Epigenetics and Inflammatory Biomarkers in Necrotizing Enterocolitis

Subjects: Gastroenterology & Hepatology

Contributor: Naser A. Alsharairi

Necrotizing enterocolitis (NEC) is a severe inflammatory necrosis of the distal small intestine/colon that primarily affects preterm or very low birth weight infants after the introduction of enteral feeds. Epigenetic alternation in the immature intestine, such as changes in DNA methylation and long non-coding RNA (lncRNA) patterns, may contribute to increased risk of NEC.

necrotizing enterocolitis | premature infant | short-chain fatty acids | metabolites | gut microbiota

1. Introduction

Necrotizing enterocolitis (NEC) is a severe inflammatory necrosis of the distal small intestine/colon that primarily affects preterm (less than 32 weeks' gestation) or very low birth weight (VLBW: <1500 g) infants after the introduction of enteral feeds. NEC is characterized by hyperosmolar injury and intestinal ischemia, which reduce the integrity of the epithelial barrier that is evident by peritonitis, hemodynamic instability, abdominal tenderness/cellulitis, acute feeding intolerance, bacteremia and abdominal distension [1][2]. The aetiology of NEC is not clear, but it is thought to be related to several factors, including pre-eclampsia, aberrant bacterial colonization (e.g., infection), premature rupture of the membranes, placental abruption, intrauterine growth restriction, LBW, patent ductus arteriosus, sepsis and anemia [3]. Early gut microbial dysbiosis is also implicated in disease pathogenesis, in which the gut microbiota composition of preterm infants with NEC is characterized by reduced abundances of *Bifidobacterium*, *Firmicutes* and *Bacteroidetes* and increased abundances of *Prevotella*, *Clostridioides*, *Staphylococcaceae*, *Proteobacteria*, *Enterobacteriaceae*, *Rothia*, *Streptococcus* and *Blautia* [4][5][6][7][8][9][10].

Pregnancy and lactation perform a crucial role in shaping the composition of infant gut microbiota, which is influenced by a range of pre-and post-natal factors, such as antibiotic exposure, lactation stage, gestational age, mode of feeding/delivery, diet and body mass index (BMI) [7][11][12]. Maternal diet during pregnancy and lactation has been linked to an increased risk of developing obesity and asthma in the infant's early years of life [13][14] and the mechanisms underlying such effects are postulated to be the alterations in maternal/infant gut microbiota and/or milk microbiota [12]. A high-fiber diet during pregnancy and lactation increases SCFAs production [12][13][14][15][16][17]. SCFAs are essential for differentiation of helper T cells (Th1, Th2) by their binding to G-protein coupled receptors (GPCRs), including free the fatty acid 2/3 receptor (FFAR2, FFAR3) present in the colon, thereby

maintaining gut homeostasis and regulating inflammation by reducing the expression of pro-inflammatory cytokines [15][16]. Higher levels of SCFAs detected in breastmilk may enhance the neonatal anti-inflammatory immune responses by inducing factor fork head box protein 3 ($FOXP3^+$) regulatory T (T_{reg}) cell differentiation in the gut [18]. Breastmilk is a source of secretory IgA immunoglobulin A (SIgA) and IgA-producing antibody-secreting cells (ASCs), which regulate early gut microbiota maturation and immunity by binding to SCFA-producing *Bifidobacterium* and *Lactobacillus*, resulting in reduced NEC-related inflammation in preterm infants [19]. It has been suggested that infant feeding with probiotics-supplemented formulas and solid/complementary foods alter gut microbiota composition during the first years of life [20][21][22][23]. Evidence from randomised controlled trials (RCTs) has shown that NEC-specific treatments, such as oral lactoferrin combined with probiotics and parenteral/oral supplementation with arginine, reduce the disease risk [3][24][25][26][27]. Probiotic supplementation with *Bifidobacterium* and *Lactobacillus* strains, prebiotics (e.g., HMOs), synbiotics (mixtures of probiotics and prebiotics), long chain polyunsaturated fatty acid (PUFA) and bovine colostrum were also demonstrated by a large number of human RCTs, to be effective preventive strategies for NEC, which are thought to modulate the immune response and increase the abundance of beneficial gut microbes [28][29][30][31]. Breastfeeding has been demonstrated to have a protective role against NEC due to its potential to promote the colonization of commensal bacteria and decrease the susceptibility to gut dysbiosis in premature infants [32][33].

