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

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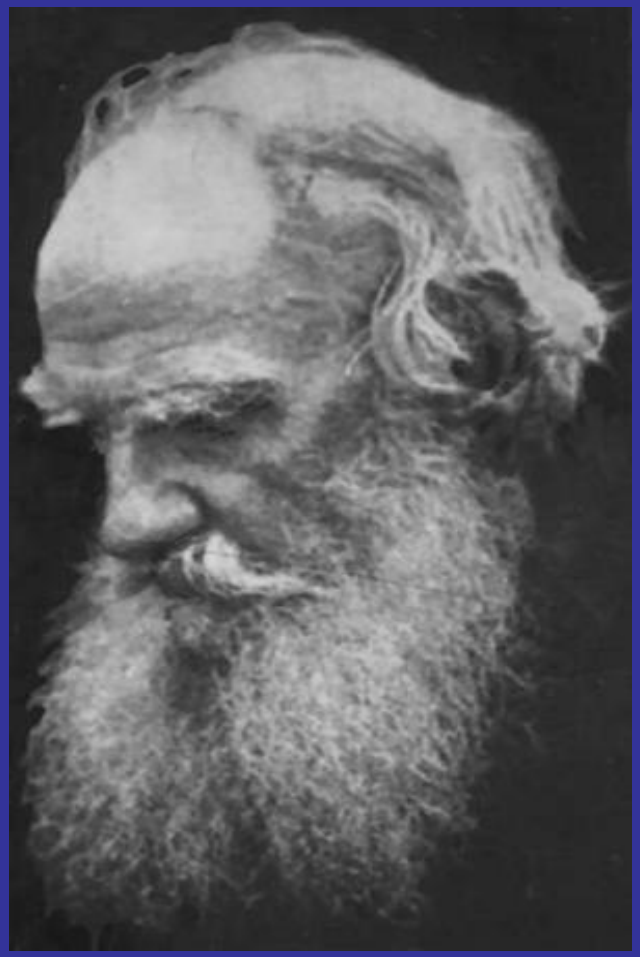
WESTERN TRAUMA ASSOCIATION MARCH 4,2010

