WAR AND PEACE AT MUCOSAL SURFACES: A “TOLL-STO(R)Y”

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Tolstoy vs Woody: War and Peace vs Love and Death
War and Peace: Silent killing
INTESTINAL EPITHELIAL BARRIER: ACTIVE NOT PASSIVE
Host Response to Breaks in the Mucosal Barrier
Mucosal defense against invading pathogens
SECRETORY IgA

- The most abundant Ab in secretions
- Polymeric form with high Ag avidity
- In addition to interact with Ag via the Fab (Ag-specific) portion of molecule, interacts with bacteria adhesins confers “innate-like properties”
SECRETORY IgA in DEFENSE of MUCOSAL SURFACES

• Prevent Pathogen Adhesion to Host Cells (immune exclusion, canonical)

• Intracellular or serosal neutralization of Ag

• Activation of Non-Inflammatory Pathways

• Homeostatic control of endogenous microflora:
Protection at Mucosal Surface: Speak Softly and Carry a Big Stick
Secretory IgA vs IgG
SIgA: Host defense without Collateral damage
BOOK / VOLUME ONE:
CYTOSKELETON, IgA AND ETHANOL
PROVERBS 21:2

• Wine is a mocker and beer a brawler; whoever “is led astray by them is a fool.”
ALCOHOL AND TRAUMA

GENE MOORE:
“THE PERFECT STORM”
ALCOHOL AND PNEUMONIA RISK

- Increased risk attributed to changes in oral flora, poor oral hygiene, and aspiration
- Previous studies: increased systemic levels of both IgA and SIgA, with deceased gut luminal IgA levels
- Alcohol effects gut cytoskeleton: increased permeability
SIgA transport experiments:

- MDCK cells transfected with the plgR for cells incubated with dIgA at 4 degrees C (receptor saturation)

- Transfected MDCK monolayers then held in incubator at 37 degrees C and IgA concentrations determined from the apical chamber
Figure 1A: Effect of Taxol Pretreatment on Transcytosis of Ethanol exposed MDCK Cells

- Control
- 1% EtOH
- 1% EtOH/Taxol

*\( p < 0.001 \) vs. Control at same time period
#\( p < 0.001 \) vs. 1% EtOH/Taxol at same time period

N = 8 in each group.
BOOK / VOLUME TWO:
INTESTINAL EPITHELIAL CELLS
AS A PROXIMAL SIGNAL IN
INFLAMMATION FROM THE GUT
NEED TO GO UPSTREAM: The Mucosal Surface
GUT and POST-INJURY MOF

• Proinflammatory cytokines released from the gut may cause barrier failure and contribute to remote organ injury and MOF.
• Enterocytes secrete a number of pro-inflammatory cytokines
Treatment Groups: Effects on PMN cytotoxic potential

- Control (Caco-2 cells under normoxic conditions)
- Caco-2 cells + H/R
- Caco-2 cells-normoxia + *E. coli* C-25
- Caco-2 cells + H/R + *E. coli* C-25
Figure 1A: Percent CD11b Expression in PMNs

*\(p<0.001\) vs. all other groups
Figure 1B: Percent CD11 Expression in PMNs in the Presence and Absence of fMLP

![Graph showing percent CD11 expression in PMNs in various conditions.](image)

- Control
- Control+fMLP
- Caco-2 HR
- Caco-2 HR+fMLP
- Caco-2+EC
- Caco-2 EC+fMLP
- Caco-2 HR+EC
- Caco-2 HR+EC+fMLP

Statistical significances:
- "p < 0.001 vs. same group without fMLP"
- "# p < 0.001 vs. Control"

Mean Receptor Density
Figure 2A: Superoxide anion Production

* p<0.001 vs. all other groups
Figure 2B: Superoxide Production in PMNs in the Presence and Absence of fMLP

Control | Control+fMLP | Caco-2 HR | Caco-2 HR+fMLP | Caco-2+EC | Caco-2+EC+fMLP | Caco-2 HR+EC | Caco-2 HR+EC+fMLP
---|---|---|---|---|---|---|---
2.3 | 3.2 | 3.1 | 6.4* | 3.3 | 7.2* | 3.5 | 10.6*  

