

Amniotic fluid: Source of trophic factors for the developing intestine

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Abstract

The gastrointestinal tract (GIT) is a complex system, which changes in response to requirements of the body. GIT represents a barrier to the external environment. To achieve this, epithelial cells must renew rapidly. This renewal of epithelial cells starts in the fetal life under the influence of many GIT peptides by swallowing amniotic fluid (AF). Development and maturation of GIT is a very complex cascade that begins long before birth and continues during infancy and childhood by breastfeeding. Many factors like genetic preprogramming, local and systemic endocrine secretions and many trophic factors (TF) from swallowed AF contribute and modulate the development and growth of the GIT. GIT morphogenesis, differentiation and functional development depend on the activity of various TF in the AF. This manuscript will review the role of AF borne TF in the development of GIT.

Key words: Amniotic; Fluid; Gastrointestinal; Factors; Tract; Trophic; Development

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Core tip: The gastrointestinal tract (GIT) is a complex system with a combination of factors being responsible for its development. Trophic factors (TF) in amniotic fluid (AF) represent an important component that affects the development and maturation of the GIT during fetal life. We highlight the various phases of GIT development, the formation/circulation of AF, various TF in AF and the respective roles they play in fetal GIT development. We also emphasize that much remains to be known about the milieu of TF within AF. We hope this article provides an insight of what is known about

such TF and what we hope to discover in the future.

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INTRODUCTION

There is a combination of many factors which are responsible for the growth and development of the gastrointestinal tract (GIT) like genetic programming, trophic factors (TF) in amniotic fluid (AF), endocrine factors like corticosteroids, growth hormone and insulin and paracrine growth factors^[1-4]. Development and maturation of GIT is a continuum and a very complex process which finally results in a mature GIT.

As a barrier to the external environment, gut epithelium must be renewed rapidly and repeatedly^[1]. Growth and renewal of gut epithelial cells is dependent on controlled cell stimulation and proliferation by a number of signaling processes and TF^[1]. The importance of AF in fetal nutrition is an acknowledged fact^[5]. It has been documented that swallowing of AF enhanced fetal gastrointestinal (GI) development^[5] in rabbits. Experiments performed in sheep showed that esophageal ligation decreases fetal intestinal growth and re-establishment of swallowing promoted growth^[5]. Surana *et al*^[6] studied the effect of AF on fetal intestinal growth in humans and found that proximal obstruction of intestines led to more growth impairment as compared to a distal obstruction. Hirai *et al*^[7] examined the trophic effects of AF, human milk and several recombinant growth factors on a human fetal small intestine cell line. They discovered that AF and human milk promoted cell growth equally^[7]. They also noted that growth factors stimulated cell growth but enhancement was less than that of AF alone^[7]. All of these suggest that AF is a source of TF for the development and maturation of intestine.

But do we know what a mature GIT is? How can we clinically differentiate immature and mature gut? What affects the maturation and development of GIT during fetal life and after birth? To answer these questions we will start with the development of fetal GIT.

FETAL GIT DEVELOPMENT

Development and maturation of GIT is a continuous process which starts during early fetal life and continues well into infancy and childhood. Now the question is what a mature GIT is and what leads to a mature GIT? To answer this question, we need to know the fetal and neonatal GIT development and maturation and what is responsible for such processes. There are five phases of fetal GIT development (Table 1 and Figure 1).

Phase 1 (embryonic phase)

This is a phase of organogenesis which begins immediately after conception and quickly leads to phase 2^[1]. In phase 1, there is formation of primitive gut. GIT undergoes very rapid growth from the week 5 of gestation onwards^[8].

Phase 2

There is selective growth and apoptosis that allows the formation of the rudimentary gut tube^[1]. The GIT undergoes rapid growth with the formation of villi and microvilli^[8-9]. During this phase an entrance and an exit site is formed (future mouth and anus respectively) and fetus starts swallowing AF, which has both physical and trophic effects on the development of GIT (Figure 2).

Phase 3 (Late gestational age)

This comprises active differentiation during late gestation when the GIT prepares for extra-uterine life^[1]. The intestinal cells actively divide causing cells to migrate up the villus and ultimately forming actively absorbing cells^[1]. This phase is also characterized by selective apoptosis at the tips and crypts of villi^[10]. After about 96 h, the villous epithelial cells slough off in the lumen of intestine where they mix with mucous and bile to become meconium^[9].

