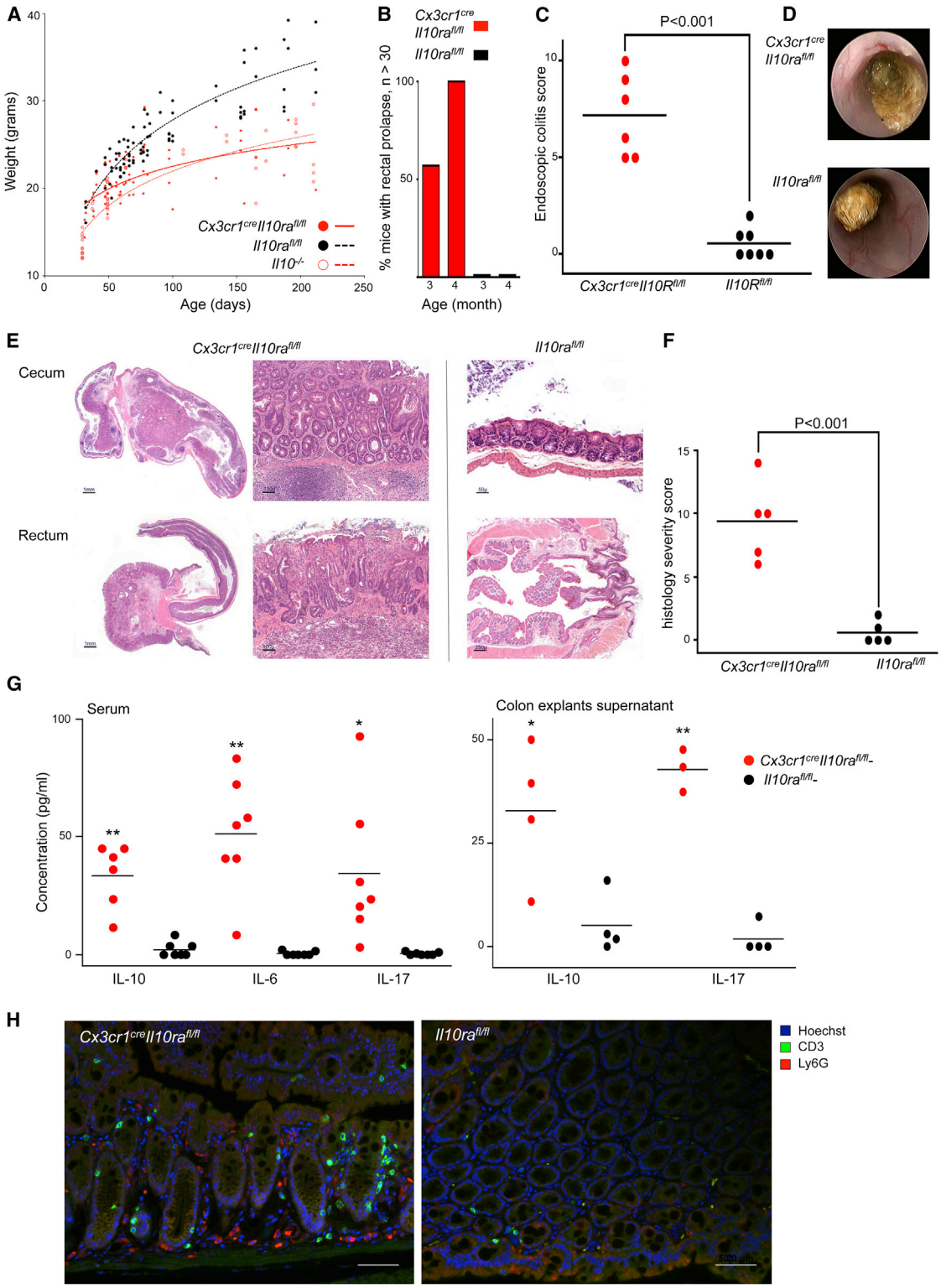

Figure 4. Mice Harbor Macrophage-Restricted IL-10Rα Mutation Developed Spontaneous Colitis and Growth Retardation

(A) Graphical summary of body mass follow up of male *Cx3cr1*^{cre}*Il10Ra*$^{fl/fl}$, *Il10ra*$^{fl/fl}$, and *Il10*$^{-/-}$ mice.