2. Epigenetics and Inflammatory Biomarkers in NEC

Epigenetic alternation in the immature intestine, such as changes in DNA methylation and long non-coding RNA (lncRNA) patterns, may contribute to increased risk of NEC. Epigenetic changes are attributed to prenatal and postnatal factors (e.g., microbiome, intrauterine infection and enteral feeding) that may affect intestinal function/structure and cause upregulation of pro-inflammatory cytokines [34][35]. DNA methylation changes in cytosine-phosphate-guanine dinucleotides (CpG) regions of NEC-related genes are related to disease risk. For example, high levels of CpG methylation in the *DNMT3A*, *TNT2/3*, *TNIP1*, *GALNT6* and *HNF4* genes have been identified in stools and colons of premature infants with NEC [36][37][38]. An association of CpG methylation in the cytokine Oncostatin M (OSM) with NEC has also been observed, which can induce intestinal inflammation [38]. The hypermethylation of four genes (*MPL*, *KDM6A*, *ZNF335* and *RASAL3*) has been reported in the intestine of neonatal NEC, which is associated with lymphocyte proliferation and intestinal epithelial permeability [39]. Analyses of CpG methylation positions in the intestinal epithelial cells of neonatal NEC revealed a significant hypermethylation in five genes (toll-like receptor 4; TLR4, *ENOS*, *EPO*, *DEFA5* and *VEGFA*) at three sites [40]. Overexpression of micro-431 (miR-431) in the intestinal tissues of neonatal NEC results in significantly inhibited *FOXA1* and *HNF4A* and increased pro-inflammatory (e.g., interleukins IL-6, IL-8, IL-10, *LGR5*, tumor necrosis factor-α; TNF-α and *PRKCZ*) gene expression in response to lipopolysaccharide (LPS) stimulation [41]. lncRNA influences the expression of mRNAs in the intestine tissues of neonatal NEC by upregulating expression levels of IL-6, IL-1β and TLR4 after LPS exposure, which induces activation of peroxisome proliferator-activated receptors (PPARs) and phosphatidylinositol-3 kinase/serine-threonine kinase (PI3K-AkT) signaling pathways, suggesting that lncRNA contributes to NEC pathogenesis [42].

NEC is characterized by decreased FOXP3$^+$ T_{reg} cell levels and gut expression of transforming growth factor β (TGF-β). Infants with NEC displayed elevated levels of nitric oxide (NO) and high cytokine expression levels with pro-inflammatory effects (e.g., Nuclear factor-$_κ$B; NF-$_κ$B, tumor necrosis factor-α; TNF-α, interferon; IFN-γ, IL-6, IL-8, IL-10, IL-1β) induced by LPS and produced by the cells of the adaptive immune system in response to colonization by pathogenic bacteria (e.g., *Staphylococcus* spp., and *Clostridium* spp.), thereby disturbing the integrity of epithelial tight junctions [43][44][45][46][47][48][49]. An experimental study has shown overexpression of TLR2 and TLR4 receptor-mediated IL-8 mRNA expression in the immature intestine of neonatal NEC [50]. Data from a human NEC experiment showed that pro-inflammatory cytokine expression of IL-1β, IL-1A, IL-6, TNF-α and IL-36 isoforms IL36A were increased in epithelial cells, whereas cytokines IL-37 and IL-22, which are considered protective, were decreased [51]. Evidence from an experimental study showed that IL-17F expression and its related pro-inflammatory C-X-C motif chemokines ligand 8 and 10 (CXCL8, CXCL10) are upregulated in the intestine of neonatal NEC [52]. A case–control study demonstrated higher levels of TNF-α, IL-8, IL-1β and lower levels of TGF-β, FOXP3$^+$ T_{reg} and IL-10 in the ileum of surgical NEC patients compared with matched controls (patients with spontaneous intestinal perforation/congenital intestinal atresia) [8]. Another case–control study showed that the levels of serum TNF-α, IL-6 and intestinal fatty acid-binding protein (I-FABP) were higher in NEC patients than non-NEC counterparts [53]. In a recent experimental study, preterm newborns displayed increased mRNA expression of fecal cytokines IL-1α/β, IL-7 and IL-12p40 [10]. This suggests that preterm infants with NEC display intestinal inflammation with markedly increased pro-inflammatory cytokines and chemokines, in which DNA methylation and lncRNA as epigenetic mechanisms are involved.