Phase 4 (Neonatal phase)

This phase begins after birth when exposure to enteral nutrition leads to more rapid mucosal differentiation and development of GIT for the extra uterine adaptation^[1]. Premature infants lack this developmental phase, which occurs during the third trimester. This is why this process is more prolonged in premature infants. This is also the phase that has the largest antigenic load presented to it in the form of dietary proteins and pathogens^[1]. During this time the gut develops the ability to distinguish between foreign pathogens and safe nutrient proteins^[11,12].

Phase 5 (Weaning phase)

This is the last phase of gut development and occurs in late infancy/early childhood^[1] as the child transitions from milk based diet to complementary diet. During this time, there is a second phase of mucosal expansion which is associated with epithelial hyperplasia that renders the gut functionally more mature. During this phase, the intestinal mucosal immunity develops the ability to differentiate between foreign pathogen and nutrient proteins^[12]. Preterm infants lack this process due to lack of exposure to AF borne TF due to premature birth. That is why premature infants are at increased risk of proliferation of pathogenic bacteria in the lumen of intestine and subsequent mucosal invasion of pathogenic microorganisms^[13]. This may significantly impact the GIT function as well systemic immune functions^[13].

Table 1 Phases of mammalian gastrointestinal development (adapted from reference 1)

Phase	Development
Phase 1	Embryonic phase of organogenesis Forms primitive GIT
Phase 2	Entrance and exit sites of GIT form Formation of rudimentary primitive GIT Formation of mouth and anus Fetal swallowing of amniotic fluid begins
Phase 3	Active differentiation Increase in cell number in crypts Cells from crypts start migrating up to the villi GIT growth is more rapid than the fetal body as a whole Growth accompanied by selective apoptosis
Phase 4	After birth, exposure to enteral nutrition Breast milk feeding – rapid mucosal differentiation and development Infancy – mucosal growth continues with deepening crypts, increasing villi (increasing width and number) and appearance of sub-mucosal folds Development of GIT mucosal immunity due to exposure to dietary antigens Mucosal immune system can distinguish between foreign pathogens and safe nutrient proteins and commensal organisms
Phase 5	Late infancy – early childhood. Transition from milk feeding to solid foods. This is second phase of mucosal immunity with epithelial (Weaning) hyperplasia with maturation of gut functions similar to older children.

GIT: Gastrointestinal tract.

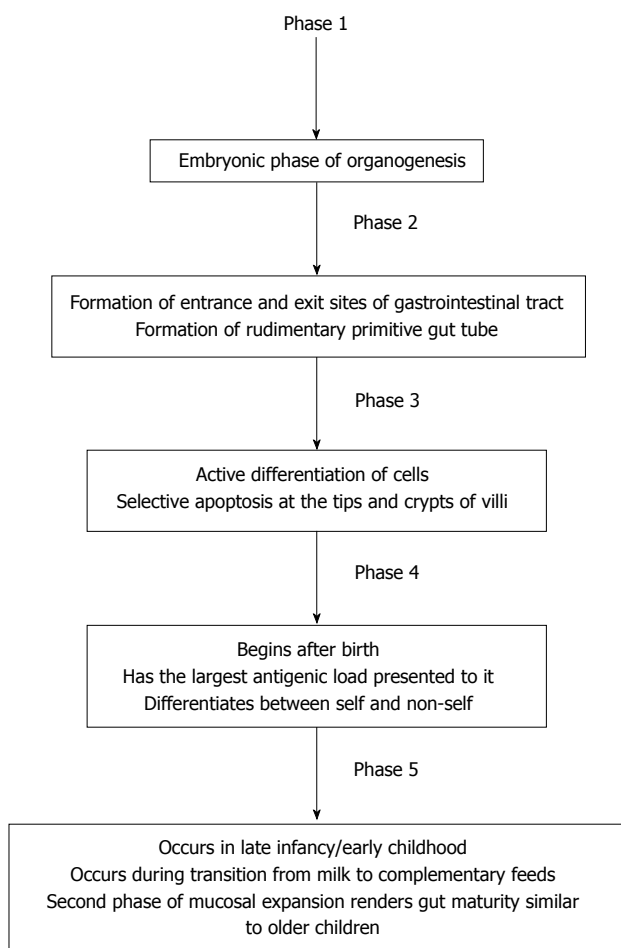


Figure 1 Various phases of mammalian gut development.