(B) Graphical summary of rectal prolapse incidence of indicated male mice.

(C) Graphical summary of endoscopic colitis grades for 6-month-old male *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice and their *Il10ra*$^{fl/fl}$ littermates. Data are representative of two independent experiments (n = 6–7).

(D) Representative colonoscopy images of indicated mice.

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(Kullberg et al., 2006). Of note, sentinels in our facility were typed *Helicobacter*-positive.

Our data establish that colon-resident macrophages require IL-10 to prevent their hyperactivation in the gut. Colonic macrophages also constitutively express IL-10 mRNA (Zigmond et al., 2012), and we have shown that BM culture-derived macrophage are subject to an autocrine regulatory loop that controls their response to LPS in vitro and has been previously noted (Pils et al., 2010a; Siewe et al., 2006). *Cx3cr1*^{cre}*IL10* $^{fl/fl}$ mice did however not develop signs of colitis. Hence, cells other than macrophages must provide the critical homeostatic IL-10 that suppresses colonic macrophage hyperactivation. Notably, IL-10 can be produced by most hematopoietic cells (Moore et al., 2001). Moreover, even certain nonimmune cells, such as intestinal stroma and epithelium, have been proposed to express IL-10 (Colgan et al., 1999; Ina et al., 2005; Poulin et al., 2012). Several studies have highlighted the critical importance of IL-10 production by CD4$^+$ T cells. Mice harboring a CD4$^+$ T cell-restricted IL-10-deficiency develop spontaneous colitis (Roers et al., 2004), similar to IL10-deficient mice. The main homeostatic IL-10 producers in the gut are, aside from CX_3CR1^{hi} macrophages, Foxp3$^-$ Type I regulatory (Tr1) cells and Foxp3$^+$ Treg cells. In the colon, IL-10-producing T regulatory cells are mainly of the FoxP3$^+$ type (Banerjee et al., 2009; Maynard et al., 2007), suggesting that Foxp3$^+$ Treg cells, rather Tr1 cells, are the prime IL-10 source preventing colitis. Supporting this notion, Rudensky and colleagues established by using *Foxp3*^{Cre}*Il10*$^{fl/fl}$ mice that mere impairment of IL-10 production by Foxp3$^+$ Treg cells is sufficient to cause spontaneous inflammation that was restricted to the large, but not small intestine (Rubtsov et al., 2008). Interestingly though, IL-10-deficient Foxp3$^+$ Treg cells retained the potential to suppress T effector cells, both in in vitro assays, as well as the CD45RB$^+$ T cell transfer model of colitis (Murai et al., 2009; Rubtsov et al., 2008). Why Foxp3$^+$ Treg cell-derived IL-10 was required to maintain gut homeostasis hence remained elusive. Our data suggest that colonic Foxp3$^+$ Treg cell-derived IL-10 is critical to ensure the homeostatic noninflammatory gene-expression signature of intestinal CX_3CR1^{hi} macrophages (Rivollier et al., 2012; Zigmond et al., 2012). In absence of Treg cell-derived IL-10, these cells respond to the abundant microbial stimuli in the gut with the production of proinflammatory cyto- and chemokines triggering inflammation. Such a scenario is supported by the fact that local macrophage elimination ameliorates gut inflammation in IL-10-deficient mice (Watanabe et al., 2003). Finally, it has been shown that Treg cells can reverse intestinal inflammation in *H. hepaticus*-infected *Rag2*$^{-/-}$ and *Tbx21*$^{-/-}$*rag2*$^{-/-}$ mice, corroborating the assumption of a T effector cell-independent regulatory role of these cells (Maloy et al., 2005).