Tolstoy vs Woody: War and Peace vs Love and Death



War and Peace: Silent killing

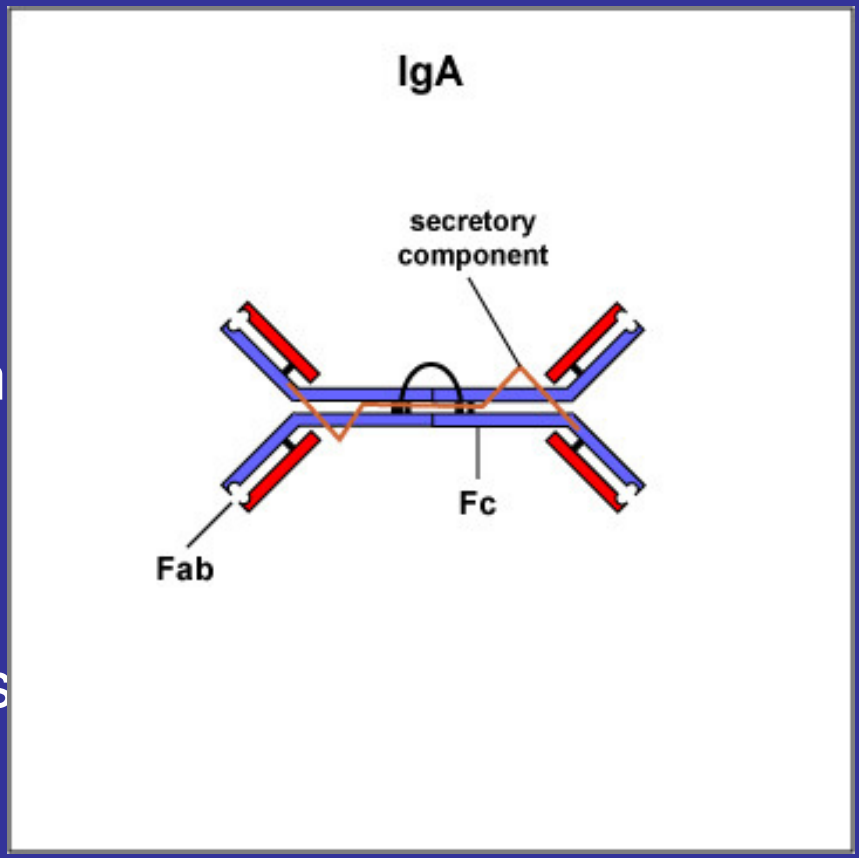
INTESTINAL EPITHELIAL BARRIER: ACTIVE NOT PASSIVE

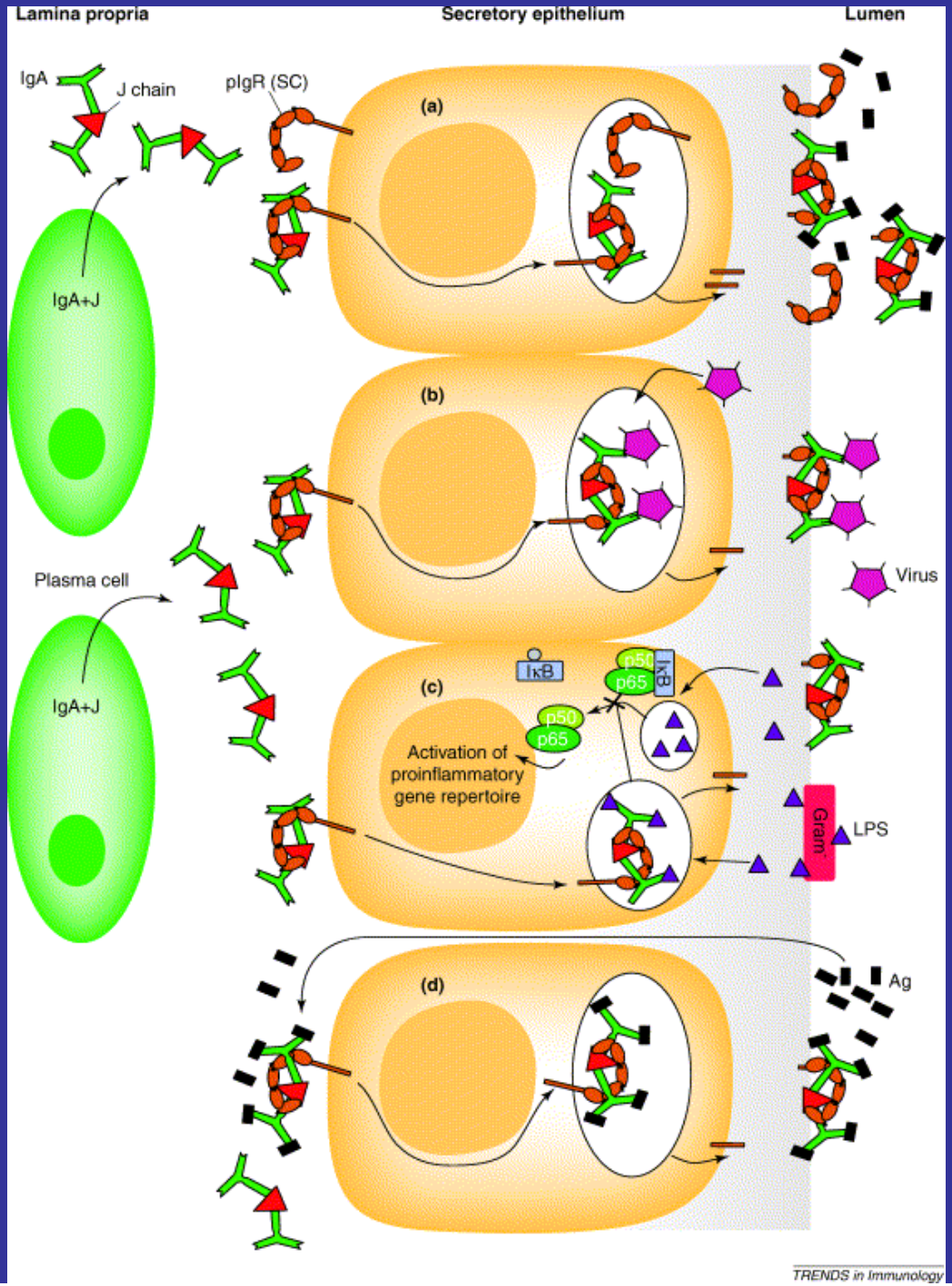
Host Response to Breaks in the Mucosal Barrier