*p<0.001 vs. Same group without fMLP  
#p<0.001 vs. Control
Figure 3B: Total Elastase Release by PMNs in the Presence and Absence of fMLP

*p<0.001 vs. groups without fMLP
Figure 4: Effect of Supernatants on PMN Apoptosis at 90 minutes

*\( p < 0.001 \) vs. Control

#\( p < 0.001 \) vs. Caco-2 HR and Caco-2+EC
Figure 5: Apoptosis of HMVEC Cells at 90 minutes

- Control: 3.58
- Caco-2 HR: 5.52*
- Caco-2+EC: 6.24*
- Caco-2 HR+EC: 0.50#

* \( p < 0.001 \) vs. Control

# \( p < 0.001 \) vs. Caco-2 HR and Caco-2+EC
Figure 6: HMVEC Cell Permeability

*p<0.001 vs. Control
#p<0.001 vs. Caco-2 HR and Caco-2 +EC
CaCO2 cell monolayers: in vitro model of the gut epithelial barrier

- CaCO2 cell monolayers incubated with normal gut flora +/- alcohol

- Cytokine release and intestinal barrier integrity responses determined
Figure I: Synergistic Effect of Ethanol and E.coli on Gut TNF-alpha Production

*\(p<0.001\) versus no EtOH and versus all groups
Figure II: Synergistic Effect of Ethanol and E.coli on Caco2 IL-6 production

Interleukin 6 (pg/mL) vs %Ethanol

- *p<0.001 versus no EtOH and versus all other groups
Figure III: Synergistic Effect of Ethanol and E.coli on Gut Permeability

*p<0.001 versus no EtOH and versus all other groups
Intestinal Epithelial Cells: orchestrating the immunoinflammatory response
BOOK / VOLUME 3: IgA AND RESPIRATORY PATHOGENS IN THE LUNG
Post-Op Pneumonia-Not Just an Epiphenomena
HOST DEFENSE OF THE LUNG
IN ICU PNEUMONIA
Humoral Immunity

- **Secretory IgA**
  - 10% of total protein in BAL fluid
  - Produced locally, plgR mediated transport
  - Anti-inflammatory properties

- **IgG**
  - 19% of total protein in BAL fluid
  - In respiratory secretions by passive transudation or through leaky epithelial boundary.
  - Has profound ability to enhance inflammatory potential
IgA Modulates Inflammatory Responses in an In Vitro Model of Pneumonia
Figure 1: Comparison of CD11 in PMNs Cocultured with Calu-3 Cells

PMN/Calu-3: 50.4
PMN/EC: 148.0
PMN/Calu-3/EC: 128.5
PMN/Calu-3/EC+IgG: 118.0
PMN/Calu-3/EC+IgA: 46.5

*p<0.001 vs. PMN/Calu-3
#p<0.001 vs. PMN/EC
N=5 for each group
Figure 3: Comparison of Elastase in PMNs Cocultured with Calu-3 Cells

* p<0.001 vs. PMN/Calu-3
# p<0.001 vs. PMN/Calu-3/EC+IgA
N=5 for each group
Figure 5: Basal Compartment IL-6 Levels

Conc. IL-6 (pg/ml)

PMN/Calu-3 39.2
PMN/EC 52.5
PMN/Calu-3/EC 50.2
PMN/Calu-3/EC+IgG 51.3
PMN/Calu-3/EC+IgA 17.0

*p<0.001 vs. PMN/Calu-3
#p<0.001 vs. PMN/Calu-3/EC+IgA
N=4 for each group
Figure 6: Basal Compartment TNF-α Levels

*p<0.001 vs. PMN/Calu-3
#p<0.001 vs. PMN/Calu-3/EC+IgA
N=4 for each group
Impact of Cleavage of IgA: Relative IgA Deficiency in Respiratory Tract/other Mucosal Surfaces

• Loss of anti-inflammatory properties/exaggerated inflammatory response by other effector cells.
• Kudsk: SIgA and bacterial pneumonia.
Relative SIgA Deficiency

• Highly dependant on structure for it’s function.

• Cleavage of antibody into Fab and Fc fragment renders it immunologically inactive.