References

1. Gregory, K.E.; Deforge, C.E.; Natale, K.M.; Phillips, M.; Van Marter, L.J. Necrotizing enterocolitis in the premature infant: Neonatal nursing assessment, disease pathogenesis, and clinical presentation. Adv. Neonatal Care 2011, 11, 155–164.
2. Patel, B.K.; Shah, J.S. Necrotizing enterocolitis in very low birth weight infants: A systemic review. ISRN Gastroenterol. 2012, 2012, 562594.
3. Rose, A.T.; Patel, R.M. A critical analysis of risk factors for NEC. Semin. Fetal Neonatal Med. 2018, 23, 374–379.
4. Torrazza, R.M.; Ukhanova, M.; Wang, X.; Sharma, R.; Hudak, M.L.; Neu, J.; Mai, V. Intestinal microbial ecology and environmental factors affecting necrotizing enterocolitis. PLoS ONE 2013, 8, e83304.
5. Unger, S.; Stintzi, A.; Shah, P.; Mack, D.; O'Connor, D.L. Gut microbiota of the very-low-birth-weight infant. Pediatr. Res. 2015, 77, 205–213.
6. Pammi, M.; Cope, J.; Tarr, P.I.; Warner, B.B.; Morrow, A.L.; Mai, V.; Gregory, K.E.; Kroll, J.S.; McMurtry, V.; Ferris, M.J.; et al. Intestinal dysbiosis in preterm infants preceding necrotizing

enterocolitis: A systematic review and meta-analysis. Microbiome 2017, 5, 31.

