Nutrition & Gut Immunity

Kenneth A. Kudsk, MD FACS
Professor of Surgery
University of Wisconsin - Madison
Madison, Wisconsin

No conflicts of interest to disclose
Survival vs. Malnutrition
(after septic peritonitis with E. coli)

- Well Nourished: 70%
- Depleted: 10%
- Depleted + Re-fed: 60%
- TPN: 0%
- TPN + Fat: 5%
Survival After Septic Peritonitis

% Survival

Day

IG-TPN
Chow
IV-TPN

% Survival

100
90
80
70
60
50
40
30
20
10
0

1
2

Day
Enteral / Parenteral Study

Septic Morbidity vs. Severity of Injury

<table>
<thead>
<tr>
<th>Injury Severity</th>
<th>Enteral</th>
<th>Parenteral</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blunt</td>
<td>16</td>
<td>10</td>
</tr>
<tr>
<td>Penetrating</td>
<td>35</td>
<td>35</td>
</tr>
<tr>
<td>ISS &lt;20</td>
<td>17</td>
<td>20</td>
</tr>
<tr>
<td>ISS &gt;20</td>
<td>34</td>
<td>35</td>
</tr>
<tr>
<td>ATI &lt;24</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>ATI &gt;24</td>
<td>27</td>
<td>21</td>
</tr>
<tr>
<td>ISS &gt;20</td>
<td>20</td>
<td>12</td>
</tr>
</tbody>
</table>

p-values:
- Blunt: p = 0.08
- Penetrating: p < 0.05
- ISS <20: NS
- ISS >20: p < 0.002
- ATI <24: NS
- ATI >24: p < 0.005
- ISS >20: p < 0.003
## INFECTIONS

<table>
<thead>
<tr>
<th></th>
<th>Enteral</th>
<th>TPN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pneumonia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all patients</td>
<td>11.8 %</td>
<td>31 %</td>
</tr>
<tr>
<td>severely inj.</td>
<td>18 %</td>
<td>56 %</td>
</tr>
<tr>
<td><strong>Abscess</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all patients</td>
<td>3.9 %</td>
<td>17.8 %</td>
</tr>
<tr>
<td>severely inj.</td>
<td>9 %</td>
<td>33 %</td>
</tr>
</tbody>
</table>
## INFECTIONS

<table>
<thead>
<tr>
<th></th>
<th>Enteral</th>
<th>TPN</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pneumonia</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all patients</td>
<td>11.8 %</td>
<td>31 %</td>
</tr>
<tr>
<td>severely inj.</td>
<td>18 %</td>
<td>56 %</td>
</tr>
<tr>
<td><strong>Abscess</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>all patients</td>
<td>3.9 %</td>
<td>17.8 %</td>
</tr>
<tr>
<td>severely inj.</td>
<td>9 %</td>
<td>33 %</td>
</tr>
</tbody>
</table>
## INFECTIONS

<table>
<thead>
<tr>
<th></th>
<th>Enteral</th>
<th>TPN</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pneumonia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>all patients</td>
<td>11.8 %</td>
<td>31 %</td>
</tr>
<tr>
<td>severely inj.</td>
<td>18 %</td>
<td>56 %</td>
</tr>
</tbody>
</table>

**WHY ????
The Principle

If nutrition support and enteral feeding *really* make a difference, there should be a reason... and it should be measurable... and it should be testable in clinical populations.
What is it? OFFENSE ? (the bacteria)
Bacterial Response to Stress

Results
MICROGRAPHS OF *E. coli*
Bacterial Gene Expression

Non-Hostile Environment

Virulence Genes Downregulated

Stressors

Genes Upregulated

Adherence Appendages
  (e.g. *Ps* Lectins)

Attachment
Stressors of Host - Microbe Status

- Antibiotics
- Vasoactive drugs
- Block gastric acid
- Opiates - Motility
- TPN
- Starvation
Mortality, Stress and Adhesion

Alverdy, Ann Surg 2000
or the defense?
DEFENSE:
Mucosal immunity
Innate immunity
Cells and molecules that defend the intestinal mucosal surface

- Lumen
- Outer mucus layer
- Inner mucus layer
- Bacteria
- Antimicrobial proteins
- Tight junction
- γδ intraepithelial lymphocyte
- Phagocytosis of invasive bacteria
- Transcytosis
- IgA
- Epithelial cell
- IgA-secreting plasma cell

Macrophage
Mucosal Immunity

Organization

Epithelium

Naïve T/B lymphocyte

PP

MLN

Thoracic duct

Blood stream

Respiratory Tract

Secretory IgA

Plasma cell

Th 2 cytokines

IgA

pIgR

Thoracic duct

Respiratory Tract

Th 2 cytokines
Feeding Models

Chow

CED

Complexity, Intermittency

ISONIT / ISOCAL

IV-TPN

IG-TPN

Route

Complexity
Feeding Models

Chow > CED > IG-TPN > IV-TPN

Degree of Enteral Stimulation

IV-TPN prevents lethal malnutrition
Mucosal Immunity

Parenteral Nutrition
(No ENT stimulus)

