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ORIGINAL RESEARCH

Resident Bacteria-Stimulated Interleukin-10-Secreting B Cells Ameliorate T-Cell-Mediated Colitis by Inducing T-Regulatory-1 Cells That Require Interleukin-27 Signaling

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SUMMARY

Regulatory mechanisms of interleukin-10 (IL10)-producing B cells in mucosal homeostasis are not fully understood. This study shows that IL10-secreting B cells activated by resident bacteria ameliorate T-cell-mediated colitis by inducing T regulatory-1 cells via an IL27-dependent mechanism.

BACKGROUND & AIMS: The regulatory roles of interleukin-10 (IL10)-producing B cells in colitis are not fully understood, so we explored the molecular mechanisms by which these cells modulate mucosal homeostasis.

METHODS: $CD4^+$ T cells from wild-type (WT), *Il10*$^{-/-}$, or *Il27ra*$^{-/-}$ mice were cotransferred with B cells from specific pathogen-free (SPF) or germ-free (GF) WT or *Il10*$^{-/-}$ mice into *Rag2*$^{-/-}$*Il10*$^{-/-}$(double-knockout) mice, and the severity of colitis and intestinal regulatory T-cell populations were characterized. In vitro, WT or *Il10*$^{-/-}$ B cells were cocultured with unfractionated, naïve or regulatory T cells plus *Il10*$^{-/-}$ antigen-presenting cells and stimulated with cecal bacterial lysate (CBL) with or without IL27 or anti-IL10R blockade. Gene expressions, cytokines in the supernatant and cell populations were assessed.

RESULTS: WT but not *Il10*$^{-/-}$ B cells attenuated T helper cell T_H1/T_H17-mediated colitis in double-knockout mice that also received WT but not *Il10*$^{-/-}$ T cells. In vitro, CBL-stimulated WT B cells secrete abundant IL10 and suppress interferon-γ (IFNγ) and IL17a-production by T cells without requiring cell contact. Although both WT and *Il10*$^{-/-}$ B cells induced $Foxp3^+CD4^+$ T-regulatory cells, only WT B cells induced IL10-producing (Foxp3-negative) T regulatory-1 (Tr-1) cells both in vivo and in vitro. However, IL10-producing B cells did not attenuate colitis or induce Tr-1 cells in the absence of T cell IL27 signaling in vivo. WT B cell-dependent Tr-1 induction and concomitant decreased IFNγ-secretion were also mediated by T-cell IL27-signaling in vitro.

CONCLUSIONS: IL10-secreting B cells activated by physiologically relevant bacteria ameliorate T-cell-mediated colitis and contribute to intestinal homeostasis by suppressing effector T cells and inducing Tr-1 cells via IL27-signaling on T cells. *(Cell Mol Gastroenterol Hepatol 2015;1:295–310; http://dx.doi.org/10.1016/j.jcmgh.2015.01.002)*

Keywords: Experimental Colitis; IBD; Immunoregulation; Regulatory B Cells.

Inflammatory bowel diseases (IBD) are chronic, T-cell-mediated intestinal disorders characterized by loss of tolerance to resident enteric bacteria and aggressive inflammatory responses.[1,2] Regulatory T cells (Treg) have a well-described role in attenuating experimental colitis and IBD. Treg help maintain intestinal homeostasis by preventing inappropriate innate and adaptive immune responses against resident bacteria. $CD4^+$ T cells that express forkhead box P3 (Foxp3) and T regulatory-1 (Tr-1) cells that lack Foxp3 expression ($Foxp3^{neg}$), but produce interleukin-10 (IL10) comprise major regulatory T-cell populations in the intestine.[3,4] $CD25^+Foxp3^+CD4^+$ Treg prevent colitis in severe combined immunodeficiency mice cotransferred with $CD45RB^{high}$ T cells,[5] and enteric bacterial antigen-specific Tr-1 cells ameliorate colitis induced by pathogenic T helper 1 (T_H1) cells.[6]