FORMATION OF AF

The first fluid to enter the developing fetal GIT is the AF^[1]. It is a bioactive medium containing proteins,

lipids and phospholipids, urea and electrolytes that is actively secreted by cells lining the amniotic cavity^[1]. During early gestation (organogenesis), AF volume increases by getting water from the mother’s plasma and is transported to the fetus through fetal membranes depending on the hydrostatic and osmotic pressure gradients. AF volume increases from 10 mL at 10 wk to around 400 mL at 20 wk gestational age. Around 8-10 wk gestation, the skin is not keratinized hence there is a bi-directional fluid diffusion between the fetus and the AF. During early gestation, AF volume and fetal size are related in a linear manner. Around this time, fetus starts passing urine and fetal swallowing also begins. Keratinization of fetal skin starts around 20 wk of gestation and is completed around 25 wk. After skin keratinization, the relationship of fetal size and AF volume is no longer linear. Around 28 wk gestation, AF volume increases to its maximum volume (about 800 mL) where it plateaus near term gestation and thereafter AF volume starts declining to 400 mL at 42 wk. Fetus swallows around 250 mL/kg/d of AF, which is the main source of AF removal^[14]. The chemical composition of AF changes with increasing gestational age. AF is not the main source of fetal nutrition; it contributes only 15% of fetal nutritional requirements^[15,16] but has a very important role in the development and maturation of gut^[1]. The important nutritional components of AF are summarized in Table 2. In the second half of pregnancy, sodium, chloride and osmolality decrease whereas urea and creatinine concentrations increase. AF composition is more regulated than the AF volume. (Refer to reference 18 for further details)^[17].

AF CIRCULATION

Figure 3 shows 5 pathways of exchange and AF circulation that have been identified^[18]. Excretion of urine

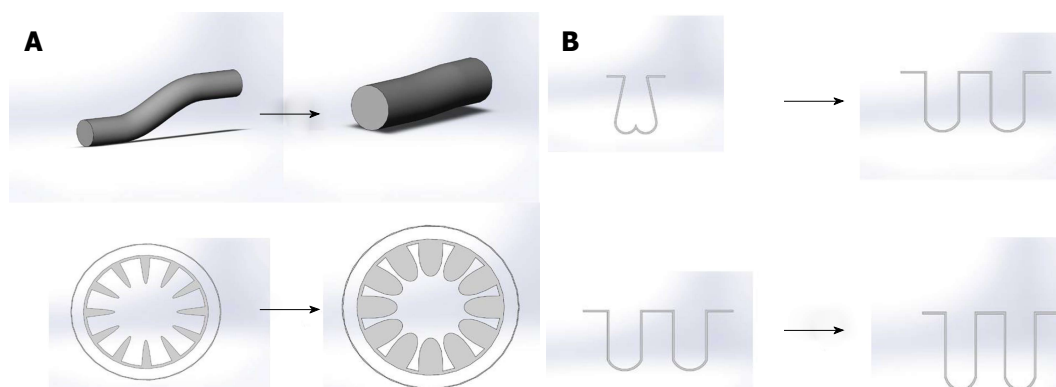


Figure 2 Two patterns of the growth of small intestine. A: Cylindrical organ growth in length and diameter; B: Luminal mucosal growth with amplification of the internal surface area by submucosal folds and villi.

Table 2 Important nutritional components of amniotic fluid

Component	Most important examples
Amino acids	Glutamine, arginine
Proteins	Lactoferrin
Minerals	Zinc, iron
Hormones	Growth hormone, prolactin
Growth factors	IGF-1, EGF

IGF-1: Insulin like growth factor-1; EGF: Epidermal growth factor.

and the secretion of oral, nasal, tracheal and pulmonary fluids predominantly accomplish production of AF^[19]. Fetal breathing movements also lead to the efflux of lung fluid into the AF but this effect is minimal^[18]. Removal of AF is predominantly accomplished by fetal swallowing. Also a significant intramembranous pathway transfers fluid and solutes from the amniotic cavity to the fetal circulation across the amniotic membranes^[20]. The trans-membranous pathway, which is the movement of AF across the fetal membranes into the maternal circulation, affects the AF volume only minimally^[18]. All of these pathways together maintain the relative stability of the AF in spite of large fluid shifts^[18].

