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551

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DEVELOPMENT OF IMMUNE FUNCTION IN THE INTESTINE AND ITS ROLE IN NEONATAL DISEASES

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The intestine is the largest immune organ in the body, with the ever-challenging task of handling large quantities of potentially harmful antigens. The intestine needs to balance the essential processes of breaking down food into macromolecules and prepare them for absorption while preventing excess amounts of antigen from passing through the small intestinal mucosa and initiating undesirable immune responses. [78] If antigens do pass through the mucosal barrier, then normal responses must occur to ensure that either systemic tolerance or appropriate immune responses occur.

The development of normal gut immune function depends on a properly functioning mucosal barrier. In addition to antigen entry, this barrier protects against microorganisms and viruses from penetrating

into the systemic circulation. [58] This article reviews the essential components of the barrier in relation to the entire immune system. These elements include the gut-associated lymphoid tissue (GALT), macromolecular uptake, bacterial adherence, and cytokine expression. Finally, gastrointestinal disorders, such as necrotizing enterocolitis, milk protein enteropathy, and bacterial infections, all of which afflict both preterm and term newborns, are discussed. The pathophysiology of these conditions is explained, emphasizing proposed pathways that stem from aberrant mucosal immune function.

ONTOGENY OF MUCOSAL IMMUNITY

In addition to the passive immune protection acquired from the mother, neonates require their own immune system to respond appropriately to foreign antigens. Gut-associated immunity develops in parallel with the other central lymphoid organs, such as the thymus. In particular, the presence of Peyer's patches parallels the development of the spleen and peripheral lymph nodes.

T lymphocytes do not populate the intestine until the thymus is mature. T-cell precursors are derived from hematopoietic stem cells, which colonize the human embryonic thymus at approximately 8 to 9 weeks of gestation (Fig. 1) , immediately before the thymic lobes fuse. [80] [82] Prothymocytes in the thymus then acquire the T-cell receptor (TCR) complex and express both the CD4 (T helper/inducer) and the CD8 (T killer/suppressor) surface molecules associated with the TCR. Subsequently, these *double-positive thymocytes*, as they are known, undergo a dual selection process. Negative selection removes T cells that react with self-antigens, and positive selection allows cells to mature that respond efficiently to foreign antigens in conjunction with host major histocompatible complex (MHC) molecules. Positive selection most likely permits the thymocyte to develop into functionally mature T cells. [82] Once mature, T cells expressing surface CD4 recognize antigen in association with class II MHC, and T cells expressing CD8 recognize antigen with class I MHC. CD4+ or CD8+ T cells are then ready to exit into the periphery; however, some evidence suggests that extrathymic maturation may

continue even after entering the peripheral circulation. [12] [83]

The spleen plays a significant role in the development of the fetal immune system. At 10 to 12 weeks' gestation, the fetal spleen contains few lymphocytes; however, by 16 to 18 weeks' gestation, moderate numbers of both T and B cells are forming around what will become active splenic germinal centers. Because in fetal life there is very little antigenic stimulation, germinal centers with secondary follicles rarely form until close to term and after birth. [22] [63]

In the intestine, the first discernible villi appear at approximately 10 weeks' gestation, with crypt formation following immediately at 10 to 11 weeks' gestation. Specialized cells, such as Paneth's cells, appear

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553

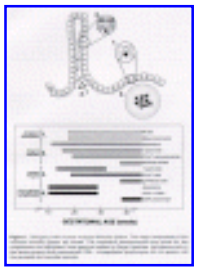

Figure 1. Ontogeny of the human mucosal immune system. The major components of the mucosal immune system are shown. The respective developmental time points for key components are highlighted. Note germinal centers in Peyer's patches. IgA plasma cells in the lamina propria and predominant CD8+ intraepithelial lymphocytes do not appear until the perinatal and neonatal periods.

554

at that same time, whereas goblet cells and endocrine cells appear at approximately 12 to 13 weeks' gestation. With the formation of columnar epithelium and microvilli at approximately 10 to 11 weeks' gestation, brush-border enzymes appear. Maltase, sucrase, and isomaltose are all at postnatal intestinal levels at as early as 15 weeks' gestation. [50] [63]

From the last trimester of development onward, the majority of GALT in small intestine is composed of three major components: the T-cell and B-cell-rich Peyer's patches, lymphoid cells of the lamina propria, and a diffuse infiltrate of intraepithelial lymphoid cells. Although the functions of these lymphoid components are interrelated in normal mucosal immune defense (see Fig. 1),they are distinct structurally and, therefore, are discussed separately.

