Early Human Development 97 (2016) 33–39

Contents lists available at ScienceDirect

Early Human Development

journal homepage: www.elsevier.com/locate/earlhumdev

Current research in necrotizing enterocolitis

Simon Eaton [a,*], Clare M. Rees [a], Nigel J. Hall [b]

[a] UCL Institute of Child Health and Great Ormond Street Hospital for Children, London, UK
[b] Faculty of Medicine, University of Southampton, Southampton, UK

ARTICLE INFO

Article history:
Received 20 January 2016
Accepted 21 January 2016

Keywords:
necrotizing enterocolitis
intestinal microbiota
premature infants
inflammation
TLR4
surgery

ABSTRACT

Despite decades of research on necrotizing enterocolitis, we still do not fully understand the pathogenesis of the disease, how to prevent or how to treat the disease. However, as a result of recent significant advances in the microbiology, molecular biology, and cell biology of the intestine of premature infants and infants with necrotizing enterocolitis, there is some hope that research into this devastating disease will yield some important translation into improved outcomes.

Contents

1. Introduction

Although necrotizing enterocolitis (NEC) is one of the most common life-threatening surgical diseases affecting neonates, we still do not completely understand the pathogenesis or how to prevent or treat the disease [1]. The high mortality (around 30% for surgical NEC [2,3] and long-term morbidity [4, 5] of survivors mandates urgent research into the pathogenesis, diagnosis, prevention and treatment of this devastating disease. There are several animal models of NEC, however, it is not completely clear how accurately these models recapitulate the human disease. This is particularly difficult as clinical NEC itself is quite variable, with some authors arguing for sub-classification into different forms reflecting the differences in disease onset and progression between a 'typical' NEC presentation in an extremely premature infant

* Corresponding author at: Department of Paediatric Surgery, UCL Institute of Child Health, 30 Guilford Street, London, WC1N 1EH, UK. Tel.: +44 020 7905 2158; fax: +44 020 7404 6181.
E-mail address: s.eaton@ucl.ac.uk (S. Eaton).

http://dx.doi.org/10.1016/j.earlhumdev.2016.01.013
0378-3782/

who has been fed enterally for a few weeks, and other presentations such as NEC in a term infant who has a cardiac defect or who has had gastroschisis [6]. As a consequence of the limitations of the animal models, it is vital to undertake clinical research studies in parallel with basic science/animal model studies, and in this brief review article, our aim is to describe some areas of current research interest; an exhaustive review of all current NEC research is unfeasible.

2. Pathogenesis

The main factors thought to be involved in the pathogenesis of NEC are: intestinal immaturity, enteral feeds, the intestinal microbiome, inflammation and local ischaemia and/or reperfusion injury. We will briefly discuss recent research in each of these areas below.

2.1. Intestinal immaturity

The fetal gut develops in an environment where exposure to microbes is limited. Therefore, premature infants are exposed to a much greater diversity and quantity of bacteria, viruses and fungi. The premature infant gut displays an excessive inflammatory response [7], and toll-like receptor 4 seems to play a key role in this inflammatory response ([8] see below). However, a difference in inflammatory response is not the only aspect of the premature infant intestine that might be affected. The neonatal gut also seems to be more susceptible to intestinal ischaemia/reperfusion injury than adult gut [9], and the activity of carbohydrate digestive enzymes is significantly lower in preterm intestine than term intestine, so that in a pig model the incidence and severity of NEC can be modulated by variation in the carbohydrate supplied [10]. Although gut motility appears is to be different in preterm and term infants [11], NEC does not seem to be related to early stooling pattern in premature infants [12]. Another potentially important difference between premature and term infants is that of maternal separation. Partial separation of mouse pups from the mother is enough to induce changes in colonic histology and permeability [13], although the relevance of these observations to NEC is unknown.