VOLUME CHANGES OF AF WITH INCREASING GESTATIONAL AGE

Table 3 summarized the volume changes of AF with increasing gestational age^[18].

Ten weeks of gestation: 25 mL (AF and fetal size related in a linear fashion in this early period^[18]. Fetal kidneys start making urine by 8 wk gestation and swallowing begins soon after).

Twenty weeks of gestation: 400 mL (Keratinization of skin is complete by 25 wk gestation and AF and fetal size lose their linear relationship).

Twenty-eight weeks of gestation: 800 mL.

Term gestation: Plateau near term gestation.

Forty-two weeks of gestation: 400 mL.

Table 3 Amniotic fluid volume changes with increasing gestational age

Gestational Age	Volume of AF
10 wk	25 mL
20 wk	400 mL
28 wk	800 mL
Term gestation	Plateau in volume of AF
42 wk	400 mL

AF: Amniotic fluid.

AF: SOURCE OF TF

It is important to note that AF is the first fluid which bathes the GIT secondary to swallowing of AF (450 mL/d to 1000 mL/d at term gestation)^[21]. Table 4 summarized the roles of various TF found in AF in intestinal development and the location of their receptors. AF serves as the physical barrier to the external environment. In the 1970's, Chochinov *et al*^[22] correlated AF borne growth hormone concentration with the development and maturation of fetal kidney. Mulvihill *et al*^[16] *in vitro* studies showed that AF and fetal bovine serum had equivalent stimulating effect on fetal gastric epithelial cells. Further Barka *et al*^[23] confirmed the trophic properties of AF. More recently, Maheshwari^[24] described the role of cytokines in AF and their role in the development of GIT. In an *in vitro* study, Hirai *et al*^[7] demonstrated the trophic effects of AF and further showed that trophic effects of AF were equivalent to breast milk. The growth of intestine occurs by duplication of intestinal crypts, which leads to cylindrical growth of small intestine (Figure 2A). During growth, the crypts gradually divide longitudinally into two daughter crypts (Figure 2B). This process is promoted by various TF like epidermal growth factor (EGF), keratinocyte growth factor, and many other TF which are present in the AF. Over the years, different investigators have found many TF in the AF. Different TF present in AF work in concert to provide bioactivity. Wagner *et al*^[25] used fetal small intestinal cells (Fhs74) and showed a synergistic relationship of EGF and transforming growth factor- α

Table 4 Roles of various trophic factors found in amniotic fluid in intestinal development and the location of their receptors

Trophic factor	Location of receptors	Role in intestinal growth
EGF	Basolateral intestinal membrane	Stimulates cell mitosis and differentiation Stimulates intestinal epithelial cell proliferation
HGF	Intestinal crypt epithelial cells and in the muscle layers of the intestine	Intestinal cell proliferation <i>in vitro</i> and has been demonstrated to induce intestinal growth in rats
TGF- α and TGF- β	Basolateral intestinal membrane	Primary role may be intestinal mucosal repair
IGF-1	Crypt cells, basolateral membrane and in the distal intestine	Primary mediator of both intrauterine and postnatal growth in mammals May be important for growth of muscle growth of distal small intestine
EPO	Apical surface of intestinal epithelial cells	Increased villus height, villus area, crypt depth and crypt epithelia cell proliferation in rat pups. <i>In vitro</i> , recombinant EPO has been shown to protect cells against mucosal injury
G-CSF	Apical regions of the intestine	Role in epithelial cell maintenance
IL family	Intestinal epithelial cells	Enhances intestinal epithelial cell restitution. Enhances the integrity of the intestinal epithelial cell junctions. Intestinal epithelial cell proliferation and increased nutrient uptake

IGF-1: Insulin like growth factor-1; EGF: Epidermal growth factor; TGF: Transforming growth factor; HGF: Hepatocyte growth factor; EPO: Erythropoietin; G-CSF: Granulocyte colony stimulating factor; IL: Interleukin.