Peyer's Patches

In fetuses, Peyer's patches appear in the distal small intestine, concentrated primarily in the ileum. Early observations reported microscopic accumulations of lymphoid cells in the small intestine after 14 to 16 weeks' gestation [19] ; however, recent immunohistochemical data demonstrated Peyer's patches as early as 11 weeks' gestation in developing fetuses. These early clusters of cells are not lymphocytes but are collections of subepithelial cells expressing CD4 antigen, which, later in development, exist as MHC class II restricted T cells. After 14 weeks' gestation, both CD4 + and CD8 + T cells can be seen within the follicles making up the Peyer's patches. [66]

For macromolecules to traverse the gut in a controlled manner,

specialized epithelial cells have evolved that overlay the developing Peyer's patches. These microfold (M) cells have scant microvilli and mucus covering their surface and a paucity of intracellular organelles, thus allowing easier access and penetration for gut macromolecules to cross the epithelial barrier. [57] In addition, M cells have a deep invagination of their basal membrane surface, into which lymphoid cells can intrude; thus, lymphocytes and antigen-presenting cells (APC) can position themselves close to the intestinal lumen. Microfold cells constitute the primary physiologic route for nonreceptor transport of macromolecules. [2] Furthermore, other studies have confirmed M cells' role in antigen transport with electron microscopy using ferritin [6] and horseradish peroxidase [53] as antigens. Whether M cells contain specific surface receptors to aid in the transport of macromolecules across the epithelium is still unknown.

By 16 to 18 weeks' gestation and throughout fetal life, larger clusters of cells can be immunohistochemically identified as containing both T and B cells, whereas CD4+ T cells are the predominant T-cell subset. IgM and IgG expressing B cells remain mingled among the T-cell population until 18 to 20 weeks' gestation, when the first evidence of discrete T-cell and B-cell areas are observed. At as early as 16 weeks' gestation, B cells, originally derived from pre-B cells in bone marrow and liver,

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are relatively mature and express cytoplasmic IgM and surface IgD. [25] [63] [66] Another distinguishing feature of fetal Peyer's patch B cells from postnatal life is the expression of CD5, a surface molecule of unknown function present on most mature T cells but on very few mature B cells. [66]

Because of the paucity of antigenic stimuli in utero, a possible function of Peyer's patches during fetal life is to serve as a site of antigen-independent B-cell proliferation. This phenomenon has been documented in other animal species and includes the exiting of B cells from Peyer's patches into the periphery. [54] Alterations in this pathway may, at least in rabbits, affect normal humoral responses. [39]

Thus, by the end of the second trimester, the Peyer's patches have developed histologically into structures that mimic those in postnatal tissues; however, whether their full immunologic potential is reached even by term is still unresolved. In fetal life, no evidence shows direct germinal center formation; however, soon after birth, the prompt development of germinal centers is ensured with a plethora of gut luminal antigens as stimuli. [10]

Lamina Propria Lymphocytes

The first evidence of lymphocytes either having immigrated to, or differentiated within, the lamina propria occurs at approximately 14 weeks' gestation. [66] The lamina propria consists of a mixture of scant B and T cells. In fetal life, its lymphocyte composition is markedly different from that of the postnatal lamina propria, which contains a majority of IgA-secreting plasma cells. Because normally almost no exogenous antigen exists in fetal life, the organized lymphoid tissue lining the gut receives little stimulation, resulting in the development of few lamina propria plasma cells. Antigenic exposure occurs at birth; and within 1 to 2 weeks, IgA-producing and IgM-producing plasma cells are evident in the lamina propria. By the sixth month of life, IgA cells predominate. [9] CD4+ T cells continue to appear

postnatally throughout the lamina propria, reaching adult numbers by the third month of life. [65]

Intraepithelial Lymphocytes

With electron microscopy, Orlic and Lev [52] demonstrated that lymphocytes appear between small intestinal epithelial cells by between 11 and 12 weeks' gestation. With a similar distribution both in fetal and postnatal life, these intraepithelial lymphocytes (IEL) are situated almost entirely in the basal portion of the epithelial layer. CD3 + expression persists after 14 weeks' gestation. In fetal life, phenotypically, only 50% of IELs express CD4 or CD8; the rest are CD4-- and CD8--. Postnatal IELs, however, are almost entirely CD8 +, [64] which is in contrast with the lamina propria and Peyer's patches, in which the majority of lymphocytes in fetal and postnatal life are CD4+. [63]

