Necrotizing Enterocolitis: The Role of the Immune System

Patricia Denning, M.D.
Associate Professor in Pediatrics
Division of Neonatology
Emory University School of Medicine
What is NEC?
What is NEC?

“Necrotizing enterocolitis is a serious disease that occurs when the intestinal tissue become damaged and begins to die. It most often affects premature infants.”
What is NEC? Symptoms?
What is NEC?

Symptoms?

- **Systemic signs**
  - Apnea/bradycardia
  - Temperature instability
  - Hypotension

- **Intestinal signs**
  - Abdominal distention/tenderness
  - Bloody stools
  - Gastric residuals/emesis
  - Absent bowel sounds
  - Peritonitis
What is NEC? Signs?
What is NEC?  
Signs?

- Laboratory
  - Metabolic acidosis
  - Neutropenia
  - Thrombocytopenia
  - DIC

- Radiologic
  - Asymmetric bowel gas pattern
  - Pneumatosis
  - Fixed bowel loop
  - Portal venous air
  - Pneumoperitoneum
What is NEC?  
Epidemiology?
What is NEC? Epidemiology?

- **Incidence:**
  - Up to 10% in VLBW
  - Inversely proportional to gestational age
    - Smaller infants also with higher morbidity and mortality
  - Rare in term infants
    - Usually associated with predisposing/underlying disorders

- **Morbidity and Mortality:**
  - Overall 15-30%
  - 20-40% require surgery
    - With up to 50% mortality
  - Survivors have worse neurodevelopmental outcome

![Graph showing incidence of NEC](image)

32-33 wks
What is NEC?
Risk factors?
What is NEC?
Risk factors?

- Prematurity
- Feeding
- Prolonged empiric antibiotic use
What Causes NEC?
“Immature intestinal host defenses predispose the premature infant gut to injury. An abnormal bacterial colonization pattern with a deficiency of commensal bacteria leads to further breakdown of these host defense mechanisms, predisposing the infant to NEC.”
What Causes NEC?

- Immature intestinal host defenses
  - Barrier function
  - Immune response

- Abnormal bacterial colonization
  - Prolonged antibiotic use increases risk
  - Probiotics decrease risk
  - Stool colonization studies show a gammaproteobacter bloom associated with NEC
What Causes NEC?

- Intestinal inflammation
- Increased intestinal injury
- Intestinal barrier damage
- Opportunistic infection
- Increased intestinal injury
- Intestinal barrier damage
What Causes NEC?

We will review the role of the immune system in NEC pathogenesis

- Role of passive immunity
- Role of physical barriers
- Role of immune cells
Role of the Immune System

What are the main arms of the immune system?
Role of the Immune System

What are the main arms of the immune system?

- Innate
- Adaptive
Role of the Immune System

What are the main arms of the immune system?

- **Innate**
  - Rapid innate immune cell recruitment and cytokine production in response to barrier threats is a highly evolutionarily conserved process critical for host protection

- **Adaptive**
What are the main arms of the immune system?

- **Innate**
  - Rapid innate immune cell recruitment and cytokine production in response to barrier threats is a highly evolutionarily conserved process critical for host protection.

- **Adaptive**
  - Adaptive immune response thought to regulate the innate response, which if left unchecked can lead to disease.
Role of the Immune System

Abbas and Litchman: Basic immunology
Role of the Immune System

Adaptive immunity is underdeveloped in the neonate
Role of the Immune System

Adaptive immunity is underdeveloped in the neonate

Thus, adaptive immunity transferred from mother to infant is meant to protect until infant’s own adaptive immunity develops (passive immunity)
Role of the Immune System: Passive Immunity
Role of the Immune System: Passive Immunity

- Placental transfer of IgG
  - Mediated by the FcRN receptor
  - Starts at 13 weeks gestation
  - Greatest amount occurs in last 4 weeks

- Secretory IgA in breast milk
  - Higher levels in preterm breast milk
  - However, oral immunoglobulin does not reduce NEC
# Role of the Immune System: Passive Immunity

<table>
<thead>
<tr>
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<th>Time of Maturation</th>
<th>Role in NEC</th>
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<td><strong>Placental transfer of IgG</strong></td>
<td>Starts at 13 weeks</td>
<td>Deficiency may predispose to NEC</td>
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<td>Mature by term</td>
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<td>Preterm infants with reduced IgG transfer</td>
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<tr>
<td><strong>Breast milk transfer of sIgA</strong></td>
<td>Preterm human milk with higher levels</td>
<td>Unclear benefit of oral IgA administration in decreasing risk of NEC</td>
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- Placental transfer of IgG:
  - Starts at 13 weeks
  - Mature by term
  - Preterm infants with reduced IgG transfer
  - Deficiency may predispose to NEC