In parallel with Treg cells, B cells contribute to intestinal homeostasis by secreting immunoglobulins that decrease mucosal translocation of luminal bacteria and producing regulatory cytokines that inhibit effector mucosal immune responses.[7] Moreover, antigen presentation by B cells promotes the differentiation of tolerogenic $CD4^+$ T cells.[8] B-cell depletion may contribute to the development of human IBD[9] and potentiate murine experimental colitis,[10] suggesting that B cells are protective in IBD. However, the

Abbreviations used in this paper: APC, antigen-presenting cell; Blimp-1, B-lymphocyte-induced maturation protein-1; CBL, cecal bacterial lysate; DKO, double-knockout; ELISA, enzyme-linked immunosorbent assay; Foxp3, forkhead box P3; GF, germ-free; HBSS, Hanks' balanced salt solution; IFN, interferon; IL, interleukin; IL10R, interleukin-10 receptor; LP, lamina propria; LPL, lamina propria lymphocytes; MLN, mesenteric lymph nodes; PBS, phosphate-buffered saline; Rag2, recombination-activating gene 2; SPF, specific pathogen-free; T_H, T helper cell; Tr-1, T regulatory-1; Treg, T regulatory; UNC, University of North Carolina; WT, wild-type.

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mechanisms by which B cells attenuate intestinal inflammation are not entirely clear.

B cells secrete IL10 as do many other cell types including Treg cells, macrophages, mast cells, epithelial cells, and dendritic cells. IL10 reduces inflammation by inhibiting effector T-cell activation.[11] IL10-deficient (*Il10*$^{-/-}$) and IL10-receptor-deficient mice develop resident enteric bacteria-dependent T_H1/T_H17-mediated colitis.[12] The role of IL10 derived from intestinal T-cells and myeloid cells in maintaining mucosal homeostasis is well studied, but relatively little is known about the importance of IL10-producing B cells in IBD and experimental colitis.[9,10,13,14] Moreover, the mechanisms of how IL10-producing B cells potentially maintain mucosal homeostasis in the intestine are poorly understood.

IL27, a member of the IL12 family, consists of Epstein-Barr virus induced gene-3 (EBI3) and p28 subunits and has pleiotropic effects on the immune system. IL27 was originally reported to induce T_H1 cell development,[15] but it can also suppress the development of T_H17 cells by decreasing *Ror*γt gene expression and increasing the number of IL10-producing Tr-1 cells but not Foxp3$^+$ Treg.[16–20] IL27 promotes the expansion of Tr-1 cells by up-regulating c-Maf and aryl hydrocarbon receptor in naïve T cells.[20] There is conflicting evidence regarding the role of IL27 in colitis, with some studies showing that IL27 has proinflammatory effects[21–23] and others demonstrating that IL27 is anti-inflammatory.[24–26] Thus, IL27 likely plays a pivotal role in regulating the delicate balance between proinflammatory T_H1/T_H17 cells and anti-inflammatory IL10-producing T-cell populations in the intestine. However, it is unknown whether IL10-producing mucosal B cells affect the regulatory or proinflammatory functions of IL27.

This study addresses the mechanisms by which IL10-secreting B cells influence regulatory T-cell differentiation and ameliorate T-cell-mediated colitis. We show that IL10-producing B cells suppress wild-type (WT) but not *Il10*$^{-/-}$CD4$^+$ T-cell-mediated colitis and are associated with increased frequency of intestinal Tr-1 cells and decreased T_H1/T_H17 cytokine profiles. Moreover, we demonstrate that regulatory functions of IL10-secreting B cells are mediated by IL27-signaling in T cells. Together, these findings elucidate novel regulatory mechanisms of IL10-secreting B cells, help explain mechanisms of mucosal homeostasis, and could be exploited to treat IBD.