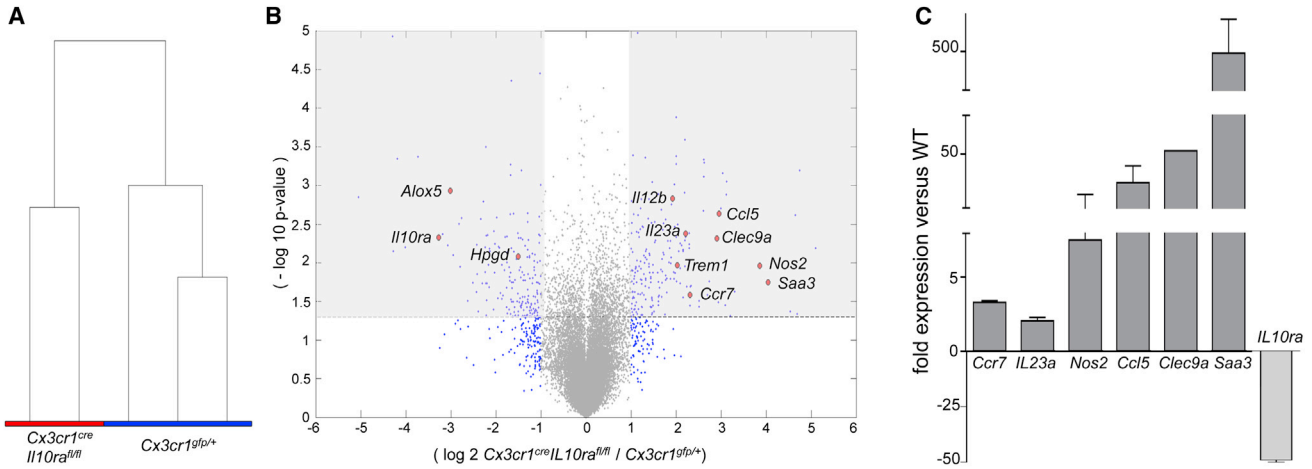
Of note, the Rudensky group established that mice harboring a Foxp3$^+$ Treg cell-restricted *Il10ra* deficiency develop spontaneous, albeit less severe colitis (Chaudhry et al., 2011). Numbers of Foxp3$^+$ Treg cells were found increased in these *foxp3*^{Cre}*Il10r* $^{fl/fl}$ mice, however, notably, these cells failed to produce IL-10. As IL-10 suppresses Th17 cells (Huber et al., 2011), this colitis phenotype had been interpreted as a failure to control T cell-mediated inflammation (Chaudhry et al., 2011). Our present results offer an alternative, though not mutually exclusive explanation. Thus, as in *foxp3*^{cre}*Il10*$^{fl/fl}$ mice, impaired production of IL-10 by Treg cells in *foxp3*^{cre}*Il10ra*$^{fl/fl}$ mice likely compromises the conditioning of Ly6C$^+$ monocytes and the establishment of noninflammatory CX_3CR1^{hi} macrophages that is critical for gut homeostasis.

This study highlights the central role CX_3CR1^{hi} macrophages play in IBD pathophysiology. IL-10Rα-deficient macrophages express a battery of proinflammatory cytokines that can trigger deleterious T cell responses, as indicated by the elevated IL-17 and IL-6 serum titers. Of note, Rag-deficient *Il10*$^{-/-}$ mice develop colitis only upon transfer of T cells (Liu et al., 2011; Murai et al., 2009) and gut inflammation in this model hence depends on effector T cells. In addition, Shouval et al. recently showed that colitis development in a model that relies an IL-10Rβ deficiency in innate immune cells requires adaptive immunity (Shouval et al., 2014).

Interestingly, our microarray gene-expression analysis revealed that intestinal CX_3CR1^{hi} macrophages of IL-10-deficient animals share an expression signature with cells isolated from animals harboring the macrophage-specific *Il10ra* ablation. Both populations upregulated mRNA, encoding CCL5 a known proinflammatory chemokine involved in human and murine colitis (Mazzucchelli et al., 1996; Kucuk et al., 2006), as well as inducible nitric oxide synthase (iNOS), a molecule produced by proinflammatory macrophages (MacMicking et al., 1997) and involved in colonic injury (Beck et al., 2004). Another important molecule upregulated by proinflammatory intestinal macrophages was p19 (*Il23a*) which heterodimerizes with p40 to create the cytokine IL-23. IL-23 is important for the activation of Th17 cells (Yen et al., 2006), which are involved in our chronic colitis model. Finally, we found that TREM-1 is expressed by both BM culture-derived IL-10Rα-deficient macrophages in vitro and intestinal macrophages that drive colitis in our mice model. TREM-1 is responsible for amplification of chronic inflammation in the intestine, and expressed by intestinal macrophages of IBD patients (Schenk et al., 2007).