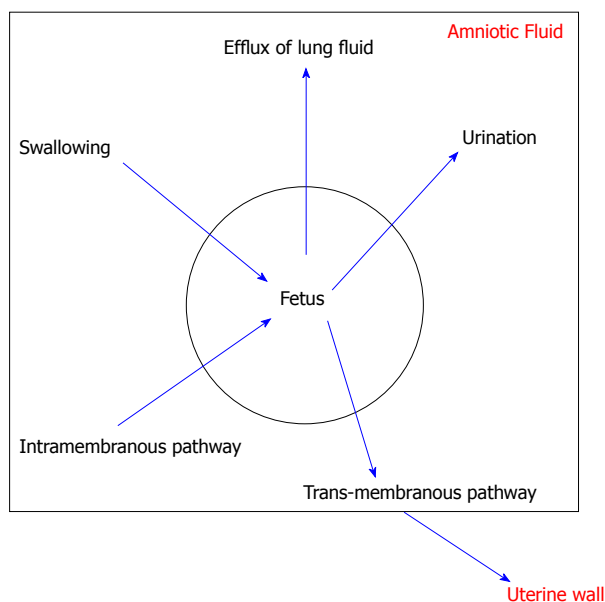


Figure 3 Various pathways of amniotic fluid circulation.

(TGF- α), which was greater than individual effect of recombinant EGF (rEGF), or TGF- α alone. Booth *et al*^[26] also showed that no single growth factor increased cell proliferation of rat intestinal epithelial cells but when rEGF, TGF- α , insulin like growth factor-1 (IGF-1) and platelet derived growth factors were combined, epithelial cell proliferation was increased significantly. How these TF in AF work- whether they work in concert or separately- is only directed by *in vitro* studies. The interplay of these TF in AF *in vivo* is not well understood. In this review, we will focus on different TF in the AF and their role in the development and maturation of the GIT.

EGF

In 1962, a growth factor was discovered from mouse saliva which could induce premature eruption of the teeth and opening of eyelids - that is why it was called EGF^[27]. EGF is a family of peptides that share structure

and affinity to the EGF receptor. The salivary glands and Brunner’s glands of duodenum in the GIT continuously secrete EGF. The EGF receptor (erbB1) is found in fetal as well adult GIT, liver and pancreas. EGF receptor levels increase in intestinal pathology like ulceration of rat oxyntic mucosa^[28]. It is a small peptide which functions as a luminal surveillance peptide that can attach to the EGF receptor on the basolateral membrane when the luminal barrier is damaged^[29]. As GIT is an important barrier to outside noxious substances, there is quick healing of injured epithelial lining by epithelial migration and proliferation, called restitution^[30]. EGF stimulates restitution of the superficial epithelial lining of GIT. It stimulates cell mitosis and differentiation, decreases acid secretion, increases bicarbonate, mucus secretions and GIT blood flow and helps in digestion by increasing amylase secretions and by increasing gastric motility. EGF is also a cytoprotective molecule that can stabilize GIT epithelial cells from agents like ethanol or non-steroidal anti-inflammatory drugs^[31]. EGF has two main physiological functions: (1) Involved in mucosal protection and healing of damaged epithelial lining; and (2) involved in digestion, absorption and transportation of nutrients.

EGF is found in significant quantities in human AF and it increases with progression of pregnancy^[32]. The suggested site of production of EGF is either the lungs or the amniotic membranes^[5]. EGF has been shown to increase DNA and glycoprotein synthesis in cultured human fetal gastric cells^[33]. The impact of EGF in AF on fetal intestinal growth is an area of active research. EGF receptors are mainly expressed on the basolateral intestinal membrane^[24]. It is largely resistant to gastric proteolysis in the preterm infant and thus remains bioavailable in the intestine^[24,34]. All in all, EGF is a potent stimulus to intestinal epithelial cell proliferation^[15] (Table 5).

In summary, EGF has mitogenic as well as nonmitogenic roles in GIT function. Further understanding of its role is required before we use it in the settings of

Table 5 Effects of epidermal growth factor on the gastrointestinal tract

Increased effect on	Possible secondary message
Proliferation	-
Bicarbonate secretion	Prostaglandins
NaCl and glucose uptake	Na ⁺ - glucose cotransporter, lipids
Mucus secretion	Prostaglandins
GI blood flow	Beta-adrenergic NO prostaglandins
Longitudinal smooth muscle contraction	Prostaglandins
Circular smooth muscle contraction	Desensitizes (not prostaglandins)
Restitution	Cell-migration prostaglandins
Permeability	-
Mucosal protection	Proliferation, polyamines, mucus, trefoil peptides
Decreased effect on	Possible secondary message
Gastric acid secretion	Protein kinase C, cAMP
Gastric emptying	-
Increased and decreased effect on	Possible secondary message
Chloride secretion	Phosphatidylinositol 3-kinase
Pancreatic amylase (3.2.1.1) secretion	cAMP phospholipase C

GI: Gastrointestinal; NaCl: Sodium chloride; cAMP: Cyclic adenosine monophosphate.

inflammation of mucosal damage such as necrotizing enterocolitis.