Materials and Methods

Mice

We purchased C57BL/6 (B6).WT, B6.*Il10*$^{-/-}$, B6.*Rag2*$^{-/-}$, and B6.*Il27ra (WSX-1)*$^{-/-}$ mice from Jackson Laboratories (Bar Harbor, ME). We purchased 129S6/SvEv (129).WT mice from Taconic Farms (Germantown, NY). The 129.*Il10*$^{-/-}$ mice were obtained from Dr Donna Rennick (DNAX Laboratories, Palo Alto, CA). The *Il10*$^{-/-}$*Rag2*$^{-/-}$ double-knockout (DKO) mice were generated by crossing B6.*Il10*$^{-/-}$ with B6.*Rag2*$^{-/-}$ mice or 129.*Il10*$^{-/-}$ with 129.*Rag2*$^{-/-}$ mice. The B6.*Il10*$^{+/EGFP}$ reporter (Vert-X) mice were obtained from Dr Christopher Karp.[27] These mice were originally maintained in the specific pathogen-free (SPF) facility at the University of North Carolina (UNC), then all 129 strains, B6.WT, *Il10*$^{-/-}$, DKO, and Vert-X mice were derived into germ-free (GF) conditions by embryo transfers, and breeding colonies were established in the GF facility at the UNC. Afterward, the mice were transferred to a SPF room and colonized with SPF feces to maintain SPF colonies. The mice used in this study were born from parents that had also been born and raised in SPF conditions over six generations, and they were used at 8 to 16 weeks of age. These studies were approved by the UNC–Chapel Hill Institutional Animal Care and Use Committee (ACUC) Protocol no. 12–300.0.

Cell Isolation

Mononuclear cells were isolated from the colon lamina propria (LP), mesenteric lymph nodes (MLN), and spleen, as described elsewhere.[13] The MLN were pressed through 70-μm filters into phosphate-buffered saline (PBS) with 2.5% fetal bovine serum (Gibco/Invitrogen, Carlsbad, CA). Spleens were mechanically dissociated, and the red blood cells were lysed with red blood cell lysing buffer (Sigma-Aldrich, St. Louis, MO). For isolation of colonic LP lymphocytes (LPL), the large intestines were washed with cold PBS, opened longitudinally, and cut into 10-mm pieces. Then the intestines were incubated in 1 mM dithiothreitol (Sigma-Aldrich) in Ca^{2+}- and Mg^{2+}-free Hanks' balanced salt solution (HBSS; Gibco/Invitrogen) for 15 minutes at room temperature. Next, the tissues were incubated in 1 mM EDTA (Sigma-Aldrich) in HBSS for 20 minutes at 37°C with shaking, which was repeated after a thorough washing. The cell suspensions were removed, and the remaining fragments were transferred to flasks containing HBSS with 1 mg/mL of collagenase type 3 (Sigma-Aldrich) and 1% penicillin-streptomycin (Gibco/Invitrogen), then stirred gently for 60 minutes at 37°C. The cell suspensions containing LPL were filtered through a nylon mesh and centrifuged, then the LPL were purified using a 44%–70% discontinuous Percoll gradient (GE Healthcare, Buckinghamshire, UK). After centrifugation at 800*g* for 20 minutes at 22°C, the mononuclear cells were collected from the interface.

Cell Purification

Splenic B cells were purified magnetically by positive selection with anti-CD19 microbeads after negative selection by a mixture of anti-CD90.2, anti-CD11c, and anti-Ter119 microbeads (Miltenyi Biotec, Auburn, CA) (greater than 99.5% pure and 90% viable). The CD4$^+$ T cells were isolated by a CD4$^+$ T-cell isolation kit (Miltenyi Biotec) (more than 94.7% pure and 95% viable). In some experiments, unfractionated CD4$^+$ T cells were further fractionated into CD25$^+$ and CD25$^-$ T cells by PE-conjugated anti-CD25 antibody with anti-PE microbeads. Red blood cell lysed-unfractionated splenocytes from *(Il10*$^{+/+}$*)Rag2*$^{-/-}$ and DKO mice were used for WT and *Il10*$^{-/-}$ antigen-presenting cells (APC), respectively (more than 88.4% CD11b$^+$).

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