We recently showed that during DSS-induced gut inflammation, acutely recruited CX_3CR1^{int} effector monocytes drive gut inflammation (Zigmond et al., 2012). In this setting, CX_3CR1^{hi} resident macrophages maintained their characteristic noninflammatory signature (Zigmond et al., 2012). This established robust resistance of this profile to acute exposure to microbial stimuli. The molecular cues that condition monocytes in the healthy gut lamina propria to adopt this signature remained

(E) Representative histological images of cecum and rectum of 6-month-old *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ and *Il10ra*$^{fl/fl}$ mice.
(F) Graphical summary of histological severity score of indicated mice (n = 5).
(G) Graphical summary of IL-10, IL-6, and IL-17 concentrations in colon explants culture supernatants determined by Multiplex assay (n = 4) and sera (n = 7) of 6-month-old *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ and *Il10ra*$^{fl/fl}$ mice.
(H) Immunofluorescence analysis for CD3 (T cells) and Ly6G (Neutrophils) done on sections from the distal colon of the indicated mice. Scale bar represents 50 μm.
Statistical comparisons were performed with the Student's t test (A, C, F, G), (*$p < 0.05$, **$p < 0.005$).



D

	Genes Up-regulated in IL10Ra-deficient macrophages															Genes Down-regulated in IL10Ra-deficient macrophages								
Cytokine/ chemokines & receptor interactions	Ccl5	Ccr7	Il23a	Il18bp	Il12b	Cxcl9	Il15ra	Il1a	Il12rb1	Il2rg	Csf3r	Ccl22	Il27	Il1rn	Inhba	Namp	Il10Ra	Bmp4	Bmp2	Pf4	Kitl				
Lectins	Clec4e	Clec9a	Clec12a	Clec5a	Reg3b	Reg3g	Cd69	Klrk1	Olr1	Sell							siglech	Reg4	CD209a	Lgals12					
Innate immunity / acute phase proteins	Irg1	Saa3	Lrg1	Lcn2																					
Pattern recognition receptors	Nlrc5	Zbp1	Nod1	Nlrp1c-ps	Tlr2	Tlr11											Cd36								
Coagulation cascade	F10	Procr	Cfb	C3													F13a1								
Solute carrier and channels	Slc7a11	Slc16a3															Slco2b1	Slc10a5	Cftr	Ano1	Kcne3	Clic6			
Lipid metabolism	Ddhd1	Fabp5	Acsl1	Scarf1	Apoc2	Abcg1	Acss2										Acsm3	Acer1	Ces1g	Ces1f	Ces2g	Lass4	Mecr	Fads3	Aadac	Hao2
Arachidonic acid metabolism	Pla2g16	Pla2g2a															Hpgd	Edn1	Ednrb	Alox5	Cyp2c55	Ltc4s	Cyp2c40			
G-protein coupled receptors	Niacr1		Gpr18	Htr7	Gpr171												Ednrb	Rgs18								
NO / Arginine metabolism	Nos2	Arg2	Ass1																						
Adhesion	Spon1	Parvg	Itgb7														Postn	Abp1	Dpt	Fn1	Hepacam2	Col14a1	Cd24a		
Antigen processing and presentation	Psmb8	Psmb9	Tap1	Tap2	Cd1d1	H2-gs10	H2-Q6	H2-Q8	H2-T22								Abcb1a	Ctse							
GTP binding / GTPase activity	Irgm1	Irgm2	Gbp5	Gbp4	Gbp2	Gbp11	Gbp8	Gbp6	Iigp1	Tgm2	Tgtp1	Mpa2l	Tac1	Ifi47	Arl5a		Tgm3								
Regulation of Macrophage activities	Cd300lf	Treml4	Pdcd1lg2	Trem1	Nr1h3	Slamf8	Slamf6	Trem3	Sirpb1b	Sirpb1a							Retnla	Fstl1	S1pr1	Lum					
Transcription factors	Stat1	Batf2	Irf7	Irf1	Tcfec												Foxf1a	Hoxb13							
Serine protease inhibitors	Serpina3f	Serpinb9	Serpinb6b														Serpinb6a								
Carbohydrate metabolism	Slc2a6	Slc2a1	Hk3	Pygl													Aldob	Npl	Pck1						
TNF receptor-signaling complex	Tnfrsf14	Tnfsf15	Mlkl	Ripk3	Ikbke																				
Cytochromes																	Cyp2c55	Cyp2f2	Cyp2s1	Cyp2c40					
Glutathione S transferase																	Gstm1	Gstm2	Gstm3	Gstm6	Gstm7	Ltc4s	Gsta4		
miscellaneous	Cst7	Tarm1	Upp1	Ifi205	Art2b	Mmp25	Sectm1a	Trpm2	Gdap10	Tm7sf4	Fam26f	Tac1	Zc3h12c	Slfn1	Cyfip2	Ly6i	Fcrls	Sycn	Fam55b	Klk1	Folr2	Wfdc2	Gsdmc4	Mep1a	Thbs4	Sult1c2
	Dram1	Ddit4	Hpse	Rsad2	Sepx1	Rnf213	Pion	Akt3	Gda	Ppt2	Lmo4	Nrg1	Slamf6	Ero1l	Tnni3	Bnip3	Selenbp1	Nov	Leprel1	Car2	Hmcn1	Cela1	Cth	Mfsd4	St6galnac2	Gas6
	Samhd1	Clcn7	Ly6a														B3gnt7	Sult1a1	Abat	Selenbp2	Ogn	Dcn	Pmp22	Muc3	Ndrg2	Efemp1
																	Mecom	Pde2a	Cd34	Sval1	Entpd5	Akr1c14	Hspa8	Fbn1	Stard8	Tmem30b
																	Sorbs2	Ehf	Lrig3	Csrp2	Ppargc1a	Atoh1	Adh1	Fermt1	Etv1	Sparc
																	Ttr	Etv1	Htr4	Tinag	Boc	Edn1	Hgfac	Maob	Myb	
																	Gne	Speer4d	Fam83b	Lphn2	Speer4e	Cachd1	Emp2	Nr5a2	Nnt	Pdgfra
																	Ocln	Gm1123	Vsig2	Add3	Wwp1	Pcolce	Reps2	Ghr		
																	Aspn	Dpep1	Gsdmc2	Pam	Scin	Cyr61	Tmem30b	Ecm1	Fhl1	
																	Sparc	B4galnt3	St3gal6	Tlll6	Aldh2	Ank	Hmcn1	Cbs	Suclg2	