- Breast milk transfer of sIgA:
  - Preterm human milk with higher levels
  - Unclear benefit of oral IgA administration in decreasing risk of NEC
Role of the Immune System: Passive Immunity

• Breast milk contains additional protective factors
  • Antimicrobial
  • Anti-inflammatory
  • Promote maturation of intestinal host defenses

• Components include
  • Nutrients
  • Bioactive proteins
  • Immunoregulatory cytokines
  • Growth factors
## Additional Protective Factors in Breast Milk

<table>
<thead>
<tr>
<th>Nutrients: Oligosaccharides</th>
<th>Mechanism of Protection</th>
<th>Role in NEC</th>
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<tr>
<td><strong>Caseins</strong></td>
<td>Promote growth of commensal bacteria</td>
<td>Oligosaccharide supplementation may reduce NEC risk</td>
</tr>
<tr>
<td></td>
<td>Stimulate increased Paneth cell and goblet cell number and possibly function</td>
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<td>May also reduce bacterial adherence to intestinal epithelia</td>
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<th>Bioactive proteins:</th>
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<tr>
<td>Lysozyme</td>
<td>Antibacterial, synergistic with lactoferrin</td>
<td></td>
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<tr>
<td>Lactoferrin</td>
<td>Antibacterial, antifungal, antiviral</td>
<td>Lactoferrin supplementation (+/- probiotics) may reduce NEC risk</td>
</tr>
<tr>
<td>PAF-AH</td>
<td>Inactivates PAF (key mediator of NEC)</td>
<td></td>
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## Additional Protective Factors in Breast Milk

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<th>Immunoregulatory Cytokines:</th>
<th>Mechanism of Protection</th>
<th>Role in NEC</th>
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<tr>
<td>IL-10</td>
<td>Anti-inflammatory cytokine important for intestinal homeostasis</td>
<td>IL-10 supplementation in animal models is protective</td>
</tr>
<tr>
<td></td>
<td>Genetic defects in IL-10R cause colitis</td>
<td>Increased IL-10 in human milk associated with a decreased risk of NEC</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Involved in regulating inflammation and wound healing</td>
<td>Low levels in human milk may predict feeding intolerance in growth restricted infants</td>
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# Additional Protective Factors in Breast Milk

## Mechanism of Protection

<table>
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<tr>
<th>Growth factors:</th>
<th>IGF family</th>
<th>EGF family</th>
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<tr>
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<td>Promotes IEC proliferation; reduces IEC apoptosis</td>
<td>Promotes IEC proliferation/differentiation/restitution and TJ expression</td>
</tr>
<tr>
<td></td>
<td>Reduces IEC autophagy</td>
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</tr>
<tr>
<td></td>
<td>Increases mucin production</td>
<td>Increases mucin production</td>
</tr>
<tr>
<td></td>
<td>Inhibits TLR-4 signaling</td>
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<tr>
<td></td>
<td>Promotes anti-inflammatory macrophages</td>
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## Role in NEC

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<td></td>
<td>IGF supplementation reduces NEC in animal models</td>
<td>Decreased EGF associated with increased NEC risk</td>
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<tr>
<td></td>
<td>EGF supplementation reduces NEC in animal models</td>
<td>EGF supplementation in humans promotes intestinal mucosa trophic effects</td>
</tr>
</tbody>
</table>
Role of the Immune System

Innate immunity:
- Epithelial barriers
- Phagocytes
- Complement
- NK cells

Adaptive immunity:
- B lymphocytes -> Antibodies
- T lymphocytes -> Effector T cells

Hours:
- 0
- 6
- 12

Days:
- 1
- 3
- 5

Time after infection:
- 1
- 3

Abbas and Litchman: Basic immunology
Role of the Innate Immune System: Physical Barriers
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- Gastric acid
  - Kills pathogens
  - H2 blockers increase NEC
Role of the Innate Immune System: Physical Barriers

- **Gastric acid**
  - Kills pathogens
  - H2 blockers increase NEC

- **Mucus**
  - Prevents epithelial contact with luminal bacteria
  - Stabilizes trophic and reparative factors (trefoil factor, EGF)
Role of the Innate Immune System: Physical Barriers