Hepatocyte growth factor

It is present in AF and human milk and is expressed in embryonic and fetal intestinal tissue^[24]. Hepatocyte growth factor (HGF) and C-met mRNA are expressed in the fetal intestine^[24]. HGF receptor, C-met - a proto-oncogene, is present on intestinal crypt epithelial cells although it is also expressed in the muscle layers of the intestine^[15]. HGF stimulates intestinal cell proliferation *in vitro* and has been demonstrated to induce intestinal growth in rats when administered in pharmacologic doses^[24]. In an animal model of NEC, we showed that oral supplementation of AF is protective against experimental NEC in a rat model of NEC (hypoxia and hypothermia model) which was mediated, at least partly, by HGF.

TGF- α

Detectable in the human GIT at 15 wk gestation^[24]. It has a structure similar to EGF and binds to the same receptor. It is found in AF and human milk. Recombinant TGF- α has been shown to elicit a synergistic trophic response on cultured intestinal cells when combined with EGF, IGF-1, FGF and HGF^[7]. However it was noted that this trophic response was not as strong as that seen with AF or human milk alone. Its primary role is believed to be in mucosal repair^[24].

TGF-beta

It belongs to a family of signaling peptides that influences the distribution of intestinal stem cells. It is found in human AF only during the late stages of gestation^[5]. It is believed to induce terminal differentiation of intestinal epithelial cells and to accelerate the rate of healing of intestinal wounds by stimulating cell migration^[5]. A role in the prevention of necrotizing enterocolitis has been suggested as well^[34]. We showed that TGF β , especially

TGF β 2, suppresses macrophage inflammatory responses in the developing intestine and protects against mucosal inflammatory injury. We further showed that enteral feeding of TGF β 2 protected mice from experimental NEC-like injury^[35].

Insulin like IGF-1

It is the primary mediator of both intrauterine and postnatal growth in mammals. Experiments have shown that enterally infused IGF-1 in sheep that have undergone esophageal ligation led to an increase in somatic growth and bowel wall thickness^[36]. The concentration of IGF-1 in human AF can reach as high as 20 ng/mL. This means that fetal swallowing near term may lead to 20 mcg of IGF-1 being ingested daily^[37].

Insulin like Growth Factor-2

It is a major modulator of early embryonic and 2nd trimester fetal growth^[5]. It is synthesized by fetal lung and likely exits the fetal lung *via* the efflux of fetal lung fluid^[5]. Insulin like growth factor-2 (IGF- II) peaks in AF at 19 wk gestation, declining thereafter^[38]. Deletion of the *IGF-II* gene expressed in the placental trophoblast led to reduced placental growth followed several days later by fetal growth restriction^[39].

Receptors to IGF- I and IGF- II are expressed on crypt cells, on the basolateral membrane and in the distal intestine^[24]. Ingested IGF is likely to remain bioactive in the intestine secondary to its relative stability and presence of milk borne protease inhibitors. Effects similar to the effects on sheep bowel described previously have been observed in human duodenal explants^[24].

Fibroblast Growth Factor

Activity has been demonstrated in AF and human milk^[7]. A study by Hirai *et al*^[7] showed that inhibition of fibroblast growth factor (FGF) activity in AF caused a 58% reduction in AF induced intestinal epithelial cell proliferation^[4], suggesting an important role in the

Table 6 Important trophic factors involved in gut development and the most relevant reference articles

Trophic factor	Ref.	n of references cited
EGF	Maheshwari (2004) ^[24]	36
	Underwood (2005) ^[18]	63
	Playford (1996) ^[29]	23
	Cummins (2002) ^[15]	108
HGF	Maheshwari (2004) ^[24]	36
	Underwood (2005) ^[18]	63
	Cummins (2002) ^[15]	108
TGF- α and TGF- β	Maheshwari (2004) ^[24]	36
	Seare (1998) ^[56]	32
	Underwood (2005) ^[18]	63
	Cummins (2002) ^[15]	108
IGF-1	Maheshwari (2004) ^[24]	36
	Underwood (2005) ^[18]	63
	Seare (1998) ^[56]	32
	Cummins (2002) ^[15]	108
Cytokines	Maheshwari (2004) ^[24]	36
	Seare (1998) ^[56]	32

IGF-1: Insulin like growth factor-1; TGF: Transforming growth factor; EGF: Epidermal growth factor; HGF: Hepatocyte growth factor.

development of the GIT.