556

THE MUCOSAL DEFENSE BARRIER

For the intestine to mount a proper immune response against potentially harmful antigens and microorganisms, a significant degree of luminal surveillance must occur, which is the capacity of the gut epithelia to modulate antigen transport to induce beneficial mucosal immune responses rather than adverse reactions. For efficient surveillance, antigens must be presented and transported across the gut in a balanced manner to prevent deleterious immune responses. Excess or inappropriate antigen transport could lead to an inappropriate response, resulting in clinical disease states. [57]

The mucosal barrier consists of several components (Fig. 2) that collectively prevent excessive immune responses. Nonimmunologic components constitute the first step in barrier function and include the following factors (Table 1): gastric pH, gastric enzymes, pancreatic exocrine products, and gut peristalsis. [57] In addition, macromolecules are restrained from reaching the intestinal microvillus surface by a glycoprotein-rich mucin layer and an intact microvillus enterocytic surface under growth factor control. Ample laboratory and clinical evidence shows that antigens can traverse the intestinal epithelium to enter the systemic circulation. [79] The immunologic components of this barrier as previously described (see Table 1) include functional intraepithelial and lamina propria T cells and mature reactive B cells. Surface IgA and IgM immunoglobulin production (see Fig. 2) constitutes a significant portion of epithelial surface protection. The Peyer's patches constitute another component of GALT that samples luminal antigens and modulates specific antibody responsiveness.

Many important aspects of the human mucosal barrier are not fully developed even by term, thereby allowing for less restricted antigen transport in neonates as compared with older children or adults. In animals, the process of decreased antigen absorption from infancy to adulthood is known as *closure*. [8] Closure initially applied only to immunoglobulin transport in the rodent intestine; however, more recently, important alterations in absorption were documented for antigens with unrecognized transport pathways, thereby broadening the concept. For example, the closure phenomenon was well demonstrated by Udall and

TABLE 1 -- COMPONENTS OF THE NEONATAL MUCOSAL DEFENSE BARRIER

Nonimmunologic	Immunologic		
Gastric acidity		Intraepithelial T cells (CD8)	
Gastric enzymes			Lamina propria T cells (CD4)
Pancreatic enzymes	B cells		
Peristalsis			Gut-associated lymphoid tissue (e.g., Peyer's patches)
Mucin layer			Secretory IgA system
Enterocytic molecules (cytokines, growth factors)		Non-IgA immunoglobulin IgE-mucosal mast-cell system	

557

Figure 2. Barriers to antigenic absorption in the intestine. Antigen entry is limited by nonimmunologic and immunologic mechanisms and by the structure of the epithelium. If potential antigens cross into the enterocyte, as they are being degraded and presented as antigens, signals are produced, causing T-cell activation and subsequent additional cytokine production and release.

colleagues, [76] who showed that bovine serum albumin (BSA) is transported from the lumen across the intestine in decreasing amounts with increasing age. In addition, later animal studies demonstrated that breast-fed rabbits had lower plasma BSA levels than did formula-fed rabbits, suggesting that breast milk may contain important factors that hasten the normal development of the mucosal barrier. [76]

> For normal production of immunoglobulin to be directed against luminal antigens, they must first cross the epithelium and interact with reactive B cells positioned in Peyer's patches or the lamina propria. [24]

558

Thus, processes that allow for normal antigen passage through the intestinal epithelium are the first steps to proper B-cell activation. As mentioned previously, M cells also can contribute to nonreceptor-mediated antigen passage through the epithelium and thus may aid in lymphocyte

activation. In contrast, T-cell responses require short peptides linked to MHCs. Antigens can be processed by the intestine in at least two ways: (1) antigen fragments can be produced directly during epithelial transit by intracellular proteolysis or (2) processed as the whole antigen once reaching APCs in the mucosal immune system. Varying immune responses likely occur depending on the pathway by which antigen is processed on entering from the gut lumen and by its presentation to the immune system. The components of these pathways are essential in understanding the mucosal immune response, and alterations in these components could cause pathologic changes.

