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# Activity of SHIP, Which Prevents Expression of Interleukin 1β, Is Reduced in Patients With Crohn's Disease

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**BACKGROUND & AIMS:** Crohn's disease (CD) is associated with a dysregulated immune response to commensal microorganisms in the intestine. Mice deficient in inositol polyphosphate 5′-phosphatase D (INPP5D, also known as SHIP) develop intestinal inflammation resembling that of patients with CD. SHIP is a negative regulator of PI3Kp110α activity. We investigated mechanisms of intestinal inflammation in *Inpp5d*$^{-/-}$ mice (SHIP-null mice), and SHIP levels and activity in intestinal tissues of subjects with CD. **METHODS:** We collected intestines from SHIP-null mice, as well as *Inpp5d*$^{+/+}$ mice (controls), and measured levels of cytokines of the interleukin 1 (IL1) family (IL1α, IL1β, IL1ra, and IL6) by enzyme-linked immunosorbent assay. Macrophages were isolated from lamina propria cells of mice, IL1β production was measured, and mechanisms of increased IL1β production were investigated. Macrophages were incubated with pan–phosphatidylinositol 3-kinase inhibitors or PI3Kp110α-specific inhibitors. Some mice were given an antagonist of the IL1 receptor; macrophages were depleted from ilea of mice using clodronate-containing liposomes. We obtained ileal biopsies from sites of inflammation and peripheral blood mononuclear cells (PBMCs) from treatment-naïve subjects with CD or without CD (controls), and measured SHIP levels and activity. PBMCs were incubated with lipopolysaccharide and adenosine triphosphate, and levels of IL1β production were measured. **RESULTS:** Inflamed intestinal tissues and intestinal macrophages from SHIP-null mice produced higher levels of IL1B and IL18 than intestinal tissues from control mice. We found PI3Kp110α to be required for macrophage transcription of *Il1b*. Macrophage depletion or injection of an IL1 receptor antagonist reduced ileal inflammation in SHIP-null mice. Inflamed ileal tissues and PBMCs from patients with CD had lower levels of SHIP protein than controls ($P < .0001$ and $P < .0002$, respectively). There was an inverse correlation between levels of SHIP activity in PBMCs and induction of IL1β production by lipopolysaccharide and adenosine triphosphate ($R^2 = .88$). **CONCLUSIONS:** Macrophages from SHIP-deficient mice have increased PI3Kp110α-mediated transcription of *Il1b*, which contributes to spontaneous ileal inflammation. SHIP levels and activity are lower in intestinal tissues and peripheral blood samples from patients with CD than controls. There is an inverse correlation between SHIP activity and induction of IL1β production by lipopolysaccharide and adenosine triphosphate in PBMCs. Strategies to reduce IL1B might be developed to treat patients with CD found to have low SHIP activity.

*Keywords:* PI3Kp110α; Signal Transduction; Inflammatory; Phosphatidylinositol.

Crohn's disease (CD) is a subtype of inflammatory bowel disease (IBD) characterized by chronic inflammation along the gastrointestinal tract.[1] IBD affects 1 in 150 people in North America and the incidence of disease is increasing in developed countries.[1,2] Although the etiology of disease remains unknown, current thinking is that CD occurs in genetically susceptible individuals due to an inappropriate immune response to intestinal flora.[3,4] Biological therapy, monoclonal antibodies directed against tumor necrosis factor (TNF)α, is effective at inducing remission and has revolutionized the treatment for CD.[5] However, some patients are refractory to biological therapy or therapy becomes ineffective because patients develop antibodies against the drug.[6,7]

The pro-inflammatory cytokine, interleukin (IL) 1β, plays a critical role in IBD pathogenesis. IL1β is secreted from intestinal tissues and macrophages isolated from patients with IBD, and IL1β levels correlate with disease severity.[8–11] IL1β acts as an alarm cytokine, initiating the inflammatory response, thus its production is tightly regulated by a 2-step process: (1) Toll-like receptor (TLR) or endogenous ligands induce *IL1B* transcription, which is translated to pro–IL1β, an inactive precursor; (2)

***Abbreviations used in this paper:*** **ATP, adenosine triphosphate; BMM, bone marrow–derived macrophage; CD, Crohn's disease; clod-lip, clodronate-containing liposomes; DSS, dextran sulfate sodium; ELISA, enzyme-linked immunosorbent assay; IBD, inflammatory bowel disease; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; PBMC, peripheral blood mononuclear cell; PI3K, phosphatidylinositol 3-kinase; SHIP, SH2 domain-containing inositolphosphate 5′-phosphatase; TLR, Toll-like receptor; TNF, tumor necrosis factor; Wm, wortmannin.**

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numerous stimuli, typically danger-associated molecular patterns, cause assembly of the inflammasome, a hetero-oligomeric protein complex, which catalyzes the processing of pro−IL1$\beta$ for secretion.[12–14] IL1$\beta$ can feedback and activate cells to induce *IL1B* transcription. Hence, monogenic gain-of-function mutations leading to increased IL1$\beta$ production cause a group of autoinflammatory diseases (periodic fever syndromes), which can be treated with anakinra, an IL1 receptor antagonist.[1,12] Intestinal inflammation is a common complication of monogenic autoinflammatory diseases and primary immune deficiencies characterized by increased IL1$\beta$ production.[1,15–17] A gene variant in *ATG16L1* (rs2241880), which has been associated with CD and high IL1$\beta$ production, and a second intronic gene variant (rs12994997), which has traditionally been associated with ATG16L1, are located adjacent to the human gene encoding SH2 domain-containing inositolphosphate 5′-phosphatase (SHIP), *INPP5D*, and may impact disease by affecting *ATG16L1* and/or *INPP5D*.[18–20] IL1$\beta$ antagonism has been used effectively to treat some genetically defined forms of very early onset IBD[21] and may be more broadly applicable for the treatment of subgroups of IBD.