Erythropoietin

It is present in AF and human milk and its receptor is present on the apical surface of intestinal epithelial cells^[24]. It resists degradation and remains bioactive in the intestine. It has been shown that administration of recombinant erythropoietin (EPO) increased villus height, villus area, crypt depth and crypt epithelial cell proliferation in rat pups^[24]. *In vitro*, recombinant EPO has been shown to protect cells against mucosal injury and there are reports that the incidence of necrotizing enterocolitis is lower in neonates who had received recombinant EPO^[40].

Granulocyte colony stimulating factor

Significant concentrations of granulocyte colony stimulating factor (G-CSF) are present in AF and human milk. Similar to EPO, it resists simulated neonatal digestion^[24]. Its receptors are mostly present in the apical regions of the intestine starting at 10 wk gestation. There is evidence that G-CSF may play a role in epithelial cell maintenance^[41].

Interleukin family

Interleukin (IL)-2 is present in AF and is believed to enhance intestinal epithelial cell restitution. There is evidence that IL-2 plays a crucial role in mucosal healing because IL-2 knockout mice develop colitis similar to human ulcerative colitis^[42]. IL-4, which is also present in AF, is believed to enhance the integrity of the intestinal epithelial cell junctions^[24]. IL-6 which is detectable in AF as early as 7 wk of gestation, may play a role in protecting intestinal cells against apoptosis secondary to hypoxia or other severe insults^[43]. IL-1 found in AF, leads to intestinal epithelial cell proliferation and increased nutrient uptake^[24]. IL-1 also induces expression of

decay accelerating factor, which is responsible for the degradation of activated complement and thus may play a protective role against complement activation^[15].

Other factors such as vascular endothelial growth factor, IL-8 and the trefoil factor family need more in-depth evaluation in terms of their respective roles in the development of the human intestine. Table 6 summarizes the important TF involved in gut development and their important references.

GIT morphogenesis, functional development and differentiation are regulated by various TF present in the AF^[44]. In contrast to adult enterocytes, fetal enterocytes hyper-react to microbial challenges hence the defense responses are inadequate^[45]. AF borne TF compensates for immaturity of enterocytes by modulating exaggerated responses of the enterocytes and by promoting immune maturation^[46]. Enterocytes are not fully mature at birth and breast milk continues to provide TF and immunomodulatory molecules which promote enterocyte development.

EVIDENCE OF AF AS A SOURCE OF TF

Recently, there have been few studies done to show the role of AF in protecting against intestinal mucosal injuries. Good *et al*^[47] showed administration of AF into fetal intestine reduced LPS-mediated signaling within the fetal intestinal mucosa by reducing expression of EGF receptor. The reduced expression of EGF receptor occurs by inhibiting toll-like receptor-4 signaling within the fetal intestine and hence attenuates experimental NEC. Sigger *et al*^[48], in a pig model of NEC showed decreased NEC by enteral administration of AF by suppressing pro-inflammatory intestinal responses. Zani *et al*^[49] demonstrated that intra peritoneal administration of AF stem cells improves survival and reduced NEC like injury in a rat model of NEC. They further showed that

pre-treatment with AF stem cells resulted in improved intestinal function, decreased intestinal inflammation and increased enterocyte proliferation. We also showed oral administration of AF protected rat pups against NEC-like mucosal injury, which is partly mediated by HGF^[50]. There is more research going in our and other laboratories mainly focusing on the role of each individual TF present in AF by blocking different TF. In summary, there is definite evidence of protective effect of AF against NEC. But still many more questions need to be answered before we can use AF in human infants.

PERSPECTIVE

GIT epithelial biology research has changed to a dynamic interface which is able to sense environmental changes and immune responses. GIT epithelial cells can differentiate even if the stimuli from the gut lumen are harmful or not. This depends upon the underlying mechanisms like immunological tolerance. These functions depend upon the developmental status of the enterocytes. The AF during fetal life and breast milk after birth, compensate for the developmental immaturity to assure the maturation of enterocytes. Understanding of the interaction between fetal AF and exposure to various TF is not only theoretical but is also beginning to modulate the clinical management of premature infants. The use of EGF and other TF present in AF, that target GIT epithelial lining, has expanded a new horizon in the management of premature infants.