2.2. Enteral feeding

Although it has long been known that firstly, NEC predominantly occurs in premature infants that have been enterally fed, and secondly that human breast milk is protective towards NEC, we do not completely understand how the type of feed interacts with other risk factors. Interestingly, the protective effect of breast milk appears to be dose related [14]. A huge array of protective factors present in breast milk has been suggested (summarized [15]) and some of these have been suggested as potential preventative measures or treatments (see below). One mechanism by which feed components could influence intestinal gene expression is epigenetics, with epigenetic changes defined as '*relating to or arising from non-genetic influences on gene expression*'. As epigenetic changes frequently involve methylation, they are potentially influenced by diet. A current area of research interest is the potential epigenetic effects of breast milk and other enteral feeds [16]. Marked epigenetic changes have been observed in the intestine of premature infants [17], and in a pig model, enteral feeding has been linked with epigenetic changes causing upregulation of pro-inflammatory genes [18]. In addition, the type of enteral feed can also interact with other risk factors described below such as the gut microbiome [19] and intestinal blood flow [20].

2.3. Intestinal microbiome

While the precise role of bacterial agents in the development of NEC is unclear, several factors implicate their involvement. Occasionally NEC is observed to occur in clusters, in which a higher than expected number of cases are observed in one centre [21]. Identical organisms are grown from babies within these clusters and the initiation of infection control measures has been shown to control such outbreaks [22]. However, different organisms are grown from separate outbreaks so it cannot be claimed that a single organism is involved in development of NEC. Bacterial involvement in the pathogenesis of NEC is also implicated by association; endotoxaemia [23,24] and positive blood cultures are common in infants with NEC and the gastrointestinal pneumatosis found in NEC contains 30% hydrogen [25], a gas produced solely by bacterial metabolism. As long ago as 1975, it was hypothesized that a dysbiosis (imbalance between protective microflora and harmful microflora) was involved in the pathogenesis of NEC [26]. The recent explosion of interest in the intestinal microbiome, and the availability of high throughput pyrosequencing techniques, has led to several relevant research studies in NEC. However, such data are very complex, and analysis of these data in a very heterogeneous disease like NEC is extremely challenging, especially where both the nosocomial microbiota and their measurement methods vary between neonatal units [27]. Nevertheless, recent studies suggested a loss in microbial diversity to occur immediately before NEC onset [28,29], with a consequent predominance of *Escherichia spp.* [28] or strict anaerobes [29].

2.4. Inflammation

Histologically, there is a massive intestinal inflammatory response in NEC. Some authors have even suggested that there may be antenatal precedents to this exaggerated inflammatory response, such as chorioamnionitis. A recent systematic review and meta-analysis of available studies concluded that chorioamnionitis with fetal involvement, and clinical chorioamnionitis both significantly increased the risk of NEC, whereas there was no increased risk from histological chorioamnionitis [30]. Differences in the immune response to mucosal damage and the microbiota may also be responsible for the exaggerated inflammatory response in NEC (reviewed [31]). Recent studies have highlighted such differences, such as those showing that intraepithelial T cell receptor γδ lymphocytes are decreased in surgical NEC specimens compared with appropriate controls [32] as are lamina propria T regulatory cells [33]. A key player in intestinal inflammation and the response to pathogens is TLR4, and recent work has shown that TLR4 signalling is important in the development of NEC [8,34–36]. Intriguingly, TLR4 signalling also links to other factors involved in the pathogenesis of NEC, such as the microcirculation ([36] see below).

2.5. Ischaemic injury

From early descriptions, ischaemia/reperfusion injury due to relative splanchnic hypoperfusion (the so called 'diving reflex') was though to play a part in the pathogenesis of NEC [26], in part due to the similarity in histological damage between intestinal ischaemic damage (such as that following mesenteric infarction) and NEC. A primary role for intestinal ischaemic damage long fell out of favour (for discussion, see [37]), but recently some evidence from both animal models and clinical studies has resurrected the potential role of intestinal ischaemia, although probably not as the sole initiating factor. Experimental studies have suggested that in NEC, there is an impairment in intestinal microcirculation [37–39] which can be improved by direct peritoneal resuscitation [40–43]. A potential role for splanchnic hypoperfusion in NEC has been suggested from a variety of clinical studies: firstly, there is a decline in mesenteric oxygenation when preterm infants are fed during red blood cell transfusion [44] (which may itself be associated with precipitation of acute NEC [45]). Secondly, there is increasing recognition that an important subset of infants with NEC have congenital cardiac disease that may predispose to splanchnic hypoperfusion [46]. Thirdly, several clinical studies have suggested that arginine and/or citrulline, amino acids which are important in production of nitric oxide and regulation of intestinal blood flow, are decreased in NEC and that supplementation of infants with arginine may prevent NEC [47–51].