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- **Epithelial barrier**
  - Apical junctional complex (APC)
  - Receptors (PRRs/TLRs) on epithelia sense pathogens
    - Signal transduction → cytokine release → attract leukocytes
Role of the Innate Immune System: Toll-like Receptors
Role of the Innate Immune System: Toll-like Receptors
Role of the Innate Immune System: Physical Barriers

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    - Signal transduction → cytokine release → attract leukocytes

- **Antimicrobial peptides**
  - Secreted by epithelial cells, neutrophils, Paneth cells
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<th>Physical Barrier Component</th>
<th>Time of Maturation</th>
<th>Role in NEC</th>
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<tr>
<td><strong>Gastric Acid</strong></td>
<td>Mature secretion by 24 weeks</td>
<td>Acid suppression associated with an increase risk of NEC</td>
</tr>
<tr>
<td><strong>Mucus layer (Goblet Cells)</strong></td>
<td>Term</td>
<td>Deficiency may predispose to NEC</td>
</tr>
<tr>
<td></td>
<td>Premature infants with immature mucus layer</td>
<td>NEC causes reduced number &amp; reduced production of mucins and trefoil factor</td>
</tr>
<tr>
<td><strong>Epithelial barrier (AJC)</strong></td>
<td>Mature structure of AJC at 12 wks gestation (in utero)</td>
<td>Immature barrier function may increase NEC risk</td>
</tr>
<tr>
<td></td>
<td>Premature infants with increased intestinal permeability</td>
<td>Breast milk and probiotics may reduce NEC risk by improving epithelial barrier function</td>
</tr>
<tr>
<td></td>
<td>Mature function at term</td>
<td></td>
</tr>
<tr>
<td><strong>Antimicrobial peptides</strong></td>
<td>Paneth cells detectable at 12 wks gestation with secretory capability at 13-20 wks</td>
<td>Deficiency of Paneth cell number and function may predispose to NEC</td>
</tr>
<tr>
<td></td>
<td>Premature infants with decreased Paneth cell number and secretory capability</td>
<td>NEC causes upregulated Paneth cell numbers but these cells are dysfunctional</td>
</tr>
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</table>
Role of the Innate Immune System: Physical Barriers

Building the healthy intestine

Healthy State

IEC (intestinal epithelial cell)
Building the healthy intestine

Healthy State

AJC (apical junctional complex)

IEC
Building the healthy intestine

Healthy State

IEC

Mucus

AJC
Building the healthy intestine

Healthy State

Commensal microbiota regulated by antimicrobial peptides

Mucus

IEC
Building the healthy intestine

Healthy State

Microbiota: *Bifidobacteria, Lactobacillus* enriched

Mucus

IEC
Building the healthy intestine

Healthy State

Breast milk-derived factors: IgA, HMO, EGF, IL-10, lactoferrin, lysozyme, TGFβ

Microbiota: *Bifidobacteria, Lactobacillus* enriched

Mucus

IEC
Role of the Immune System: Innate Immune Cells
Role of the Immune System: Innate Immune Cells

- Intraepithelial lymphocytes (γδ T cells)
  - One of the earliest innate immune cells recruited to the gut
  - Fortifies the epithelial barrier via IL-17A and EGF
  - Reduced number and function seen in human NEC
Building the Healthy Intestine

Healthy State

Breast milk-derived factors: IgA, HMO, EGF, IL-10, lactoferrin, lysozyme, TGFβ

Microbiota: *Bifidobacteria, Lactobacillus* enriched

Mucus

IEC

γδ IEL

IL-17

EGF
Role of the Immune System:
Innate Immune Cells

- Natural killer cells (NK cells)
  - Protect intestinal barrier
  - Regulate inflammation
  - Reduced number in premature infants may predispose to NEC
Building the Healthy Intestine

Healthy State

Breast milk-derived factors: IgA, HMO, EGF, IL-10, lactoferrin, lysozyme, TGFβ

Microbiota: Bifidobacteria, Lactobacillus enriched

Mucus

IEC

γδ IEL

IL-17 EGF IL-22

NK cell
Role of the Immune System: Innate Immune Cells

- Neutrophils (PMNs)
  - Recruited during epithelial damage
    - Kills invading microbes
    - However, can cause bystander tissue damage
    - Also mediates repair after epithelial damage via IL-22 secretion
    - May mediate barrier fortification during initial bacterial colonization
  - Early neutropenia may correlate with increased NEC risk
Building the Healthy Intestine