Epithelium

PP

Naïve T/B lymphocyte

MLN

Thoracic duct

Blood stream

Respiratory Tract

Secretory IgA

Plasma cell

Th 2 cytokines

IgA

plgR

IgA

Thoracic duct

Secretory IgA

Parenteral Nutrition
(No ENT stimulus)
IgA Levels

Intestinal

TPN days

0 1 2 3 4 5

Respiratory

TPN days

0 1 2 3 4 5

* p < 0.05 vs Day 0

Route of Diet and Established Respiratory Immunity

% Viral Shedding

% Mortality at 48hrs (Ps. Pneumonia)

Chow  CED  IV-TPN

Chow  CED  TPN  Non-immune


Mucosal Immunity: Cell Entry

- Naïve T/B lymphocyte
- Epithelium
- PP
- plgR
- MLN
- Blood stream
- Thoracic duct
- Respiratory Tract
- Secretory IgA
- Parenteral Nutrition (No ENT stimulus)
- Secretory IgA
- IgA
- Plasma cell
- Th 2 cytokines
- LPL
Entry into GALT: Cell/ Peyer’s Patch Interactions

α4β7
L-selectin

ICAM-1
MAdCAM-1
Entry into GALT: Cell/ Peyer’s Patch Interactions

α4β7
L-selectin

ICAM-1
MAdCAM-1

Peyer’s patch cells

Chow
Blockade

0 20 40 60 80 100 120 (%)

(%)
Entry into GALT: Cell / Peyer’s Patch Interactions

ICAM-1

L-selectin

MAdCAM-1

α4β7

L-selectin

Peyer’s patch cells

Blockade

Chow

α4β7
Entry into GALT: Cell/ Peyer’s Patch Interactions

ICAM-1

MAdCAM-1

α4β7

L-selectin

Peyer’s patch cells

Blockade

Chow

α4β7

L-selectin

(%)
Entry into GALT: Cell/ Peyer’s Patch Interactions

α4β7
L-selectin
ICAM-1
MAdCAM-1

Peyer’s patch cells

Blockade

Chow α4β7 L-selectin MAdCAM-1

(%)

0 20 40 60 80 100 120
Entry into GALT: Cell/Peyer’s Patch Interactions

Peyer’s patch cells

Blockade

Chow  α4β7  L-selectin  MAAdCAM-1  ICAM-1

(%)
MAdCAM-1 Expression in PP

- MAdCAM-1 Expression (mgAb/Total PP)
- Hrs of TPN
- Hrs of Refeeding

± SEM *p<.04 vs 0 and 4 hrs
± SEM †p<.02 vs 0 hrs

Lymphocyte Mass: PP & LP

% of Chow

Chow 1 2 3 4 5

TPN Day

Chow Recovery

GALT T Cells in Small Intestine of Adults: Enteral Fed (21) vs. Without (6)

Okamoto, Fukatsu et al JPEN 2005; 29:56
GALT T Cells in Small Intestine of Adults: Enteral Fed (21) vs. Without (6)

Okamoto, Fukatsu et al JPEN 2005; 29:56
What happens to the cytokines?

Normal control of IgA by T cell cytokines

Th1

IFN$\gamma$

TNF$\beta$

- IgA +

Th2

IL-4

IL-5

IL-6

IL-10
What happens to the cytokines?

Control of IgA by T cell cytokines: IV-TPN

IFN\(\gamma\)  
TNF\(\beta\)  

-  

IgA  

+  

Th1  

Th2

IL-4  
IL-5  
IL-6  
IL-10
Mucosal Immunity: IgA Transport

- Parenteral Nutrition (No ENT stimulus)
- Secretory IgA
- plgR
- PP
- Naïve T/B lymphocyte
- MLN
- Th 2 cytokines
- LPL
- Plasma cell
- Blood stream
- Respiratory Tract
IgA Transport through the Epithelial Cell

Mucosal epithelial cell

Lumen

Lamina propria

Plasma cell

Secretory IgA

IgA-plgR complex

Dimeric IgA
Intestinal pIgR Expression

* p<0.05 vs. Chow
† p<0.05 vs. CED

Mucosal Immunity

Epithelium

PP

Plasma cell

IgA transport

MLN

LPL

Thoracic duct

Blood stream

Respiratory Tract

Naïve T/B lymphocyte

Parenteral Nutrition (No ENT stimulus)

Secretory IgA

IgA

plgR

Th 2 cytokines

Thoracic duct

Parenteral Nutrition

(No ENT stimulus)
Does any of this happen in humans?

