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Cytokines and growth factors in the developing intestine and during necrotizing enterocolitis

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ABSTRACT

Cytokines and growth factors play diverse roles in the uninflamed fetal/neonatal intestinal mucosa and in the development of inflammatory bowel injury during necrotizing enterocolitis (NEC). During gestational development and the early neonatal period, the fetal/premature intestine is exposed to high levels of many "inflammatory" cytokines and growth factors, first via swallowed amniotic fluid *in utero* and then, after birth, in colostrum and mother's milk. This article reviews the dual, seemingly counter-intuitive roles of cytokines, where these agents play a "trophic" role and promote maturation of the uninflamed mucosa, but can also cause inflammation and promote intestinal injury during NEC.

Introduction

Cytokines and growth factors have a complex, multifaceted role in intestinal development and the pathogenesis of NEC. The fetal/premature intestine is exposed to high levels of many growth factors and so-called "inflammatory" cytokines, first via swallowed amniotic fluid (AF) *in utero* and then, after birth, in colostrum and mother's milk. In addition, the midgestation intestine constitutionally expresses many of the same cytokines at levels much higher than in the adult intestine. These data indicate important physiological and developmental roles of these cytokines/growth factors, and indeed, many preclinical and early clinical studies show a protective role of enterally administered cytokines against NEC. However, the onset of NEC also triggers a prominent mucosal and systemic cytokine response, and there is extensive preclinical evidence informing about the pathological footprint of these cytokines and supporting suppression of cytokine expression/signaling to attenuate NEC-like injury. This article reviews these dual, seemingly counter-intuitive roles of cytokines, where cytokines may play a "trophic" role and promote maturation of the uninflamed mucosa, but can also cause harm and promote intestinal injury during NEC. In the following sections, we have defined "cytokines" in a

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generic sense—as small, peptide extracellular-signaling molecules encompassing diverse subgroups: chemokines, colony-stimulating factors, growth factors, interleukins, interferons, and tumor necrosis factors. In addition to information from our own studies, we performed a literature search using the databases PubMed, EMBASE, and Scopus. To minimize bias, keywords from PubMed's Medical Subject Heading thesaurus were shortlisted prior to the actual search and combined with text words likely to be used in titles and abstracts.

Cytokines in the developing intestine

The developing intestine expresses many "pro-inflammatory" cytokines at levels much higher than in the adult intestine (Fig. 1). These cytokines originate in the enterocytes and macrophages, which express high levels of several innate response receptors and transcriptional regulators such as the nuclear factor-kappa B (NF-κB) and the activator protein 1 (AP-1) complex.[1–7] In addition, the fetal intestine is developmentally-deficient in anti-inflammatory signaling mediators such as the single Ig interleukin-1-related receptor (SIGIRR), interleukin-1 receptor-associated kinase (IRAK)-M, tumor necrosis factor-alpha-induced protein 3 (TNFAIP3), and the Toll-interacting protein (TOLLIP).[8] Some reports also show low levels of the inhibitor of κB (IκB).[9]

In addition to the high endogenous cytokine production, the fetal intestine is also exposed to the cytokines and growth factors present in AF. Starting at 8–11 weeks' gestation, the fetus ingests increasing amounts of AF that may reach 500 mL/d in the 3rd trimester.[10] Using a fetal sheep model, Trahair et al.[11,12] showed that esophageal ligation (to block AF ingestion) during midgestation caused mucosal atrophy, villus blunting, and enterocyte abnormalities, such as effacement of microvilli, glycogen accumulation, cellular extrusion, and lysosomal dysgenesis. Consistent with these findings, human neonates with congenital intestinal obstruction also show villus blunting and shallow, poorly organized crypts distal to the site of obstruction.[13] In Trahair's model, the mucosal defects were gradually reversed following the removal of esophageal ligatures and restitution of AF ingestion but not by the infusion of Ringer's lactate, indicating that the "trophic" effects of AF were secondary to the bioactive molecules present in AF and not merely due to the flow of fluid through the gut lumen.[14]