IBD susceptibility genes
UC susceptibility genes

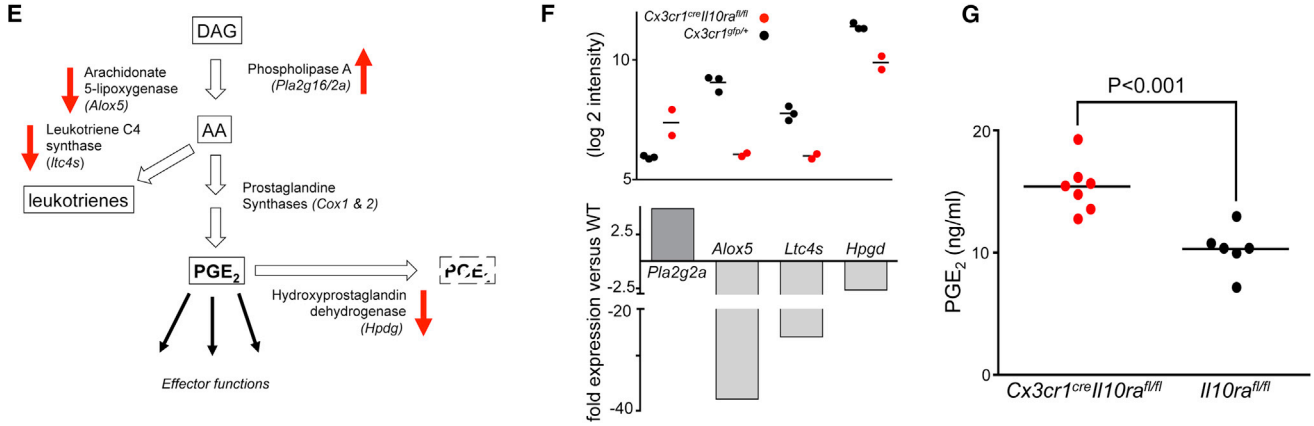


Figure 5. Gene-Expression Profiling of IL-10 Receptor Deficient Lamina Propria Macrophages

(A) Hierarchical clustering of the samples of the microarray data obtained from sorted colonic lamina propria macrophages of *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ and *Cx3cr1*$^{gfp/+}$ mice. Each sorting was done from a pool of 4–6 mice. The clustering was performed on the log 2 intensities, with Pearson dissimilarity as a distance measure.