Figure 4

Okamoto, Fukatsu et al JPEN 2005; 29:56
Does any of this happen in injured humans?
Human Lung Response After Injury

Epithelial Lining Fluid (Vol)  IgA / ELF  Total IgA

Control  Control  Control
Human Lung Response After Injury

Epithelial Lining Fluid (Vol)

IgA / ELF

Total IgA

Control | Trauma | Control | Trauma | Control

* indicates significant difference.
Human Lung Response After Injury

**Epithelial Lining Fluid (Vol)**
- Control
- Trauma

**IgA / ELF**
- Control
- Trauma

**Total IgA**
- Control
- Trauma
Mouse Respiratory IgA Response to Trauma

* p<0.05 vs all other groups

* p<0.05 vs no blockade

Respiratory IgA (ng)

0 h
4 h
8 h w/ TNF blockade
24 h

0 h
4 h
8 h
24 h

*
Murine IgA response to injury after PN?

Completely stopped!
DEFENSE:
Mucosal immunity
Innate immunity
Cells and molecules that defend the intestinal mucosal surface
Gut innate immunity

- Paneth cells are secretory epithelial cells located at the end of intestinal crypts.
- Paneth cells secret the antimicrobial proteins lysozyme, defensins, and secretory phospholipase A2 type IIA (sPLA2-IIA) into the crypt lumen.
**Phospholipase A2**

- 5 major families; ≥ 30 different enzymes

- Secretory PLA2 (sPLA2)
  - ~ 10 sPLA2 enzymes; Ca^{2+} dependent
  - Catalytically → Phospholipid Release
    - Inflammatory Regulation (Host)
    - Bactericidal (Gut Lumen)
  - Non-Catalytically → Prime Neutrophils
SIWF sPLA2 activity

Unpaired t-test, Means ± SE

p<0.05
Intestinal Fluid sPLA2 Activity

* vs. Chow, p < 0.001
† vs. PN, p ≤ 0.001
Comparison of bactericidal activity in chow and PN culture medium

ANOVA, Means ± SE, *p<0.05 vs. HBSS, †p<0.05 vs. PN-LPS0, ‡p<0.05 vs. PN-LPS10
Can we reproduce GALT effects in people who can’t be fed enterally by adding something to the TPN?
Can we reproduce GALT effects in people who can’t be fed enterally by adding something to the TPN?

i.e. is it the lack of enteral feeding or is it the TPN itself?
Can we reproduce GALT effects in people who can’t be fed enterally by adding something to the TPN?

Neuropeptides
Enteric Nervous System & Mucosal Immunity

Bombesin (BBS)

Gastrointestinal neuropeptide

Gastrin-releasing peptide analogue

Stimulates gut hormone release
- gastrin, CCK, neurotensin

Bombina bombina
Mucosal Immunity on Bombesin

Epithelium

PP

MAdCAM-1

Naïve T/B lymphocyte

MLN

Thoracic duct

Blood stream

Respiratory Tract

Secretory IgA

Secretory IgA

IgA

plgR

Th 2 cytokines

Plasma cell

LPL

Respiratory Tract

Secretory IgA
T cell phenotype changes

LP CD4⁺CD25⁺LPAM-1⁺

LP

% of lymphocytes

Chow
PN
PN-BBS

P<.05

with PN/DES
to Chow levels with bombesin

No CD8⁺ T cell changes anywhere

Almost all increase is Tregs
LP B cell phenotype changes

Unactivated naïve (IgD⁺)
- with PN/DES
- to Chow levels with bombesin

Ag-stimulated (IgD⁻LPAM-1⁺)
- with PN/DES
- to Chow levels with bombesin

Effector/memory (CD44⁺)
- with PN/DES
- to Chow levels with bombesin
SIWF sPLA2 activity

p<0.05

Unpaired t-test, Means ± SE
sPLA$_2$ in Paneth Cell Granules: Chow, PN, PN+BBS
BOMBESIN

Lamina propria:

- Increases T (Tregs) & B cells
- Increases IgA- Airway and SI
- Reverses PN-induced defect in bacterial and viral immunity
- Increases sPLA2 in Paneth cell
- After PN but not SIWF- bethanecol necessary as well
Systemic effects with lack of enteral stimulation?
Cytokines and GALT Function

GALT

Mucosa

Endothelium

IgA

IL-4

IL-10

IFN_\gamma

ICAM-1

ICAM-1
Vascular Function + IV-PN

Mucosa

Endothelium

GALT

IL-4
IL-10

IFNγ

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O

+O
Gut PMN Priming (hemorrhagic shock) → Lung PMN Localization → Pulmonary injury

2^{ND} Insult

Does IV-PN prime the cells to a second insult?

Feed Chow, IG-TPN or IV-TPN 5 days

Add stress: 15 minutes of gut ischemia and 2 hours of reperfusion
Route of Feeding: Effects After Gut I/R Injury

15 min I/R Mortality at 72 hrs

PERMEABILITY
- Lung
- Liver

* Indicates significant difference.
Lung Stain for CD18 (activation) after I/R Injury

Chow

IV-TPN

IG-TPN