APUD (Amine Precursor Uptake and Decarboxylation) cells are responsible for synthesis of biological amines and peptides and accumulation of them in the cytoplasm in the form of secretory granules of "neuroendocrine type"^[51]. Originally all APUD cells were believed to be derived from the neural crest. However recently it has been proposed that APUD cells of the GIT originate from neuroendocrine-programmed ectoblast^[52]. Andrew *et al.*^[53] in 1975 had found APUD cells in the intestinal groove of the chick, disappearing on the day 3 but reappearing by the day 12 onwards. It was hypothesized that the early arising APUD cells are precursors to one or more islet types. It was confirmed in 2007 that AF harbors a heterogeneous mixture of these multipotent stem cells^[54]. APUD cells harvested from AF may very well prove to be helpful in the repair of damaged intestinal epithelial cells following disease processes.

LIFE AFTER AF: HUMAN MILK AND THE GIT ADAPTATION

After fetal maturation, human milk is essential for the neonate's development. Human milk also has the potential to stimulate cell growth and repair and improve immune-competence^[55,56]. Human milk contains growth factors, which are similar in nature to that contained in AF. It thus stimulates cells to grow and undergo

reparative processes^[1]. The neonatal GIT undergoes rapid growth and maturational changes after birth. The tight junctions of the GIT mature and there is selective entrance of nutrients and exclusion of pathogens^[57,58]. There is development of an effective mucosal barrier, immaturity of which may result in clinical disease states such as NEC. This intestinal mucous provides a protective interface between the internal and external milieu^[1]. Thus a functionally and developmentally mature gut has effective barrier function, epithelial integrity and also adequate mucous production. The role of AF as a source of TF for this above-mentioned development cannot be underscored.

UNANSWERED QUESTIONS

As discussed above, there are so many TF present in AF but the functions and significance of each individual TF remain incompletely understood. Fetal swallowing of AF is very important for the development and maturation of GIT. But some infants are born with esophageal atresia and other conditions leading to malabsorption while other infants have normal functioning GIT. This means there is a way other than just swallowing of AF by which the intestine is exposed to these TF. There is a strong theoretical possibility that TF in AF may protect the preterm infants against NEC and or increase intestinal recovery during the healing phase of NEC^[50,59]. Could early feeding of harvested human AF or simulated AF be used in preterm infants at risk for NEC or during recovery phases of NEC? Sullivan *et al.*^[60] showed that enteral feeding of "simulated AF" containing G-CSF and EPO was well tolerated in preterm infants and the same group further showed^[61] that simulated AF was also tolerated by infants who were recovering from NEC.

Do individual TF work separately or work together in concert? What is the most important TF in AF? Can enough AF be harvested at the time of elective cesarean section deliveries? Would harvested AF remain sterile after storage and safe to be used in preterm infants? Could harvested AF be pasteurized without inactivation of TF? Porter *et al.*^[62] have shown stability of TF in AF after storage. As in utero, fetus can absorb large volumes of AF but would infants with short gut syndrome or gastroschisis be able to tolerate enteral AF infusion which will prevent or decrease villous atrophy? Early trophic feeding in preterm infants is well established. Extremely low birth weight infants are not fed for the first few days. Would feeding AF during this time be beneficial to these infants? So there are many questions which are not well understood regarding how AF borne TF work together and lead to the development of a mature GIT.

FUTURE DIRECTIONS

Many questions about AF remain unanswered. The functions and significance of individual growth factors in human AF remain incompletely described^[18]. There

is also much to be learned about the immunoprotective properties of AF. Future directions include research into the use of synthetic or harvested AF as enteral infusions to promote the development of the GI tract in premature infants or infants recovering from NEC^[18]. Which TF should be included in the simulated AF? The important question of whether TF would survive the process of storage and freezing is yet to be answered. Although many TF have been elicited and described in human AF, many remain to be understood completely and even more remain to be discovered. More *in vivo* studies are required with individual TF as well with a combination of different TF. At the same time, *in vivo* studies are required to see the role of individual growth factors in AF by blocking different TF. At present, AF remains a potential fluid with possible benefits to extremely premature infants until a definitive beneficial role is confirmed by more research and until its safety is proven.

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