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unknown. The present study defines IL-10 as a critical homeostatic conditioning factor in this process. Future experimentation should address whether exposure of the cells to this cytokine results in lasting epigenetic modifications or whether IL-10 is continuously required to maintain the noninflammatory state of CX_3CR1^{hi} macrophages.

The gene signature of IL-10Rα-deficient macrophages could include potential targets for future therapeutic IBD intervention. A lead to these approaches could be the prominent impact on lipid and eicosanoid metabolism we observe in macrophages that are blind to IL-10. Of note, a link between IL-10 and PGE_2 has been previously noted in studies involving cultured human macrophages (Antoniv et al., 2005). Moreover, also the IL-10-dependent bacterial clearance in a meningitis model is associated with the potential of this cytokine to suppress PGE_2 (Mittal et al., 2010) and inflammatory monocytes were shown to directly inhibit neutrophil activation in a PGE_2-dependent manner (Grainger et al., 2013). However, prostaglandins have pleiotropic functions and can exert both pro- and anti-inflammatory functions (Dey et al., 2006). Successful therapeutic manipulation of the PGE_2 system for IBD management will hence require additional insights into eicosanoid activities in the healthy and diseased gut context.

Collectively, our results establish intestinal CX_3CR1^{hi} macrophages as key drivers of IL-10 deficiency-based gut inflammation. The gene-expression profiles of IL-10Rα-deficient macrophages reported here and future epigenetic profiling should provide candidate molecules taking part in the early events of the pathology that might open new avenues for the research of the pathogenesis of the disease and could serve as potential targets for therapeutic manipulations aiming at restoration of gut homeostasis.

EXPERIMENTAL PROCEDURES

Mice

Il10$^{-/-}$ mice (Kühn et al., 1993) were crossed to *cx3cr1*^{gfp} mice (Jung et al., 2000) to obtain *Il10*$^{-/-}$*cx3cr1*$^{gfp/+}$ mice. *Cx3cr1*^{cre} mice (Yona et al., 2013) were crossed to *Il10*^{fl} and *Il10ra*^{fl} mice (Roers et al., 2004; Pils et al., 2011b) to obtain *cx3cr1*^{cre}*Il10*$^{fl/fl}$ and *cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ mice. All animals were on C57Bl/6 background. Animals were maintained under specific pathogen-free conditions and handled according to protocols approved by the Weizmann Institute Animal Care Committee as per international guidelines.

Cell Isolation, Flow Cytometry Analysis, and Sorting of Intestinal Macrophages

Isolation of colonic lamina propria cells was performed following a method established previously (Zigmond et al., 2012). Antibodies used for colonic lamina propria staining included: CD45 (30-F11), CD45.2 (104), Ly6C (HK1.4), CD11c (N418), CD11b (M1/70), IAb (AF6-120.1), CD64 (54-5/7.1), CD24 (M1/69), CD40 (3/23), CD3 (145-2C11), TCRb (H57-597), CD4 (GK1.5), CD25 (PC61), and CD8 (53-6.7) all from BioLegend; CD103 (M290) and CCR7 (4B12) BD Bioscience; F4/80 (CI:A3-1) Serotec and Trem1 (174031) R&D; and FoxP3 (FJK-165) eBioscience. For intracellular staining of FoxP3, fixation and permeabilization was done with BD Cytofix/Cytoperm kit according to manufacturer's protocol. Cells were analyzed with a LSRFortessa flow cytometer (BD) or sorted with a FACSAria machine (BD). Flow cytometry analysis was done with the FlowJo software.

