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CELLULAR AND MOLECULAR GASTROENTEROLOGY AND HEPATOLOGY

ORIGINAL RESEARCH

Neonatal Colonic Inflammation Epigenetically Aggravates Epithelial Inflammatory Responses to Injury in Adult Life

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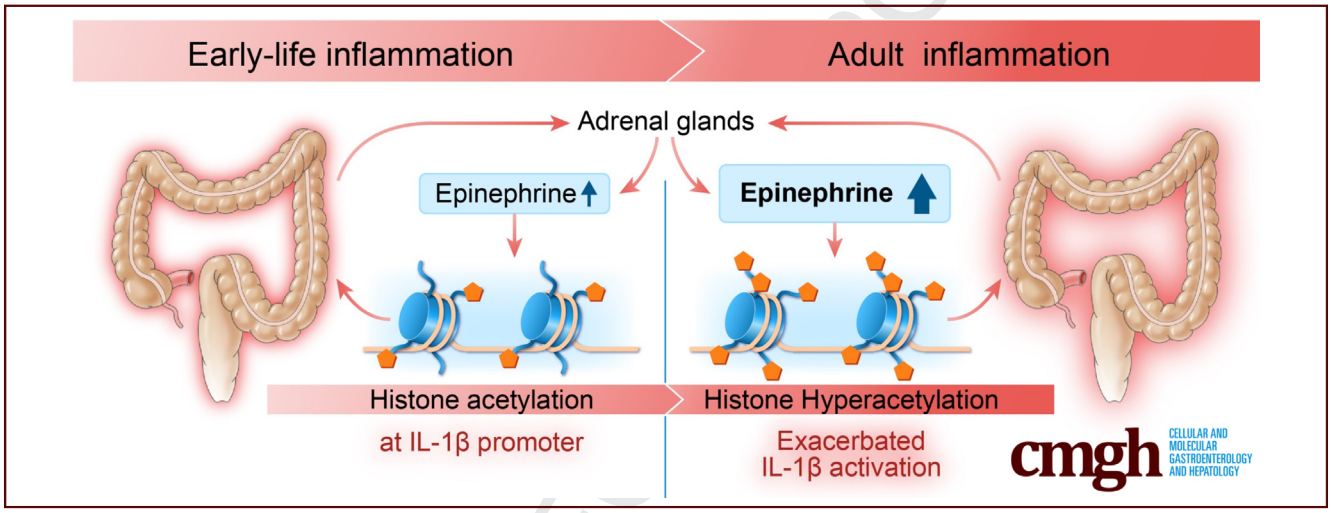

SUMMARY

This investigation showed that neonatal inflammation increases susceptibility to inflammatory bowel disease by epigenetically sensitizing the interleukin 1β promoter for exacerbated overexpression when exposed to another episode of inflammation later in life. Propranolol might mitigate against the inflammatory bowel disease susceptibility by reversing epigenetic modifications.

BACKGROUND & AIMS: Early life adversity is considered a risk factor for the development of gastrointestinal diseases, including inflammatory bowel disease. We hypothesized that early life colonic inflammation causes susceptibility to aggravated overexpression of interleukin (IL)1β.

METHODS: We developed a 2-hit rat model in which neonatal inflammation (NI) and adult inflammation (AI) were induced by trinitrobenzene sulfonic acid.

RESULTS: Aggravated immune responses were observed in NI + AI rats, including a sustained up-regulation of IL1β and Q6 other cytokines. In parallel with exacerbated loss of IκBα expression, NI + AI rats showed hyperacetylation of histone H4K12 and increased RelA binding on the *IL1B* promoter, accompanied by high levels of norepinephrine/epinephrine. Propranolol, a β-blocker, markedly ameliorated the inflammatory response and IL1β overexpression by mitigating against epigenetic modifications. Adrenalectomy abrogated NI-induced disease susceptibility whereas yohimbine sensitized the epithelium for exacerbated immune response. The macrophages of NI rats produced more IL1β than controls after exposure to lipopolysaccharide (LPS), suggesting hypersensitization; incubation with LPS plus Foradil, a β2-agonist, induced Q7 a greater IL1β expression than LPS alone. Epinephrine and Foradil also exacerbated LPS-induced IL1β activation in human THP-1–derived macrophages, by increasing acetylated H4K12, Q8 and these increases were abrogated by propranolol.

CONCLUSIONS: NI sensitizes the colon epithelium for exacerbated IL1β activation by increasing stress hormones that induce histone hyperacetylation, allowing greater access of nuclear factor-κB to the *IL1B* promoter and rendering the host susceptible to aggravated immune responses. Our findings suggest that β blockers have a therapeutic potential for inflammatory bowel disease susceptibility and establish a novel paradigm whereby NI induces epigenetic susceptibility to inflammatory bowel disease. *(Cell Mol Gastroenterol Hepatol 2018;■:■–■; https://doi.org/10.1016/j.jcmgh.2018.02.014)*

Keywords: Early-Life Adversity; Inflammatory Bowel Disease; Epinephrine; Histone Acetylation; NF-κB. Q9

Inflammatory bowel disease (IBD) is a chronic, progressive, relapsing, and immunologically mediated disorder that often targets the young and remains a lifelong affliction. Epidemiologic studies have suggested that the incidence of IBD is increasing worldwide.[1–3] Current models of human IBD posit that the inflammatory pathogenesis arises from, and is perpetuated by, interactions between host genetic and immune factors, gastrointestinal microbes, and environmental triggers.[4] Accumulating clinical evidence has shown that early life infection is a risk factor for the development of pediatric and adult IBD,[5–12] and gastrointestinal infection in adolescents and adults is a trigger for its onset or exacerbation.[13–17] However, it is unclear how these events cause an aggravated and prolonged immune response, which is the hallmark of IBD.