7. Underwood, M.A.; Mukhopadhyay, S.; Lakshminrusimha, S.; Bevins, C.L. Neonatal intestinal dysbiosis. J. Perinatol. 2020, 40, 1597–1608.
8. He, Y.; Du, W.; Xiao, S.; Zeng, B.; She, X.; Liu, D.; Du, H.; Li, L.; Li, F.; Ai, Q.; et al. Colonization of fecal microbiota from patients with neonatal necrotizing enterocolitis exacerbates intestinal injury in germfree mice subjected to necrotizing enterocolitis-induction protocol via alterations in butyrate and regulatory T cells. J. Transl. Med. 2021, 19, 510.
9. Liu, X.-C.; Du, T.-T.; Gao, X.; Zhao, W.-J.; Wang, Z.-L.; He, Y.; Bao, L.; Li, L.-Q. Gut microbiota and short-chain fatty acids may be new biomarkers for predicting neonatal necrotizing enterocolitis: A pilot study. Front. Microbiol. 2022, 13, 969656.
10. Lemme-Dumit, J.M.; Song, Y.; Lwin, H.W.; Hernandez-Chavez, C.; Sundararajan, S.; Viscardi, R.M.; Ravel, J.; Pasetti, M.F.; Ma, B. Altered gut microbiome and fecal immune phenotype in early preterm infants with leaky gut. Front. Immunol. 2022, 13, 815046.
11. Yao, Y.; Cai, X.; Ye, Y.; Wang, F.; Chen, F.; Zheng, C. The role of microbiota in infant health: From early life to adulthood. Front. Immunol. 2021, 12, 708472.
12. Alsharairi, N.A. The infant gut microbiota and risk of asthma: The effect of maternal nutrition during pregnancy and lactation. Microorganisms 2020, 8, 1119.
13. Alsharairi, N.A. The role of short-chain fatty acids in mediating very low-calorie ketogenic diet-infant gut microbiota relationships and its therapeutic potential in obesity. Nutrients 2021, 13, 3702.
14. Alsharairi, N.A. The role of short-chain fatty acids in the interplay between a very low-calorie ketogenic diet and the infant gut microbiota and its therapeutic implications for reducing asthma. Int. J. Mol. Sci. 2020, 21, 9580.
15. Zheng, N.; Gao, Y.; Zhu, W.; Meng, D.; Walker, W.A. Short chain fatty acids produced by colonizing intestinal commensal bacterial interaction with expressed breast milk are anti-inflammatory in human immature enterocytes. PLoS ONE 2020, 15, e0229283.
16. Ziętek, M.; Celewicz, Z.; Szczuko, M. Short-chain fatty acids, maternal microbiota and metabolism in pregnancy. Nutrients 2021, 13, 1244.
17. Łoniewski, I.; Skonieczna-Żydecka, K.; Stachowska, L.; Fraszczyk-Tousty, M.; Tousty, P.; Łoniewska, B. Breastfeeding affects concentration of faecal short chain fatty acids during the first year of life: Results of the systematic review and meta-analysis. Front. Nutr. 2022, 9, 939194.
18. Chun, J.; Toldi, G. The impact of short-chain fatty acids on neonatal regulatory T cells. Nutrients 2022, 14, 3670.

19. Guo, J.; Ren, C.; Han, X.; Huang, W.; You, Y.; Zhan, J. Role of IgA in the early-life establishment of the gut microbiota and immunity: Implications for constructing a healthy start. Gut Microbes. 2021, 13, 1908101.
20. Di Profio, E.; Magenes, V.C.; Fiore, G.; Agostinelli, M.; La Mendola, A.; Acunzo, M.; Francavilla, R.; Indrio, F.; Bosetti, A.; D'Auria, N.; et al. Special diets in infants and children and impact on gut microbioma. Nutrients 2022, 14, 3198.
21. McKeen, S.; Young, W.; Mullaney, J.; Fraser, K.; McNabb, W.C.; Roy, N.C. Infant complementary feeding of prebiotics for the microbiome and immunity. Nutrients 2019, 11, 364.
22. Differding, M.K.; Benjamin-Neelon, S.E.; Hoyo, C.; Østbye, T.; Mueller, N.T. Timing of complementary feeding is associated with gut microbiota diversity and composition and short chain fatty acid concentrations over the first year of life. BMC Microbiol. 2020, 20, 56.
23. Fabiano, V.; Indrio, F.; Verduci, E.; Calcaterra, V.; Pop, T.L.; Mari, A.; Zuccotti, G.V.; Cokugras, F.C.; Pettoello-Mantovani, M.; Goulet, O. Term infant formulas influencing gut microbiota: An overview. Nutrients 2021, 13, 4200.
24. Mitchell, K.; Lyttle, A.; Amin, H.; Shaireen, H.; Robertson, H.L.; Lodha, A.K. Arginine supplementation in prevention of necrotizing enterocolitis in the premature infant: An updated systematic review. BMC Pediatr. 2014, 14, 226.
25. Shah, P.S.; Shah, V.S.; Kelly, L.E. Arginine supplementation for prevention of necrotising enterocolitis in preterm infants. Cochrane Database Syst. Rev. 2017, 4, CD004339.
26. Pammi, M.; Suresh, G. Enteral lactoferrin supplementation for prevention of sepsis and necrotizing enterocolitis in preterm infants. Cochrane Database Syst. Rev. 2020, 3, CD007137.
27. Al-Alaiyan, S.; Abdulaziz, N.; Alkohlani, A.; Almairi, S.O.; Al Hazzani, F.; Binmanee, A.; Alfattani, A. Effects of probiotics and lactoferrin on necrotizing enterocolitis in preterm infants. Cureus 2021, 13, e18256.
28. Zhou, P.; Li, Y.; Ma, L.-Y.; Lin, H.-C. The role of immunonutrients in the prevention of necrotizing enterocolitis in preterm very low birth weight infants. Nutrients 2015, 7, 7256–7270.
29. Johnson-Henry, K.C.; Abrahamsson, T.R.; Wu, R.Y.; Sherman, P.M. Probiotics, prebiotics, and synbiotics for the prevention of necrotizing enterocolitis. Adv. Nutr. 2016, 7, 928–937.
30. Nolan, L.S.; Rimer, J.M.; Good, M. The role of human milk oligosaccharides and probiotics on the neonatal microbiome and risk of necrotizing enterocolitis: A narrative review. Nutrients 2020, 12, 3052.
31. Campos-Martinez, A.M.; Expósito-Herrera, J.; Gonzalez-Bolívar, M.; Fernández-Marin, E.; Uberos, J. Evaluation of risk and preventive factors for necrotizing enterocolitis in premature newborns. A systematic review of the literature. Front. Pediatr. 2022, 10, 874976.