Bone-Marrow-Derived Macrophages

BM cells were harvested from the femora and tibiae and enriched for mononuclear cells on a Ficoll density gradient. Cells were isolated by MACS cell separation with CD115-biotin (AFS98 Biolegend) and streptavidin-conjugated magnetic beads (Miltenyi). CD115 positive fractions were cultured in FCS (10%), L-glutamine (1%), Sodium-pyrovate (1%) and pen-strep (1%), in the presence of 10 ng/ml recombinant CSF-1 (Peprotech). At day 3, half the medium was replaced and on day 7 cells were either stimulated with 100 ng/ml LPS (Sigma) or 100 ng/ml LPS and 10 ng/ml recombinant IL-10 (Peprotech) or left as control.

DSS-Induced Colitis Model and Murine Colonoscopy

Mice received one cycle (7 days) of dextran sulfate sodium salt (DSS) (MP Biomedicals, C-160110) treatment 2% in drinking water. To score colitis severity, we used a high-resolution murine video endoscopic system, consisting of a miniature probe (1.9 mm outer diameter), a xenon light source, a triple chip HD camera, and an air pump ("Coloview," Karl Storz) to achieve regulated inflation of the mouse colon. Digitally recorded video files were processed with Windows Movie Maker software (Microsoft). Endoscopic quantification was graded as previously described (Becker et al., 2005).

Statistical Analysis

Data were analyzed by ANOVA followed by Bonferroni's multiple comparison test or by unpaired, two-tailed t test with GraphPad Prism 4. Data are presented as mean ± SEM; values of $p < 0.05$ were considered statistically significant.

ACCESSION NUMBERS

The microarray data are available in the Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/gds) under the accession number GSE56444.

SUPPLEMENTAL INFORMATION

Supplemental Information includes four figures, two tables, and Supplemental Experimental Procedures and can be found with this article online at http://dx.doi.org/10.1016/j.immuni.2014.03.012.

(B) Volcano plot of the statistical significance (-log 10 p value) against the log 2 ratio between colonic macrophages of IL-10Rα deficient and WT mice, based on the microarray data. Hallmark genes are indicated. Genes with fold change below or above 2 are in blue. Statistically significant genes are observed above the dashed horizontal line, which corresponds to a p value of 0.05.

(C) Graphical summary of qPCR analysis showing the mRNA ratios of indicated genes between colonic lamina propria macrophages sorted from *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ and *Cx3cr1*$^{gfp/+}$ mice. Result represent mean of three independent experiments. Results are shown as mean ± SEM.

(D) List of 156 upregulated and 176 downregulated genes in *CX*$_3$*CR1*^{cre}*Il10ra*$^{fl/fl}$ colonic resident macrophages segregated into biological and functional categories. Genes that were upregulated in GWASs of IBD and UC patients (Jostins et al., 2012) are marked in red and green, respectively. Enrichment p values for the overlap between the upregulated genes of IBD or UC associated genes are 0.005 and 0.045 respectively (calculated with the hypergeometric distribution).

(E) Schematic of arachidonate acid (AA) pathway indicating enzymes controlling leukotriene versus prostaglandine synthesis; diacylglycerol (DAG). Red arrows indicate genes whose expression was altered in IL-10Rα-deficient macrophages (see also D).

(F) Graphical summary of Log2 intensity of the indicated genes (top graph), based on the microarray data comparing gene expression in IL-10Rα deficient (red dots) and WT colonic macrophages (black dots), and qPCR analysis showing the mRNA ratio of indicated molecules between *Il10ra*$^{-/-}$ and WT resident macrophages sorted from the colonic lamina propria (bottom graph).

(G) Graphical summary for PGE_2 ELISA analysis of colon explant supernatants from *Cx3cr1*^{cre}*Il10ra*$^{fl/fl}$ and *Il10ra*$^{fl/fl}$ mice. Data are pool of two independent experiments of (n = 3–4). Statistical comparisons were performed with the Student's t test.

ACKNOWLEDGMENTS

We would like to thank all members of the Jung laboratory for discussion, the staff members of the Weizmann sorting facility for technical support, and Ron Rotkopf for advice concerning statistics. This work was supported by the BSF, the Helmsley Foundation, and a research grant from the Lord Alliance Weizmann Manchester Life Science Programme (to S.J. and W.M). S.J. was a Helmsley Scholar at the Crohn's & Colitis Foundation. C.R.W. and W.M received funding from the European Community's Seventh Framework Programme (FP7/2012-2017) under grant agreement n° 305564 (SysmedIBD).

Received: September 30, 2013
Accepted: March 18, 2014
Published: May 1, 2014

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