Because fetuses make little immunoglobulin, most circulating antibody is passively acquired from the mother as IgG. In utero, this transport occurs through an IgG Fc receptor, which appears on the placental epithelium in the last trimester. The timing of this transport process is one reason why preterm infants have significantly diminished humoral responses. [22] In many animals, the transfer of IgG occurs from breast-milk intake through the small intestine. This transport process is controlled by the presence of receptors that bind the Fc portion of this large immunoglobulin molecule. Recently, Israel and colleagues [33] demonstrated the existence of IgG receptors on fetal intestine during early gestation, suggesting that IgG may directly bind to small intestinal enterocytes, resulting in IgG absorption across the gut lumen to the systemic circulation. This observation may represent an alternative pathway for the acquisition of maternal antibodies by the fetus in early gestation.

The principal immunoglobulin of mucosal defense is IgA. Typically coating the intestinal villi see (Fig. 2) , IgA serves as the first line of immunologic defense to prevent the transfer of antigens from the lumen across the gut. Associated functions include the inhibition of attachment and penetration of bacteria and toxins. [56] In the absence of sIgA, as eloquently described by Cunningham-Rundles and colleagues, [20] excessive antigen uptake occurs, leading to increased circulating immune complexes and precipitating antibodies to absorbed cow's-milk proteins.

Effective gut immune responses require the assistance of CD4+ T lymphocytes. For these T cells to work effectively, exogenous antigen must be presented by APCs. [56] [77] The exogenous antigen must be internalized by the APC, digested, and then a peptide portion of the antigen linked to a surface glycoprotein (MHC class II or HLA-D in humans) that ultimately interacts with the appropriate TCR. Macrophages, dendritic cells, and B cells serve as APCs because of their ability to express MHC class II. In the small intestine, epithelial cells (enterocytes) also express MHC class II determinants and may serve as antigen presenting cells (Fig. 3) (Figure Not Available) . Enterocytes isolated from human intestine present antigen effectively to activated T cells. [47] This observation has led to the hypothesis

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Figure 3. (Figure Not Available) Model of antigen presentation by the enterocyte. Macromolecules can enter membrane-bound organelles of the enterocyte. Instead of being destroyed, antigen is degraded and processed within the endosomal component into fragments that can bind to MHC Class II on the inner membrane of the components. From there, they are presented on the basolateral surface of the cell (**From** Sanderson IR, Walker WA: Uptake and transport of macromolecules by the intestine: Possible role in clinical disorders [an update]. Gastroenterology 104:622-639, 1993; with permission.)

that enterocytic MHC class II molecules may present processed antigen from the cytoplasm of the enterocyte through the basolateral surface to

intraepithelial or lamina propria lymphocytes localized just below the intestinal epithelium (see Fig. 3 (Figure Not Available)). MHC class II expression is increased in several conditions, including Crohn's disease, in which enhanced expression occurs on enterocytes from inflamed areas [60] ; however, to the authors' knowledge, heightened enterocytic MHC class II expression has not been reported in any neonatal conditions and is an important area for future research.

CYTOKINES AND GROWTH FACTORS IN THE MUCOSAL IMMUNE SYSTEM

The term *cytokine* refers to proteins secreted by cells in response to a variety of inducing stimuli. These molecules, in turn, influence the behavior of target cells via a surface receptor. Three types of cytokinecell interactions exist, with a response coming only from cells expressing suitable receptors. Autocrine stimulation occurs when the secreting cell

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possesses receptors to its own cytokines. Localized paracrine stimulation occurs if a secretory cell influences adjacent cells toward expressing the cytokine receptor. And finally, cytokines may be secreted into the bloodstream to interact with other target cells expressing receptors positioned elsewhere in the body, [67] similar to the classic endocrine stimulation by hormones. Cytokines are divided into major classification categories that include growth factors, small proteins that are able to promote proliferation and differentiation of various cell types. Epidermal growth factor is one such factor, named for its ability to stimulate proliferation of basal epithelial cells of the skin and intestinal mucosa. [57] Cytokines that are produced by or act on cells of the immune system are sometimes referred to as *lymphokines*. This important group includes the interleukins (IL), which are cytokines secreted by activated T lymphocytes to act principally on lymphocytes but also can affect other cells to produce biologic responses, [22] [83] such as the increased production and secretion of mucus by gut goblet cells. [57] Finally, there are colony-stimulating factors, which are proteins that directly stimulate the growth and differentiation of specific lineages of hematopoietic cells. [22] [80] Their specific actions are not further discussed because they are outside of the scope of this review.

