Antigen-Presenting Cell Production of IL-10 Inhibits T-Helper 1 and 17 Cell Responses and Suppresses Colitis in Mice

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BACKGROUND & AIMS: Mice that are deficient in interleukin (IL)-10 develop colitis, mediated by T-helper (Th)1 and Th17 cells, and IL-10–producing regulatory T (Treg) cells suppress colitis, implicating IL-10 in maintaining mucosal homeostasis. We assessed the relative importance of immunoregulatory IL-10 derived from T cells or from antigen presenting cells (APCs) in development of intestinal inflammation. **METHODS:** $CD4^+$ cells from germ-free (GF) or specific pathogen-free (SPF) *IL-10*$^{-/-}$ or wild-type mice were injected into *IL-10*$^{-/-}$, *Rag2*$^{-/-}$ mice or *Rag2*$^{-/-}$ mice that express IL-10. After 6–8 weeks, we evaluated inflammation, spontaneous secretion of cytokines from colonic tissue, and mRNA levels of the transcription factor T-bet and the immunoregulatory cytokine transforming growth factor (TGF)-β. $CD4^+$ T cells were co-cultured with bacterial lysate-pulsed APCs and assayed for cytokine production, FoxP3 expression, and TGF-β-mediated Smad signaling. **RESULTS:** $CD4^+$ cells from GF or SPF *IL-10*$^{-/-}$ or wild-type mice induced more severe colitis and higher levels of inflammatory cytokines in *IL-10*$^{-/-}$, *Rag2*$^{-/-}$ mice than in IL-10-replete, *Rag2*$^{-/-}$ mice. Co-cultures of *IL-10*$^{-/-}$ or wild-type $CD4^+$ T cells plus bacterial lysate-pulsed APCs from *IL-10*$^{-/-}$ mice contained more interferon (IFN)-γ, IL-12/23p40, and IL-17 than co-cultures of the same T cells plus APCs from wild-type mice. $CD11b^+$ APCs were required for these effects. Blocking IL-10 receptors increased production of IFN-γ and IL-12/23p40 whereas exogenous IL-10 suppressed these cytokines. IL-10–producing APCs induced TGF-β-mediated, retinoic acid-dependent, differentiation of $FoxP3^+$ Treg cells, whereas blocking the retinoic acid receptor, in vitro and in vivo, reduced proportions of $FoxP3^+$ Treg cells. **CONCLUSIONS: IL-10 produced by APCs regulates homeostatic T-cell responses to commensal bacteria.**

Keywords: Mouse Model; Inflammatory Bowel Disease; Immune Response; Microbiota.

Inflammatory bowel diseases (IBDs), including Crohn's disease and ulcerative colitis, are T-cell–mediated inflammatory disorders induced by aggressive mucosal $CD4^+$ cell responses to commensal enteric bacteria.[1–3] Interleukin (IL)-10, in combination with transforming growth factor β (TGF-β), is a key inhibitor of effector T-cell activation and mediator of mucosal homeostasis.[1,4,5] IL-10 and IL-10–receptor (IL-10R) β–deficient mice spontaneously develop T-helper (TH)1/TH17-mediated colitis when normal microbiota are present,[6,7] whereas recombinant IL-10 delivered by parenteral injection or genetically engineered enteric bacteria prevents the onset of experimental enterocolitis[8–10] and improves epithelial barrier properties.[11] Furthermore, polymorphisms of either the IL-10 or IL-10R genes are associated with ulcerative colitis[12] and early onset pediatric Crohn's disease.[13] Mutations in IL-10R A and B associated with severe, early onset fistulizing Crohn's disease mediate loss of functional IL-10 signaling and signal transducer and activator of transcription 3 phosphorylation.[13] Thus, IL-10 has a crucial role in maintaining intestinal homeostasis.[4]