32. Xu, W.; Judge, M.P.; Maas, K.; Hussain, N.; McGrath, J.M.; Henderson, W.A.; Cong, X. Systematic review of the effect of enteral feeding on gut microbiota in preterm infants. J. Obstet. Gynecol. Neonatal Nurs. 2018, 47, 451–463.
33. Davis, J.A.; Baumgartel, K.; Morowitz, M.J.; Giangrasso, V.; Demirci, J.R. The role of human milk in decreasing necrotizing enterocolitis through modulation of the infant gut microbiome: A scoping review. J. Hum. Lact. 2020, 36, 647–656.
34. Lu, L.; Claud, E.C. Intrauterine inflammation, epigenetics, and microbiome influences on preterm infant health. Curr. Pathobiol. Rep. 2018, 6, 15–21.
35. Nair, J.; Maheshwari, A. Epigenetics in necrotizing enterocolitis. Curr. Pediatr. Rev. 2021, 17, 172–184.
36. Good, M.; Chu, T.; Shaw, P.; McClain, L.; Chamberlain, A.; Castro, C.; Rimer, J.M.; Mihi, B.; Gong, Q.; Nolan, L.S.; et al. Global hypermethylation of intestinal epithelial cells is a hallmark feature of neonatal surgical necrotizing enterocolitis. Clin. Epigenet. 2020, 12, 190.
37. Good, M.; Chu, T.; Shaw, P.; Nolan, L.S.; McClain, L.; Chamberlain, A.; Castro, C.; Gong, Q.; Cooksey, K.; Linneman, L.; et al. Neonatal necrotizing enterocolitis-associated DNA methylation signatures in the colon are evident in stool samples of affected individuals. Epigenomics 2021, 13, 829–844.
38. Good, M.; Chu, T.; Shaw, P.; Nolan, L.S.; Wrobleski, J.; Castro, C.; Gong, Q.; DeWitt, O.; Finegold, D.N.; Peters, D. Selective hypermethylation is evident in small intestine samples from infants with necrotizing enterocolitis. Clin. Epigenet. 2022, 14, 49.
39. Lu, L.; Fan, J.; Xu, W.; Cui, X.; Hu, S.; Guo, T.; Lv, Z. DNA methylome mapping identifies epigenetic abnormalities in intestinal lymphocyte regulation in human necrotizing enterocolitis. Dig. Dis. Sci. 2022, 67, 4434–4443.
40. Klerk, D.H.; Plösch, T.; Verkaik-Schakel, R.N.; Hulscher, J.B.F.; Kooi, E.M.W.; Bos, A.F. DNA methylation of TLR4, VEGFA, and DEFA5 is associated with necrotizing enterocolitis in preterm infants. Front. Pediatr. 2021, 9, 630817.
41. Wu, Y.Z.; Chan, K.Y.Y.; Leung, K.T.; Lam, H.S.; Tam, Y.H.; Lee, K.H.; Li, K.; Ng, P.C. Dysregulation of miR-431 and target gene FOXA1 in intestinal tissues of infants with necrotizing enterocolitis. FASEB J. 2019, 33, 5143–5152.
42. Chen, W.; Yan, X.; Tian, T.; Yan, R.; Wang, X.; Yu, Z.; Li, Y.; Zhang, L.; Han, S. Integrated analysis of a lncRNA-mRNA network reveals a potential mechanism underlying necrotizing enterocolitis. Mol. Med. Rep. 2020, 22, 423–435.
43. Hunter, C.J.; Upperman, J.S.; Ford, H.R.; Camerini, V. Understanding the susceptibility of the premature infant to necrotizing enterocolitis (NEC). Pediatr. Res. 2008, 63, 117–123.