The mechanisms by which T lymphocytes develop the capacity to produce trophic lymphokines during intrathymic and extrathymic development as well as in the gastrointestinal tract are poorly understood. Some investigators report that neonatal T cells have a restricted capacity to express specific lymphokines, such as IL-4, [44] and interferon (IFN), [81] strongly suggesting that neonates have markedly reduced cellular and humoral responses, predisposing them to severe, life-threatening infections.

It is well recognized that immunoglobulin class expression is influenced by cytokines that either stimulate B-cell precursors directly or mediate immunoglobulin class switching. [81] Also, the mucosal immune system differs from other aspects of the lymphoid system by the pre-dominance of IgA in its secretions. Cytokines produced by cells in intestine can influence an enhanced IgA production. Although several cytokines have been characterized that stimulate IgA secretion, no direct evidence for in vivo stimulation has been shown in humans.

One cytokine proposed to stimulate IgA secretion is IL-5. This factor was first isolated from mouse T-cell supernatants and was shown in vitro to cause a fourfold to fivefold increase in IgA secretion in Peyer's patch B-cell cultures stimulated with the mitogen lipopolysaccharide (LPS). [7] This increase was augmented further by the addition of IL-4, which alone had no effect on endogenous IgA production. Both cytokines are produced by a subset of helper T cells, Th2 cells. [49] Moreover, evidence in vivo suggests that IL-5 can upregulate IgA secretion; transgenic mice carrying the IL-5 transgene have increased levels of IgA and IgM. [74]

In early studies to determine the role of cytokines on IgA production, IL-1, IL-3, granulocyte-macrophage colony stimulating factor, and

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IFN all were found to have no effect on LPS-stimulated B cells; however, IL-2 produced a twofold to threefold enhancement of IgA secretion in similar cell culture systems. [41] To the authors' knowledge, however, no in vivo data have been reported yet on IL-2 and IgA production.

Other cytokines thought to augment IgA secretion and possibly enhance mucosal defense include IL-6 [41] and transforming growth factor (TGF). [17] Again, both of these pleiotropic factors increase IgA production in LPS-stimulated Peyer's patch B-cell cultures. In addition, further studies demonstrated the additive effect on IgA production of IL-2 with TGF was much greater than the combined effects of these cytokines alone.

Overall, Peyer's patches preferentially generate precursors of IgA-secreting cells. The cytokine environment then helps dictate the maturation process of these B cells with subsequent IgA production. Although the precursors of IgA-secreting cells are generated preferentially in the Peyer's patches, maturation of these cells leading to IgA secretion also may occur in other sites, for example, the lamina propria, an IgA-affector site. [73]

BACTERIAL ADHERENCE AND MUCOSAL IMMUNITY

The first step in gaining access to the human host for bacteria and other pathologic microorganisms includes successful adhesion to the intestinal surface. The tendency for bacteria to grow attached to surfaces has long been recognized. [70] Moreover, the crucial role of adherence in intestinal virulence was demonstrated initially in piglet diarrheal studies caused by *Escherichia coli*. The virulence was associated with a DNA plasmid encoding the K88 fimbrial adhesin (adherence molecule) and exotoxin. [62] Deletion of the plasmid abolished virulence.

Bacteria stimulate cytokine production by the host. Molecules such as endotoxin produced by microorganisms can activate cytokine production in cells such as lymphocytes, fibroblasts, and monocytes. [70] In fact, many studies have confirmed that septic symptoms from bacterial gut invasion can be prevented by molecules that directly inhibit tumor necrosis factor, IL-1, [75] and possibly IL-6 production. [45] The attachment of bacteria to epithelial cell lines is key for cytokine responses. In some in vitro studies, attaching bacteria to the gut epithelia elicited a greater cytokine response in these cell lines than did nonattaching bacteria. [45] [70] Overall, it has been well

demonstrated that bacterial adherence properties influence the epithelial cytokine response.

