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Altered Macrophage Function Contributes to Colitis in Mice Defective in the Phosphoinositide-3 Kinase Subunit p110δ

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BACKGROUND & AIMS: Innate immune responses are crucial for host defense against pathogens but need to be tightly regulated to prevent chronic inflammation. Initial characterization of mice with a targeted inactivating mutation in the p110δ subunit of phosphoinositide 3-kinase (PI3K p110$\delta^{D910A/D910A}$) revealed defects in B- and T-cell signaling and chronic colitis. Here, we further characterize features of inflammatory bowel diseases in these mice and investigate underlying innate immune defects. **METHODS:** Colons and macrophages from PI3K p110$\delta^{D910A/D910A}$ mice were evaluated for colonic inflammation and innate immune dysfunction. Colonic p110δ messenger RNA expression was examined in interleukin (IL)-$10^{-/-}$ and wild-type germ-free mice during transition to a conventional microbiota. To assess polygenic impact on development of colitis, p110$\delta^{D910A/D910A}$ mice were backcrossed to IL-$10^{-/-}$ mice. **RESULTS:** A mild spontaneous colitis was shown in PI3K p110$\delta^{D910A/D910A}$ mice at 8 weeks, with inflammation increasing with age. An inflammatory mucosal and systemic cytokine profile was characterized by expression of IL-12/23. In PI3K p110$\delta^{D910A/D910A}$ macrophages, augmented toll-like receptor signaling and defective bactericidal activity were observed. Consistent with an important homeostatic role for PI3K p110δ, wild-type mice raised in a germ-free environment markedly upregulated colonic PI3K p110δ expression with the introduction of the enteric microbiota; however, colitis-prone IL-$10^{-/-}$ mice did not. Moreover, PI3K p110$\delta^{D910A/D910A}$ mice crossed to IL-$10^{-/-}$ mice developed severe colitis at an early age. **CONCLUSIONS: This study describes a novel model of experimental colitis that highlights the importance of PI3K p110δ in maintaining mucosal homeostasis and could provide insight into the pathogenesis of human inflammatory bowel disease.**

Keywords: Inflammatory Bowel Diseases; Innate Immunity; PI3-Kinase; Enteric Microbiota.

BASIC–ALIMENTARY TRACT

The pathogenesis of the human inflammatory bowel diseases (IBDs) Crohn disease and ulcerative colitis is complex, with abnormal immune responses in genetically susceptible individuals eliciting uncontrolled intestinal inflammation.[1] Genetic variants that confer susceptibility to Crohn disease highlight the importance of innate immune interactions with the enteric microbiota in controlling inflammation.[1] Commensal and pathogenic bacteria are recognized through conserved molecular microbial patterns by pattern recognition receptors, of which toll-like receptors (TLRs) form integral components.[2] Signaling through TLRs leads to the activation of nuclear factor κβ, culminating in the induction of inflammatory cytokines including interleukin (IL)-12/23 and tumor necrosis factor (TNF). This inflammatory response is essential for the eradication of infectious microorganisms; however, excessive and prolonged activation can be detrimental to the host. Although mechanisms by which the host distinguishes commensal from pathogenic bacteria are not well defined, under normal conditions TLR signaling initiated by the enteric microbiota is protective.[3]

Phosphoinositide-3 kinases (PI3Ks) have emerged as important regulators of TLR signaling.[4,5] Class I_A PI3Ks are a family of heterodimeric enzymes consisting of a regulatory subunit (p85, p55, or p50) and a catalytic subunit (p110α, p110β, p110δ).[6] Whereas p110α and p110β are expressed ubiquitously, the p110δ isoform is highly expressed in leukocytes.[7] The clearest role of PI3K in chronic inflammation is described in a mouse harboring a point mutation in the p110δ catalytic subunit of PI3K (p110$\delta^{D910A/D910A}$).[8] These mice demonstrate B- and

Abbreviations used in this paper: BMM, bone marrow–derived macrophage; ELISA, enzyme-linked immunosorbent assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GF, germ-free; hpf, high-power field; IFN, interferon; IL, interleukin; LPMC, lamina propria mononuclear cell; LPS, lipopolysaccharide; MAP, mitogen-activated protein; PCR, polymerase chain reaction; PI3K, phosphoinositide 3-kinase; SPF, specific pathogen-free; sBLP, synthetic bacterial lipoprotein; TLR, toll-like receptor; TNF, tumor necrosis factor; WT, wild-type.

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T-cell defects including improper maturation, defective antigen receptor signaling, and impaired humoral immune responses. Notably, these mice spontaneously develop chronic segmental colonic inflammation.

However, effects of the PI3K p110δ subunit on innate immune responses in mucosal inflammation remain uncharacterized. In this study, we further describe the development of chronic IBD in PI3K p110$\delta^{D910A/D910A}$ mice and investigate the role of PI3K p110δ in the regulation of TLR signaling and bactericidal pathways in macrophages.