• Potential sources
  – PMN/macrophages
  – bacteria
IgA Cleavage
<table>
<thead>
<tr>
<th>Gram pos. resp. isolates</th>
<th>Non- resp. gram neg. isolates</th>
<th>Gram neg. resp. isolates</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Figure 6: SDS-PAGE of Bacterial Isolates</td>
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</tbody>
</table>

- 200,000
- 97,400
- 66,200
- 31,000
- 14,400
VAP: Summary from 24 Studies (1,689 episodes and 2,490 pathogens)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Frequency (%)</th>
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<tbody>
<tr>
<td>P. aeruginosa</td>
<td>24.4</td>
</tr>
<tr>
<td>Acinetobacter sp.</td>
<td>7.9</td>
</tr>
<tr>
<td>Stenotrophomonas</td>
<td>1.7</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>14.1</td>
</tr>
<tr>
<td>Haemophilus sp.</td>
<td>9.8</td>
</tr>
<tr>
<td>Other species all less than 8%</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: IgA protease activity among respiratory isolates ($10^4$ organisms) at 12 hrs.

**Intact IgA**

- **Klebsiella sp.** 108.0*#
- **Enterobacter sp.** 111.7*#
- **E. coli** 380.4*#
- **Acinetobacter sp.** 83.5*
- **Pseudomonas sp.** 10.4

*p<0.001 vs. Pseudomonas, #p<0.001 vs. Acinetobacter
Indirect Effector Functions of sIgA

• Interact of IgA with innate defense factors
  – Complement
  – Potentiate nonspecific antibacterial factors in exocrine secretions
  – Mucin
  – Interact with B and T lymphs, macrophage, PMN’s and others

• End results: IgA is relatively ineffective or directly antagonistic compared with IgG or IgM
The Relative Roles of Bacteria and Host Inflammatory Cells in SLgA Degradation
Figure 1: Effect of Primed and Activated PMNs on IgA Cleavage

- IgA Control: 432.9
- PMN+IgA: 416.4
- Activated PMN+IgA: 407.9

Legend:
- Intact IgA
- SC Fraction
Figure 4: Effect of Bacterial Isolates on IgA Cleavage

* indicates p<0.001 vs. all other groups.
Purpose

• To compare the ability of SlgA vs. IgG to modulate PMN production of proinflammatory cytokines and chemotactic potential.

• To compare the sequence of addition of Ig isotypes SlgA and IgG on cytokine production in the cell culture system.

• To examine the effect of the sequence of Ig isotype exposure on modulating PMN chemotactic ability in vitro.
Regional Differences in *Ig isotypes* at Respiratory Surfaces

- SIgA > IgG
- SIgA ≤ IgG
Figure 1: Effect of the sequence of exposure by IgA and IgG to E. coli mediated IL-6 production by monocyte-PMN cultures

- E. coli: 1387
- E. coli + IgA: 107*
- E. coli + IgG: 1456
- E. coli + IgA-IgG: 314* &
- E. coli + IgG-IgA: 814* &

*p<0.001 vs. E. coli, &p<0.001 vs. E. coli + IgA, #p<0.001 vs. IgA-IgG sequence

N = 4 for all groups
Figure 3: Effect of the sequence of exposure by IgA and IgG to E. coli mediated TNFα production by monocyte-PMN cultures

* p<0.001 vs. E. coli, & p<0.001 vs. E. coli+IgA, # p<0.001 vs. IgA-IgG sequence

N = 4 for each group
Figure 5: Effect of the sequence of exposure by IgA and IgG to E. coli mediated IL-8 production by monocyte-PMN cultures

*p<0.001 vs. E. coli, &p<0.001 vs. E.coli + IgA, #p<0.001 vs. IgA-IgG sequence

N = 4 for each group
Figure 6: Effect of the sequence of exposure by IgA and IgG to LPS mediated IL-8 production by monocyte-PMN cultures.