Anti-Adhesive Mucosal Defense Mechanisms

The development of soluble or insoluble gut luminal factors that prevent bacterial adhesion and invasion is one method to prevent the unwanted seeding of gut microorganisms into the circulation. Nature,

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at least, has produced many such antiadhesive factors; IgA is one such important molecule. This secretory immunoglobulin (sIgA), which overlays the small intestinal surface, acts to protect the mucosa. By occupying receptor binding sites on bacterial fimbriae, the sIgA molecules may prevent the subsequent attachment by bacteria to enterocyte surface receptors. [3] In contrast with this observation, clinical studies have demonstrated animals that have diminished mucosal antibody production and yet do not necessarily have increased mucosal infections, [61] suggesting that the mucosal defense barrier is composed of multiple highly functioning mechanisms to aid in this protection (see Fig. 2).

Human breast milk contains numerous beneficial immune and nonimmune factors. For example, nonimmunoglobulin glycoproteins have been demonstrated to have bacterial antiadhesive properties. [70] Although the mechanisms of inhibition of these pathways have not yet been elucidated, Schroten and colleagues [59] demonstrated that human milk glycoconjugates can inhibit directly the attachment of pathogenic *E coli* to mucosal epithelial cells, thereby suggesting an additional benefit for breast milk in neonates.

NEONATAL DISEASE AND GUT-ASSOCIATED IMMUNE FUNCTION

In this section, three common and sometimes life-threatening conditions that afflict premature and term neonates are discussed. Emphasis is placed on the immunologic basis for the pathophysiology of these disease states.

Necrotizing Enterocolitis

Necrotizing enterocolitis (NEC) is one of the leading causes of morbidity and mortality in newborns, with some reports estimating greater than 10% incidence among very low birth weight (VLBW) infants weighing less than 1500 g and with an associated mortality rate as high as 35% among those afflicted with this condition. [68] Although the etiology is still unclear, many risk factors have been associated with this gastrointestinal condition, including prematurity and enteral feeding. Other risk factors stemming from prematurity include asphyxia, patent ductus arteriosus, bacterial infections, and gut hypoxia (see Fig. 3 (Figure Not Available)). Fulminant NEC is characterized by feeding intolerance; bloody stools; intestinal inflammation; and pneumatosis intestinalis, which, if progressive, leads to gut perforation, peritonitis, shock, and death.

The research into understanding the pathophysiology of NEC has been extensive. Significant studies have demonstrated that newborn infants not only have systemic immunologic deficiencies but also gastrointestinal mucosal immune impairments that may predispose them to aberrant gut bacterial overgrowth and subsequent mucosal

inflammation

563

and injury. [13] These impairments are most likely exacerbated by hypoxia and are affected by malabsorbed high-osmolarity food substrates in the intestinal tract (Fig. 4). Some of the transient immunodeficiencies in newborns, especially preterm infants, include reduced numbers of B cells in their intestinal mucosa, decreased IgA levels, and fewer intestinal T cells. Although intestinal T-cell function is not completely understood, these lymphocytes may be needed to preserve the integrity of the mucosal surface. In addition, strong evidence shows that the mucosa in preterm infants' intestinal tract is more permeable to bacteria, carbohydrates, and proteins than in older infants and children. [55] For example, lactulose, albumin, and lactoglobulin permeability (measurements of small intestinal integrity) are higher as measured by serum levels of>

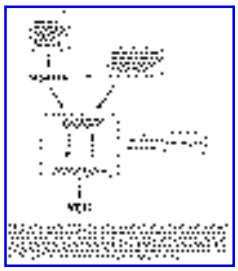

Figure 4. The causes of NEC. Risk factors associated with prematurity lead to general ischemia in the intestinal tract. Then, compounded by the problems of immune immaturity, bacterial invasion, and formula feeds, the cycle of loss of the mucosal epithelial barrier and mucosal inflammation occurs. This cycle also can be initiated by any of these factors alone. It is this cycle that eventually produces the clinical signs of NEC. Important cytokines, such as TNF-alpha PAF, and IL-6, all have been found to be highly expressed.