44. Frost, B.L.; Jilling, T.; Caplan, M.S. The importance of pro-inflammatory signaling in neonatal NEC. Semin. Perinatol. 2008, 32, 100–106.
45. De Plaen, I.G. Inflammatory signaling in Necrotizing enterocolitis. Clin. Perinatol. 2013, 40, 109–124.
46. Hunter, C.J.; De Plaen, I.G. Inflammatory signaling in NEC. Role of NFKB and cytokines. Pathophysiology 2014, 21, 55–65.
47. Nino, D.F.; Sodhi, C.P.; Hackam, D.J. Necrotizing enterocolitis: New insights into pathogenesis and mechanisms. Nat. Rev. Gastroenterol. Hepatol. 2016, 13, 590–600.
48. Mohankumar, K.; Namachivayam, K.; Ho, T.T.; Torres, B.A.; Ohls, R.K.; Maheshwari, A. Cytokines and growth factors in the developing intestine and during necrotizing enterocolitis. Semin. Perinatol. 2017, 41, 52–60.
49. Niemarkt, H.J.; De Meij, T.G.; van Ganzewinkel, C.-J.; de Boer, N.K.H.; Andriessen, P.; Hütten, M.C.; Kramer, B.W. Necrotizing enterocolitis, gut microbiota, and brain development: Role of the brain-gut axis. Neonatology 2019, 115, 423–431.
50. Nanthakumar, N.; Meng, D.; Goldstein, A.M.; Zhu, W.; Lu, L.; Uauy, R.; Llanos, A.; Claud, E.C.; and Walker, W.A. The mechanism of excessive intestinal inflammation in necrotizing enterocolitis: An immature innate immune response. PLoS ONE 2011, 6, e17776.
51. Cho, S.X.; Rudloff, I.; Lao, J.C.; Pang, M.A.; Goldberg, R.; Bui, C.B.; McLean, C.A.; Stock, M.; Klassert, T.E.; Slevogt, H.; et al. Characterization of the pathoimmunology of necrotizing enterocolitis reveals novel therapeutic opportunities. Nat. Commun. 2020, 11, 5794.
52. Tremblay, E.; Ferretti, E.; Babakissa, C.; Burghardt, K.M.; Levy, E.; Beaulieu, J.-F. IL-17-related signature genes linked to human necrotizing enterocolitis. BMC Res. Notes 2021, 14, 82.
53. Li, Z.; Sheng, L. Significance of dynamic evolution of TNF-α, IL-6 and intestinal fatty acid-binding protein levels in neonatal necrotizing enterocolitis. Exp. Ther. Med. 2018, 15, 1289–1292.