IL-10 is produced by T cells, certain B cells, macrophages, dendritic cells (DCs), and keratinocytes. This cytokine's immunosuppressive and anti-inflammatory activities are mediated through signal transducer and activator of transcription 3[14] and act directly on macrophages and DCs to inhibit IL-12 secretion and down-regulate the expression of major histocompatibility complex (MHC) class II and costimulatory molecules.[4,15–17] In addition, IL-10 directly affects T-cell effector function by inhibiting T-cell cytokine secretion and proliferation.[18–20]

Multiple studies have implicated regulatory $CD4^+$ T-cell subsets producing IL-10 in mucosal homeostasis.[21–25] In vivo neutralization of IL-10 or transfer of IL-10$^{-/-}$ $CD45RB^{low}$ $CD4^+$ cells prevented inhibition of colitis by regulatory cells in the $CD4^+CD45RB^{hi/low}$ T-cell cotransfer severe combined immunodeficiency (SCID) mouse model[21]; however, IL-10–deficient $CD25^+CD4^+$ cells, although less effective than IL-10–sufficient cells, nevertheless partially reverse colitis in the T-cell transfer model.[22] Selective deletion of IL-10 in $CD4^+$ cells induces colitis,[23] and ablation of IL-10 in forkhead box P3 (FoxP3)-express-

Abbreviations used in this paper: APC, antigen-presenting cell; CBL, cecal bacterial lysate; DC, dendritic cell; ELISA, enzyme-linked immunosorbent assay; FoxP3, forkhead box P3; GF, germ-free; IFN, interferon; IL, interleukin; IL-10R, interleukin-10 receptor; ko, knockout; MHC, major histocompatibility complex; MLN, mesenteric lymph nodes; RA, retinoic acid; Rag2, recombination-activating gene 2; SPF, specific pathogen free; TGF, transforming growth factor; TH, T helper cell; Treg, T regulatory; wt, wild type.

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ing T cells also generates mild colitis.[24] Furthermore, IL-10–secreting $CD4^+$ T regulatory (Treg) cells recognizing colonic bacterial antigens prevent colitis induced by bacterial antigen-specific $CD4^+$ cells.[25] IL-10 derived from cells other than T cells may be of importance in immunity to certain pathogens and regulation of colitis.[26–28] However, the relative functional role of IL-10 produced by antigen-presenting cells (APCs) vs T lymphocytes in mucosal immunoregulation remains uncertain.

We performed an in-depth analysis of the innate and acquired immune response in IL-10 normal (wild type [wt]) recombination-activating gene 2 ($Rag2^{-/-}$) or IL-10–deficient (knock-out [ko]) $Rag2^{-/-}$ recipients of IL-10 wt or IL-10 ko $CD4^+$ cells. We assessed functional activities of IL-10 derived from T cells vs APCs in suppressing pathogenic TH1/TH17 immune responses to antigens of commensal intestinal microbiota in vivo and in vitro. Our results suggest that production of IL-10 by APCs is a key determinant in preventing the onset of bacterial antigen-driven $CD4^+$ cell TH1/TH17-mediated chronic experimental colitis. Our studies mechanistically extend the recent observation that myeloid cell production of IL-10 maintains in vivo expression of FoxP3 during intestinal inflammation and prevents colitis in the $CD45RB^{high}$ $CD4^+$ cell transfer model.[29]

Materials and Methods

Mice

IL-10 ko mice (129S6/SvEv background) and $Rag2^{-/-}$ mice (129S6/SvEv background) (Taconic Farms, Germantown, NY) were crossed to obtain IL-10 ko/$Rag2^{-/-}$ double-deficient mice, which lack T and B cells and IL-10 production. Germ-free (GF) mice were derived and maintained in the University of North Carolina National Gnotobiotic Rodent Resource Center, Chapel Hill, North Carolina.