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”




Lamina propria
Secretory epithelium
Lumen
IgA
J chain
pIgR (SC)
(a)
IgA+J
(b)
Plasma cell
Virus
IgA+J
(c)
IκB
p50
p65
IκB
p50
p65
Activation of
proinflammatory
gene repertoire
Gram⁻
LPS
(d)
Ag
TRENDS in Immunology

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"

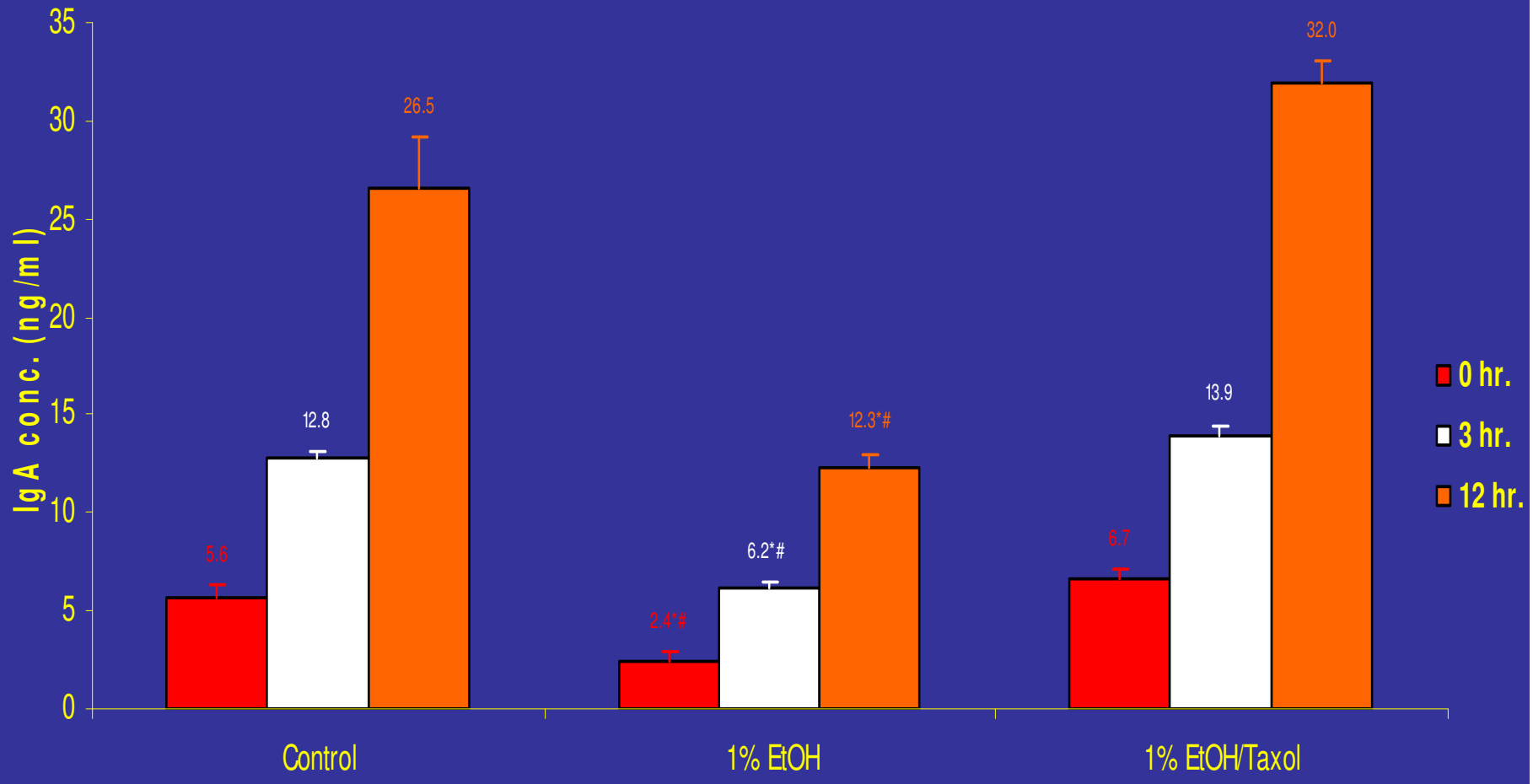
ALCHOHOL AND PNEUMONIA RISK

- 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 pIgR 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