564

these proteins in preterm infants fed with fortified cow's-milk formulas. Increased intestinal permeability to macromolecules stemming from impaired mucosal defenses has been regarded as a possible mechanism contributing to more generalized bowel mucosal injury and fulminant NEC. [13]

The proposed relationship between cytokines, aberrant T-cell activation, and the pathogenesis of NEC is not entirely new. Biochemical mediators possibly associated with NEC are listed in Table 2 . Harris and others have shown a marked increase in IL-6 in the plasma of infants with NEC (see Fig. 4), whereas levels of the potent proinflammatory cytokine tumor necrosis factor (TNF) were elevated only slightly compared with controls. [28] In normal small intestine, Tan and colleagues [72] demonstrated that Paneth's cells are the major source of TNF transcripts, and in the small intestine of patients with NEC, there is a significant increase in TNF messenger RNA formation in Paneth's cells and abundant increase in infiltrating eosinophils and macrophages, suggesting that TNF may have an important role in the pathophysiology of NEC. In addition, Lionetti and colleagues [46] proposed that abnormally activated T cells in the lamina propria could lead to mucosal tissue destruction, similar to the pathology observed in infants with NEC. Other key studies have linked the destructive action of TNF with the lipid-derived platelet activating factor (PAF) to an NEC-like pathology in animals also (see Fig. 4). [13] [14] [15] [29]

Several experimental therapies have been proposed that either may help prevent or be used to treat NEC. Antenatal steroids (see Table

2) decreased the incidence of NEC in rat pups [32] and in many large multicentered, blinded trials of preterm infants, [5] suggesting that glucocorticoids may help to accelerate intestinal maturation, decrease inflammation, or both. [32] In addition, Eibl and colleagues [23] in Austria showed that oral administration of IgA-IgG may prevent the development of NEC in VLBW infants, most likely from a combination of antiadherence of bacteria by IgA on the intestinal mucosal surface and by the opsonizing and antitoxic properties of IgG.

One mechanism previously proposed to prevent NEC is to strengthen and protect the epithelial barrier in the neonatal intestinal tract. [13] Mediators, such as epidermal growth factor (EGF), have been

TABLE 2 -- BIOCHEMICAL MEDIATORS IN NEC

Protective	Associated
Nitric oxide	Endothelin-1
Platelet-activating factor antagonists	Platelet activating factor
Acetylhydrolase	Tumor necrosis factor
Corticosteroids	Leukotriene C4
IgG	Oxygen radicals
IgA	Interelukin-6
Epidermal growth factor	Thromboxane B2

shown both in vitro and in animal models to enhance proliferation of intestinal mucosal cells; however, animal and human studies using EGF and other intestinal trophic factors to prevent NEC have not been successful thus far. [69] There is definitely a great need to identify new therapeutic agents for newborns, especially preterm infants, that may induce intestinal epithelial cell proliferation and regeneration, with the added benefit of protecting the intestine from the pathologic insults leading to NEC.

Milk Protein Enteropathy

Food protein enteropathy is a disease of infancy and early childhood in which a hypersensitivity reaction to food proteins produces varying degrees of damage to the intestinal mucosa and mucosal dysfunction. In early infancy, the best example of food protein gastroenteropathy is that induced by cow's milk protein. [40] Soy protein enteropathies also occur but with much less frequency and damage. [1]

The usual symptoms of milk protein enteropathy consist of diarrhea, vomiting, and failure to thrive. Significant malabsorption results in microcytic hypochromic anemia. Symptoms usually present within the first 6 months of introducing milk formula feeds. Resolution of symptoms usually occurs after removing all milk from the diet. [48]

Although the mechanism of disease is unknown, the symptoms usually resolve with increasing age, supporting the theory that immaturity of the infant intestinal mucosa and mucosal immune system may seem to have a significant role. Delays in maturation of

the normal mucosal defense barrier may lead to the priming of the newborn immune system, with subsequent heightened sensitization to intact food antigens on re-exposure. [51] Other studies have postulated that milk protein enteropathy may follow an acute bout of gastroenteritis [34] or result, more likely, from transient decreased levels of sIgA in infancy, which would allow more milk antigens to cross the epithelia. [31]

The role of antibody production in milk protein enteropathy also is uncertain. In the first few days of life, infants rarely exhibit measurable serum antibodies against cow's milk proteins; however, by age 1 to 2 years, almost all formula-fed infants have measurable levels of serum IgA and IgG against these proteins. Total serum IgE is often normal, and IgE against specific milk proteins rarely is evident. [21]

Neonatal Intestinal Infections

Many diverse bacteria and viruses can elicit intestinal infections in the newborn period (Table 3) that manifest themselves often with severe diarrhea and colicky abdominal pain. If allowed to progress, severe dehydration, malnutrition, and even death follow in these affected newborns. Instead of mentioning all implicated pathogens, the common