Healthy State

Breast milk-derived factors: IgA, HMO, EGF, IL-10, lactoferrin, lysozyme, TGFβ

Microbiota: Bifidobacteria, Lactobacillus enriched

Mucus

IEC

γδ
IEL

IL-17
EGF
IL-22

NK cell

PMN
Role of the Immune System: Antigen Presenting Cells

Antigen Presentation

1. A phagocyte "eats" a bacteria.
2. Parts of the bacteria (antigen) goes to the surface of the phagocyte.
3. The phagocyte presents the antigen to a helper T cell.
4. The helper T cell is activated.
Role of the Immune System: Antigen Presenting Cells

- Macrophages (Mφs)
  - Homeostatic resident macrophages recruited during fetal development (anti-inflammatory)
    - Important for tolerance to commensal bacteria
  - Blood monocytes recruited during epithelial damage differentiate into M1 macrophages
    - Secrete pro-inflammatory cytokines: IL-1β, IL-6, IL-8,
Role of the Immune System: Antigen Presenting Cells

Classical Activation
IFN\(\gamma\), LPS, TNF\(\alpha\)

Alternative Activation
IL-4, IL-13, IL-10, TGF\(\beta\)

M1
The Dark Side
ROS, RNS, TNF\(\alpha\), IL-1, IL-6, IL-12, IL-23, chemokines
Cytotoxicity, Tissue Injury

M2
The Jedi Order
IL-10, TGF\(\beta\), PDGF, VEGF, EGF, arginase
Immune Suppression, Tissue Repair

The Force
Role of the Immune System: Antigen Presenting Cells

- Macrophages (Mφs)
  - Homeostatic resident macrophages (M2) recruited during fetal development (anti-inflammatory)
    - Important for tolerance to commensal bacteria
    - Secrete IL-10, TGFβ
  - Blood monocytes recruited during epithelial damage differentiate into M1 macrophages
    - Secrete pro-inflammatory cytokines: IL-1β, IL-6, IL-8, IL-12, TNFα

- Dendritic cells (DCs)
  - Also promote tolerance during steady-state by inducing T regulatory cells
Building the Healthy Intestine

Healthy State

Breast milk-derived factors: IgA, HMO, EGF, IL-10, lactoferrin, lysozyme, TGFβ

Microbiota: *Bifidobacteria, Lactobacillus* enriched

Mucus

IEC

γδ IEL

IL-17 EGF IL-22

IL-10

TGFβ

NK cell

PMN

M2 Mφ
Role of the Immune System: Adaptive Immune Cells

Extracellular bacteria
Fungi
Autoimmunity

IL-21
IL-17a
IL-17f
IL-22
(IL-10)

RORγt/Stat3

TGFβ (IL-1)+
IL-6,21,23

Th17

IFNγ+IL-12

Th1

T-bet/Stat4

Naïve CD4 T

GATA-3/Stat5

IL-4+IL-2

IL-4
IL-5
IL-13
IL-25
Amphiregulin
IL-10

Extracellular parasites
Allergy and asthma

Immune tolerance
Lymphocyte homeostasis
Regulation of immune responses

Intracellular pathogens
Autoimmunity

IFNγ
IL-2
LTα
(IL-10)
Role of the Immune System: Adaptive Immune Cells

- CD4+ T Lymphocytes
  - TGF-β and IL-10 promote homeostasis by inducing T regulatory cells (Treg)
  - IL-1β, IL-8, TNF, IL-12 cause inflammation by inducing Th1 and Th17 cells
Role of the Immune System: Adaptive Immune Cells

- CD4+ T Lymphocytes in NEC
  - Th17 cells enriched in NEC tissue
    - Recruited in response to TLR-4 activation
  - Treg cells reduced in NEC tissue
Building the Healthy Intestine

Healthy State

Breast milk-derived factors: IgA, HMO, EGF, IL-10, lactoferrin, lysozyme, TGFβ

Microbiota: Bifidobacteria, Lactobacillus enriched

Mucus

IEC

γδ IEL

IL-17
EGF
IL-22

DC

IL-10

TGFβ

NK cell

PMN

Treg

M2 Mφ
Healthy State

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Microbiota: *Bifidobacteria, Lactobacillus* enriched

Mucus

IEL γδ IL-17 IL-22 EGF

DC IL-10 TGFβ

NK cell PMN Treg M2 Mφ Th17

NEC

Formula feeding

Microbiota: γ-proteobacter enriched, UPEC

Mucus

IEL TLR4+ IEC

Blood monocyte

M1 Mφ

PMN Treg

IL-1, IL-6, IL-8, IL-12, TNF