Early postnatal life is a uniquely vulnerable period, characterized by "epigenetic plasticity," in which neonates are susceptible to environmental influences that induce durable-epigenetic changes that persist in the adult.[18,19] It now is well recognized that adverse early life events have an important role in perinatal programming and maturation of the immune system that make the host susceptible to complex diseases,[20–24] including IBD.[8,10,18] However, the molecular mechanisms by which adverse early life experiences predispose to IBD remain unknown.

A variety of cytokines, including interleukin (IL)1, have been implicated in the pathogenesis of IBD.[25] The IL1 family of cytokines comprises 11 proteins (IL1F1–IL1F11) encoded by 11 distinct genes in human beings and mice. IL1-type cytokines are major mediators of innate immune reactions, and blockade of IL1 by the IL1 receptor antagonist has shown an essential role of IL1 in a number of human autoinflammatory diseases.[26] IL1β, a proinflammatory cytokine with a wide range of systemic and local effects, has received considerable attention as a potential mediator of inflammatory cell infiltration and mucosal barrier disruption that accompanies gut inflammation.[27] It can modulate the function of both immune and nonimmune cells. IL1β also appears to promote inflammation by stimulating the production of other cytokines (eg, IL6) and chemokines (eg, CXCL1, CXCL8, IL8).[28–30] Stimulation with IL1β promotes the activation and effector functions of dendritic cells, macrophages, and neutrophils.[31] It also induces neutrophilia and promotes neutrophil migration.[32] IL1β promotes T-cell activation and survival,[33] and acts in concert with other proinflammatory cytokines to promote the differentiation of CD4+ Th17 cells.[34–37] Because of the potent inflammatory activity of IL1β, tight mechanisms are in place to regulate its secretion. However, our understanding of IL1β activation in the pathogenesis of IBD is limited, and it is unclear whether early life adversity, such as neonatal colonic inflammation, aggravates IL1β overexpression to exacerbate immune responses when subjected to a second inflammatory insult later in life.

The present investigation sought to test the hypothesis that neonatal colonic inflammation epigenetically aggravates IL1β activation in rat colon epithelium when the host is exposed to a second episode of inflammation as an adult. Our findings provide compelling evidence that neonatal colonic inflammation triggers aberrant increases in norepinephrine and epinephrine to enhance histone acetylation at the *IL1B* gene promoter. Notably, the altered chromatin status persists, facilitating nuclear factor-κB (NF-κB) recruitment and IL1β overexpression when subjected to an additional insult in adult life.

Materials and Methods

Reagents

Propranolol hydrochloride was purchased from Tocris Bioscience (Bristol, UK). Epinephrine, norepinephrine, lipopolysaccharide (LPS, from Escherichia coli O111:B4, cat. L4391), formoterol fumarate dihydrate (Foradil), phorbol 12-myristate 13-acetate (PMA), sodium butyrate, yohimbine, and 2,4,6-trinitrobenzene sulfonic acid (TNBS) were from Sigma (St. Louis, MO).

Cell Culture

THP-1 cells were purchased from ATCC (Manassas, VA) and maintained in RPMI 1640 medium with 2 mmol/L L-glutamine, 10% fetal bovine serum, and 0.05 mmol/L 2-mercaptoethanol. To induce differentiation, THP-1 cells seeded at 2×10^5 cells/mL were incubated with 100 nmol/L PMA for 3 days.

Animals and Procedures

Male Sprague Dawley rat littermates were used in the preclinical studies. Five-day-old and 6-week-old Sprague Dawley rats were purchased from Harlan Laboratories (Houston, TX). The work was approved by the Institutional Animal Care and Use Committee at The University of Texas Medical Branch at Galveston.

Rat littermates were divided randomly into 4 groups: (1) vehicle treatment in both neonatal and adult-life stages (controls, Ctl); (2) sham treatment as neonates followed by an inflammatory insult as adults (adult inflammation [AI]) (Ctl + AI); (3) neonatal inflammatory insult (neonatal inflammation [NI]) and then sham treatment as adults; and (4) NI plus AI in combination (NI + AI) (Figure 1*A*). Experimenters were blinded to treatment assignment. To induce neonatal inflammation, TNBS (130 mg/kg, 2.86 mg for a 22-g pup, dissolved in 200 μL saline containing 10% ethanol) was injected intraluminally 2 cm into the colon of

Abbreviations used in this paper: **AI, adult inflammation; ChIP, chromatin immunoprecipitation; Ctl, control; H4K12ac, _____; HDAC, histone deacetylase; IBD, inflammatory bowel disease; IκB, _____; IL, interleukin; LPS, lipopolysaccharide; MPO, myeloperoxidase; mRNA, messenger RNA; NF-κB, nuclear factor-κB; NI, neonatal inflammation; PCR, polymerase chain reaction; PMA, phorbol 12-myristate 13-acetate; RelA, _____; RNAP II, RNA polymerase II; THP-1, _____; TNBS, 2,4,6-trinitrobenzene sulfonic acid; Tnf, tumor necrosis factor.**

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