After birth, the neonatal intestine continues to be exposed to high concentrations of cytokines in colostrum and maternal milk. Colostrum and milk, which are apocrine secretions, contain a wide range of cytokines and other intracellular proteins involved in innate/adaptive immunity.[15] Table 1 summarizes current evidence on cytokines expressed in the fetal/neonatal intestine, AF, and colostrum/milk, and their known functions in intestinal development.[16–38] With an ever-increasing number of such agents, criteria have been suggested to identify factors involved in intestinal growth/development[39,40]: (1) expression in milk or in the intestinal mucosa at the time of growth, (2) expression of cognate receptor(s) during these periods, (3) reduction of growth *in vivo* with removal of the relevant growth factor, (4) precocious growth/maturational effects by early administration, and (5) growth/maturational effects *in vitro*.

Fetal enterocytes express the receptors for diverse cytokines, chemokines, epidermal growth factor (EGF), hepatocyte growth factor (HGF), FGF, insulin-like growth factors, chemokines, erythropoietin, and granulocyte-colony stimulating factor (G-CSF). Supporting evidence from *in vitro* and animal models suggests that many of these agents can survive digestion and increase enterocyte proliferation, migration, and differentiation, prevent apoptosis, and promote mucosal restitution in the small intestine.[40–43]

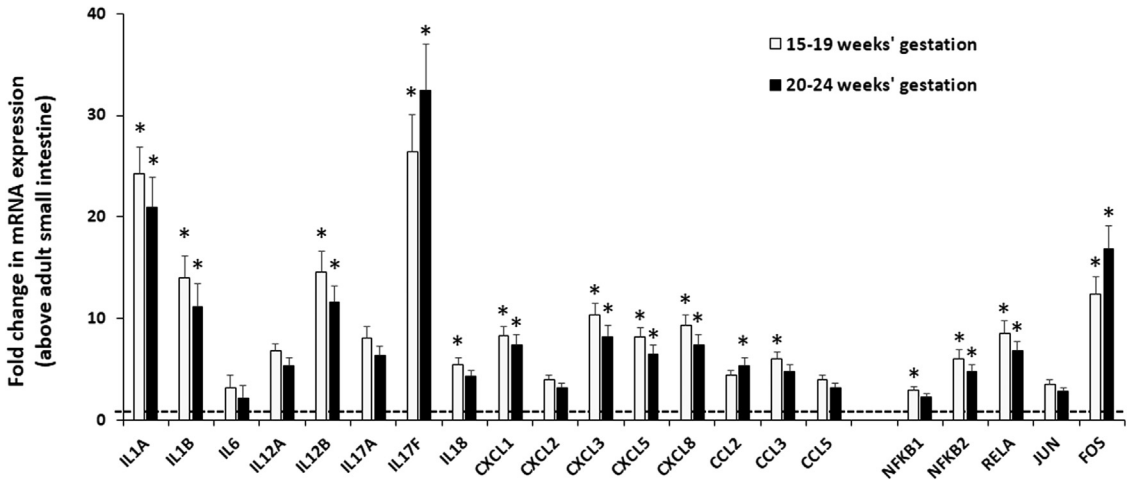

Fig. 1 – Human fetal intestine expresses several inflammatory cytokines and transcriptional regulators at higher levels than in the adult intestine. Bar diagram (means + standard errors) show fold change in mRNA expression (normalized against the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase). Broken line represents mRNA levels in the adult intestine. De-identified human fetal intestine was obtained at medical terminations ($n = 8$/group) and the adult intestine from subjects undergoing bariatric surgery ($n = 5$). The study was deemed to be "no human subjects" research by the Institutional Review Board. Crossing-threshold cycle numbers in the 2 groups were compared by Mann–Whitney *U* test. $^{*}P < 0.05$.