Phosphatidylinositol 3-kinase (PI3K) is critical in cellular processes, including growth, differentiation, proliferation, and inflammation.[22] Class I PI3Ks are heterodimeric enzymes composed of a regulatory subunit; class IA contains a catalytic subunit, p110$\alpha$, p110$\beta$, or p110$\delta$, and class IB contains the p110$\gamma$ catalytic subunit.[22] PI3Kp110 catalytic subunits have overlapping and unique functions downstream of different receptors.[23] SHIP is a hematopoietic-specific negative regulator of class I PI3K. SHIP antagonizes PI3K activity by dephosphorylating the PI3K-generated second messenger, PI(3,4,5)$P_3$.[24] Myeloid cells from SHIP-deficient mice are hyperproliferative[25] and hyperresponsive to growth factor, immune, and inflammatory stimuli.[26–30]

We, and others, have reported that SHIP$^{-/-}$ mice develop spontaneous CD-like intestinal inflammation.[31,32] We investigated the cause of intestinal inflammation in SHIP$^{-/-}$ mice to determine the contribution of SHIP$^{-/-}$ macrophages to pathology and to validate these findings in CD patients. We report that ileal macrophages from SHIP$^{-/-}$ mice produce high levels of IL1$\beta$ caused by increased class I PI3Kp110$\alpha$-driven *Il1b* transcription. Macrophage depletion or treatment with anakinra, an IL1 receptor antagonist, reduced ileal inflammation in SHIP$^{-/-}$ mice. Human subjects with CD had decreased SHIP protein levels and activity in ileal biopsies despite increased numbers of SHIP-expressing immune cells in tissues. Subjects with CD also had decreased SHIP activity in their PBMCs, which inversely correlated with the ability of their PBMCs to produce IL1$\beta$.

## Materials and Methods

Descriptions of methods described previously are included in the Supplementary Material.

### Mice

Mice heterozygous for SHIP expression (*Inpp5d*$^{+/-}$) on a mixed C57BL/6×129Sv background (F2 generation) were used to generate SHIP$^{+/+}$ and SHIP$^{-/-}$ littermates for experiments.[31] Mice used for experiments were between 4 and 8 weeks of age. Mice were housed in the Animal Research Centre at the Child & Family Research Institute, which is specific-pathogen free. Experiments were performed in accordance with Canadian Council on Animal Care guidelines (protocol numbers A09-0027 and A09-0032).

### Macrophage Derivation and Isolation

Bone marrow macrophages (BMMs) were derived from BM aspirates of femura and tibiae from SHIP$^{+/+}$ and SHIP$^{-/-}$ mice, as described previously.[28] Ileal macrophages were prepared from lamina propria cells and selected using the mouse monocyte enrichment kit (StemCell Technologies, Vancouver, BC, Canada), as described previously.[33] Macrophage populations were ≥95% F4/80$^{+}$Mac-1$^{+}$.

### Cell Stimulations

Cells were plated at a density of 0.5 × $10^6$ cells/mL and stimulated with 10 ng/mL lipopolysaccharide (LPS) (*Eschericia coli* serotype 127:B8, Sigma-Aldrich, St Louis, MO) for 5 hours, 5 mM adenosine triphosphate (ATP) for 1 hour, or LPS for 5 hours +ATP for the final 1 hour. Cell supernatants were harvested and clarified by centrifugation. Inhibitors were added to cultures 30 minutes before addition of LPS or ATP. Commercially available inhibitors, controls, and final concentrations of each were: glybenclamide (100 $\mu$M; Sigma-Aldrich, St Louis, MO), Z-YVAD-fmk (40 $\mu$M; Sigma-Aldrich), LY303511 (14 $\mu$M; Calbiochem, San Diego, CA), LY294002 (14 $\mu$M; Calbiochem), dimethyl sulfoxide (0.1%), and wortmannin (Wm; 50 nM; Calbiochem). Isoform-specific PI3K inhibitors were synthesized as described previously and used at a concentration of 10 $\mu$M.[30,34]

### Cytokine Measurements

Cytokine measurements were performed on clarified full-thickness tissue homogenates from mice or supernatants using enzyme-linked immunosorbent assays (ELISAs) according to the manufacturer's instructions. ELISA kits for mouse IL1$\alpha$, IL1$\beta$, IL1ra, and IL6, and human IL1$\beta$ were from BD Biosciences (Mississauga, ON, Canada); the IL18 ELISA kit was from MBL International (Woburn, MA).

### Gene Expression Analysis

RNA was prepared from mouse tissue or cells using the NucleoSpin RNA II Total RNA Isolation Kit (Macherey-Nagel, Bethlehem, PA) and reverse transcribed using Superscript II (Invitrogen, Burlington, ON). Gene expression was measured by quantitative polymerase chain reaction using the AB Applied Biosystems Taqman Universal Master Mix II (Life Technologies, Burlington, ON). IL1$\beta$ (*Il1b*) gene expression was normalized to ribosomal protein RPLP0 (*Rplp0*). Primer/probe sequences are in Supplementary Material.

### Macrophage Depletion and Anakinra Treatment

Macrophages were depleted from mouse ilea using clodronate-containing liposomes (clod-lip), as described previously.[35,36]

Anakinra was injected intraperitoneally into mice daily at a dose of 150 mg/kg. Mice were treated either prophylactically