- LPS: 2528 pg/ml
- LPS+IgA: 213 pg/ml
- LPS+IgG: 2226 pg/ml
- LPS+IgA-IgG: 1018 pg/ml
- LPS+IgG-IgA: 1534 pg/ml

*p<0.001 vs. LPS, &p<0.001 vs. LPS + IgA, #p<0.001 vs. IgA-IgG sequence
N = 4 for each group
Figure 7: Effect of monocyte supernatants co-cultured with E. coli and IgA and IgG on chemotaxis of PMN

*\( p < 0.001 \) vs. E. coli and E. coli + IgG-IgA

\( N = 4 \) for each group
Proximal airway

Distal airway

SLgA Ab function-
To prevent Ag interaction with respiratory epithelium

Mitigate inflammatory side effects of other immune effector cells.
RECENT STUDIES

• WTA : 2009 Crested Butte
  Decreased survival and greater lung inflammation in survivors: PlgR KO mice

• SIS : April 2010: Increased inflammatory potential after exposure to “Virulent Strains” of Pseudomonas and Acinetobacter (CLEAVAGE of SIgA)
BOOK / VOLUME 4: PATHOGEN RECEPTOR RECOGNITION: A TOLL BRIDGE BETWEEN INNATE AND ADAPTIVE ARMS OF THE IMMUNE SYSTEM
Toll-Like Receptor Associated Molecules
Disparate Effects of Bacteria and Toll-Like Receptor Dependant Bacterial Ligand Stimulation on IgA Transcytosis
Purpose

To study the effect of gram negative or gram positive bacteria and Toll-Like receptor bacterial ligand pathways on IgA transcytosis.
Figure 1: IgA Transcytosis in HT-29 Cells Following Stimulation with G- or G+ Bacteria and TLR ligands

![Graph showing IgA transcytosis](image)

- **Control**: 2.6, 14.5
- **ΔE.coli**: 3.8, 28.7*
- **LPS**: 5.3, 34.1*
- **ΔS.aureus**: 4.7, 15.9
- **PGN**: 4.4, 15.7

* *p<0.001 vs. Control

N = 4 for each group
Figure 8: Densitometry Determination of plgR Expression at Timed Intervals Following Stimulation with LPS

*p<0.001 vs. control and other timepoints
N = 4 for each group
Conclusions

- Stimulation by gram negative bacteria led to increased IgA transcytosis.

- Stimulation by the TLR-4 ligand, LPS, also led to increased IgA transcytosis in this model.

- The disparate effects between gram negative and gram positive bacteria and TLR-4 vs. TLR-2 pathways may have significant implications in the host response at mucosal surfaces.
Organ Specific Innate Immune Response: Does injury reset the TLR rheostat?

<table>
<thead>
<tr>
<th>compartment</th>
<th>microbial contact</th>
<th>PRR sensitivity</th>
</tr>
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<tbody>
<tr>
<td>blood</td>
<td>none</td>
<td>high every microbial contact indicates danger</td>
</tr>
<tr>
<td>airways, skin</td>
<td>frequent</td>
<td>regulated certain microbial load might be tolerated</td>
</tr>
<tr>
<td>gut</td>
<td>permanent</td>
<td>suppressed tolerance is dominating</td>
</tr>
</tbody>
</table>
Gazing into the Mucus layer: The Epilogue
HOMEOSTATIC CONTROL OF GUT FLORA AND IgA

- Endogenous commensal bacteria elicit production of both microbe-specific and natural polyreactive IgA (+/- T-cell depend)
- SIgA contributes to “host-parasite mutualism” the homeostatic balance which controls the degree of bacterial colonization in the gut
- Mutualism is dependent on “natural – polyreactive Ab, (cross-reactive for #s of redundant Ag on commensal bacteria
- IMPAIRED WITH GUT I/R (ACUTE) OR IBD(CHRONIC)?
PASSIVE IMMUNIZATION WITH SIgA at MUCOSAL SITES

- GUT: Administer SIgA with Biologic Fluids via enteral route: problems with stomach acid
- Upper Airways: SIgA administer via nose drops or aerosol
- Importance of Mucus at mucosal sites for anchoring SIgA
OTHER POTENTIAL ROLES FOR USE OF SIgA

• Active or Passive Immunization against viral or bacterial infections contracted at mucosal sites (influenza, Shigella, AIDS)

• Addition of SIgA containing biologics (Colostrum or artificial) to control diarrhea, including C-diff, and to improve enteral feeding tolerance in the ICU