Materials and Methods

Mice

PI3K p110$\delta^{D910A/D910A}$ mice were on the C57BL/6 background. C57BL/6 wild-type (WT) and IL-$10^{-/-}$ mice were obtained from Jackson Laboratories (Bar Harbor, ME). Mice were housed in specific pathogen-free (SPF) conditions in accordance with guidelines from the American Association for Laboratory Animal Care and Research. Germ-free (GF) 8-week-old 129 Sv/Ev WT and IL-$10^{-/-}$ mice were provided by the University of North Carolina Gnotobiotic Facility. Mice were colonized with SPF enteric microbiota at 8 weeks of age with a microbiota isolated from WT mice raised in SPF conditions.[9] PI3K p110$\delta^{D910A/D910A}$ and IL-$10^{-/-}$ homozygous mice were crossed and offspring were genotyped for PI3K p110$\delta^{D910A/D910A}$ and IL-$10^{-/-}$ mutations. For F2 breeding, mice homozygous for one mutation and heterozygous for the other mutation were bred and mice homozygous for both PI3K p110$\delta^{D910A/D910A}$ and IL-$10^{-/-}$ were identified. All experimental mice were genotyped by polymerase chain reaction (PCR) screening before tissue collection and littermates used as controls. The institutional animal care and use committees of the University of Pittsburgh and the University of North Carolina approved all methods used in this study.

Reagents

Flagellin was purchased from Invivogen (San Diego, CA). CpG DNA was obtained from Integrated DNA Technologies (Coralville, IA). Synthetic bacterial lipoprotein (sBLP) was purchased from EMC Microcollections (Tübingen, Germany). Peptidoglycan and lipopolysaccharide (LPS) from *Salmonella enteritidis* were purchased from Sigma (St Louis, MO). LPS was repurified by modified phenol extraction as previously described.[10] Granulocyte-macrophage colony–stimulating factor and macrophage colony-stimulating factor were obtained from Peptotech Inc (Rocky Hill, NJ), and interferon (IFN)-γ was purchased from R&D Systems (Minneapolis, MN).

Cell Isolation

Splenocytes and bone marrow–derived macrophages (BMMs) were cultured as described.[11] Lamina propria mononuclear cells (LPMCs) were isolated from mouse colons by an enzymatic method as previously described.[12] LPMCs were separated into $CD11b^+$ cells using anti-CD11b microbeads (Miltenyi Biotec, Auburn, CA).

Enzyme-Linked Immunosorbent Assays

Murine IL-12 p40, IL-12 p70, IL-10, IFN-γ, and TNF immunoassay kits (R&D Systems) and IL-23 (eBioscience, San Diego, CA) were used according to manufacturers' instructions. IL-17, MIP1a, RANTES, KC, and granulocyte colony–stimulating factor levels were determined by multiplex enzyme-linked immunosorbent assay (ELISA; Luminex, Austin, TX). Phosphorylation levels of pAKT were determined using a cell-based ELISA (Superarray Bioscience Corp, Frederick, MD).

Western Blot

Western blot analyses were performed on whole cell extracts as described.[11] Antibodies to phospho (p)-JNK, p-p38, p-ERK, JNK2, p38, ERK, nuclear factor κB p65, and PI3K p110δ were obtained from Santa Cruz Biotechnology (Santa Cruz, CA), and p-p65 was obtained from Cell Signaling (Danvers, MA).

Nitrite Determination

Nitrite was assayed by a standard Greiss reaction adapted to a microplate system.

Real-Time Reverse-Transcription PCR Analysis

Quantitative real-time reverse-transcription PCR was performed as described.[13] Primer sequences are available on request.

Colonic Tissue Explant Cultures and Histology

Colonic explant cultures were performed as described previously.[13] Slides were prepared for H&E staining and histologic analysis was performed by a pathologist blinded to the study groups (A.R.S.) using established criteria for IL-$10^{-/-}$ mice (and PI3K p110$\delta^{D910A/D910A}$/IL-$10^{-/-}$ mice).[13]

Histologic Scoring of PI3K p110$\delta^{D910A/D910A}$ Mice

A histologic scoring system was developed to assess colonic inflammation based on the characteristics of this model. The criteria used to classify histology into grades 0 to 4 were as follows. Grade 0 was defined as (1) presence of ≤1 mitosis in the colonic crypts per 10 high-power fields (hpfs), (2) no epithelial hyperplasia, and (3) no neutrophils in the lamina propria. Grade 1 was established if fewer than 2 of the following criteria were found: (1) presence of epithelial hyperplasia, (2) presence of more than 2 mitoses/10 hpf in the colonic crypts, (3) any apoptotic body in the colonic crypts, (4)