Transfer of $CD4^+$ Cells and Treatment of Recipient Mice

Specific pathogen–free (SPF) IL-10 ko Rag-$2^{-/-}$ and IL-10 wt $Rag2^{-/-}$ mice were injected intraperitoneally with 5×10^5 $CD4^+$ cells from spleens of either GF or SPF IL-10 wt or IL-10 ko donors. In a separate experiment, recipients of SPF IL-10 wt $CD4^+$ T cells were given 100 μg of LE540 (Wako Pure Chemical Industries, Ltd, Osaka, Japan) or vehicle (1:1 dimethyl sulfoxide plus soybean oil) by gavage 2 days before T-cell transfer, and then every other day for the 2-week duration of the experiment.

Analysis of Inflammation

See Veltkamp et al[30] and the Supplementary Materials and Methods section for more detail.

Cell Preparation, Purification, and Culture

See the Supplementary Materials and Methods section for more detail.

Cytokine Measurements

To detect production of interferon (IFN)-γ, IL-12/23p40, IL-10, or IL-17, enzyme-linked immunosorbent assays (ELISAs) were performed in triplicate using R&D Systems (Minneapolis, MN) products. See the Supplementary Materials and Methods section for more detail.

Real-Time Polymerase Chain Reaction

See the Supplementary Materials and Methods section for more detail.

Western Blot Analysis

Wild-type $CD4^+$ cells and IL-10 ko or IL-10 wt APCs were mixed, stimulated with cecal bacterial lysate (CBL) (10 μg/mL) in the presence or absence of TGF-β1, and phosphorylated Smad3 was evaluated as described in the Supplementary Materials and Methods section.

Flow Cytometry

See the Supplementary Materials and Methods section for more detail.

Statistical Analysis

We used Prism 5 software (GraphPad, San Diego, CA) to compare means between 2 groups with 2-tailed, unpaired Student t tests; comparisons of means from multiple groups were analyzed with one-way analysis of variance and the Bonferroni post test. P values less than .05 were considered significant.

Results

Production of IL-10 by Both Non-T cells and by $CD4^+$ Cells Determines Susceptibility to Chronic Colitis

To directly assess the in vivo contribution of IL-10 derived from $CD4^+$ cells vs non-T cells in the development of colitis, we transferred $CD4^+$ cells from SPF IL-10 ko mice with active colitis or from normal wt IL-10–producing donor mice into IL-10 ko $Rag2^{-/-}$ or IL-10 wt $Rag2^{-/-}$ recipients. IL-10 ko $CD4^+$ cell–reconstituted IL-10 ko recipients (ko→ko) displayed severe colitis compared with IL-10 wt recipients of IL-10 ko $CD4^+$ cells (ko→wt) (Figure 1*A*). Clinically, IL-10 ko recipient mice showed severe chronic diarrhea. Histologic features of colitis in IL-10 ko recipients appeared typical of that in IL-10 ko mice with robust lamina propria and submucosal infiltration of mononuclear cells, crypt abscesses, marked crypt hyperplasia, and near-total goblet cell depletion (Figure 1*A*). Some mice developed mucosal ulcerations. Blinded histologic inflammatory scores confirmed more severe inflammation in IL-10 ko vs IL-10 wt recipients ($P < .001$) that received IL-10 ko $CD4^+$ cells (Figure 1*B*). IL-10 ko recipient mice reconstituted with $CD4^+$ cells from normal IL-10–producing wt mice developed moderate intestinal inflammation (wt→ko), whereas IL-10 wt mice reconstituted with IL-10 wt $CD4^+$ cells did not develop colitis (wt→wt). Spontaneous IFN-γ production by colonic explants was significantly higher in IL-10 ko recipients reconstituted with ko or wt $CD4^+$ cells compared with IL-10 wt recipient mice (Figure 1*C*). Similarly, colonic IL-12/23p40 production was increased in IL-10 ko recipients reconstituted with $CD4^+$ cells compared with IL-10 wt recipients (data not shown). These results show a crucial