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TABLE 3 -- PATHOGENS ASSOCIATED WITH NEONATAL INTESTINAL INFECTIONS

Bacteria	Viruses
Escherichia coli	Enterovirus
Staphylococcus epidermidis	Rotavirus
Clostridium difficile:	Adenovirus
Salmonella	
Shigella	
Campylobacter	
Yersinia enterocolitica	

immune-mediated mechanism of virulence is discussed. Moreover, bacterial enteric pathogens are more thoroughly understood than viral or even parasitic infectious agents with respect to the manner in which they interact with the host intestinal mucosa.

Among the most recognized virulent factor of bacterial enteropathogens are fimbrial attachment factors that promote adherence to gut epithelia [38] and cytotoxic enterotoxins that lead to increased intestinal secretion (diarrhea). [43] Expression of key outer membrane proteins also allows for bacterial internalization by epithelial cells, [30] and certain secreted cytotoxins suppress protein synthesis, which leads to mucosal epithelial cell death. [11]

Studies of the differences in bacterial enterotoxin receptor expression during human ontogeny also have been conducted. Chu and Walker $r:16 concluded that differences in bacterial enterotoxin receptor expression, as measured by the number of receptors and receptor

affinity on the microvillus membrane of enterocytes, may account for an increased or decreased intestinal responsiveness to specific bacterial toxins in the small intestine. Moreover, fetal and postnatal age-related differences in the structure and function of membrane receptors for bacterial toxins have been analyzed [42] and suggest that an increased receptor affinity for a specific enterotoxin may enhance the intestinal secretory response of enterocytes after exposure to that toxin.

Almost all bacterial and viral enteropathogens stimulate the intestinal immune system, with the first evidence being the detection of IgA-secreting cells in the peripheral blood at the onset of an infection. [35] Subsequently, pathogenic antigens induce the production of specific sIgA antibodies. These mucosal antibodies have an enhanced capability to protect the gut mucosa as shown experimentally, whereby passively administered breast milk IgA immunoglobulin concentrates conferred a very high level of protection in adults challenged with enterotoxigenic *E coli* or *Shigella*. [71] Moreover, antibodies targeted against bacterial enteropathogens often are detectable in the serum even after a noninvasive infection, such as with cholera. [26] Interestingly, infections in the neonatal period with intracellular bacteria, although rare in occurrence, can elicit strong MHC class I restricted cytotoxic lymphocyte responses previously reserved to viral infections. [36]

567

Immunoglobulin therapy for neonatal enteric infections has been proposed; however, its role is still uncertain. Only one clinical study has shown some promise with immunotherapy. Barnes and colleagues [4] demonstrated that administration of intramuscular immunoglobulin given concurrently with feeds during the first week of life for 75 low birth weight infants with endemic rotavirus was associated with delayed excretion of rotavirus and milder symptoms of disease. Overall, the majority of studies using prophylactic immunoglobulin have been disappointing, because the risk for systemic infection from enteric infections is very high in hospitalized, high-risk infants. As mentioned previously, the best way to provide oral immunoglobulins is in the form of breast milk, which contains both characterized and noncharacterized anti-infectious agents. [4] [27]

SUMMARY

This review has traced the ontogeny of the human mucosal immune system, speculating that appropriate gut immune responses are essential in preventing many significant neonatal enteric diseases. Because the gastrointestinal tract serves as the portal of entry for many potential antigens, its mucosal immune function is essential in controlling antigenic responses and ensuring systemic tolerance. A thorough understanding of the development of the entire immune system is essential in defining intestinal mucosal immune function. From the protective barrier covering the enterocyte to the intraepithelial T lymphocytes, these components work together to limit antigen passage from the gut lumen to the underlying immune cells and, thus, promote normal immunity and tolerance. When abnormalities exist or when this immune barrier has not matured fully, conditions afflicting newborns, especially preterm infants, occur. Necrotizing enterocolitis, milk-protein enteropathy, and enteric bacterial infections are only three clinical examples of how aberrant gut immune-mediated defenses may have a significant role in their pathogenesis. In clinical practice, it is not only important to recognize

these conditions at their onset but also to understand the basis for the underlying illness and identify newborns who are at an increased risk of acquiring them.

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