*p<0.001 vs. Control at same time period

#p<0.001 vs. 1% EtOH/Taxol at same time period

BOOK / VOLUME TWO: INTESTINAL EPITHELIAL CELLS AS A PROXIMAL SIGNAL IN INFLAMMATION FROM THE GUT

NEED TO GO UPSTREAM: The Mucosal Surface

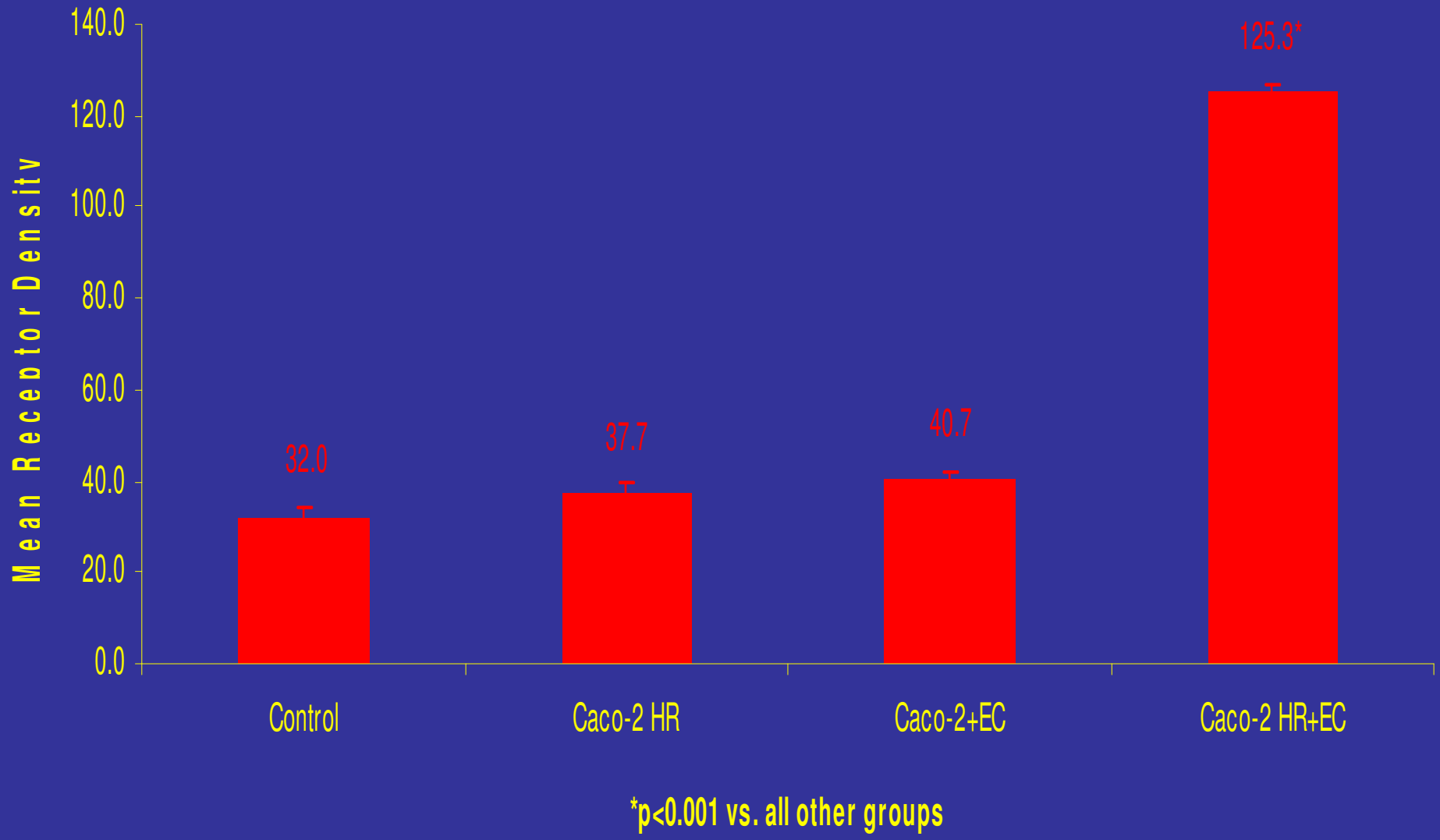
GUT and POST-INJURY MOF

- 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 cytoxic potential

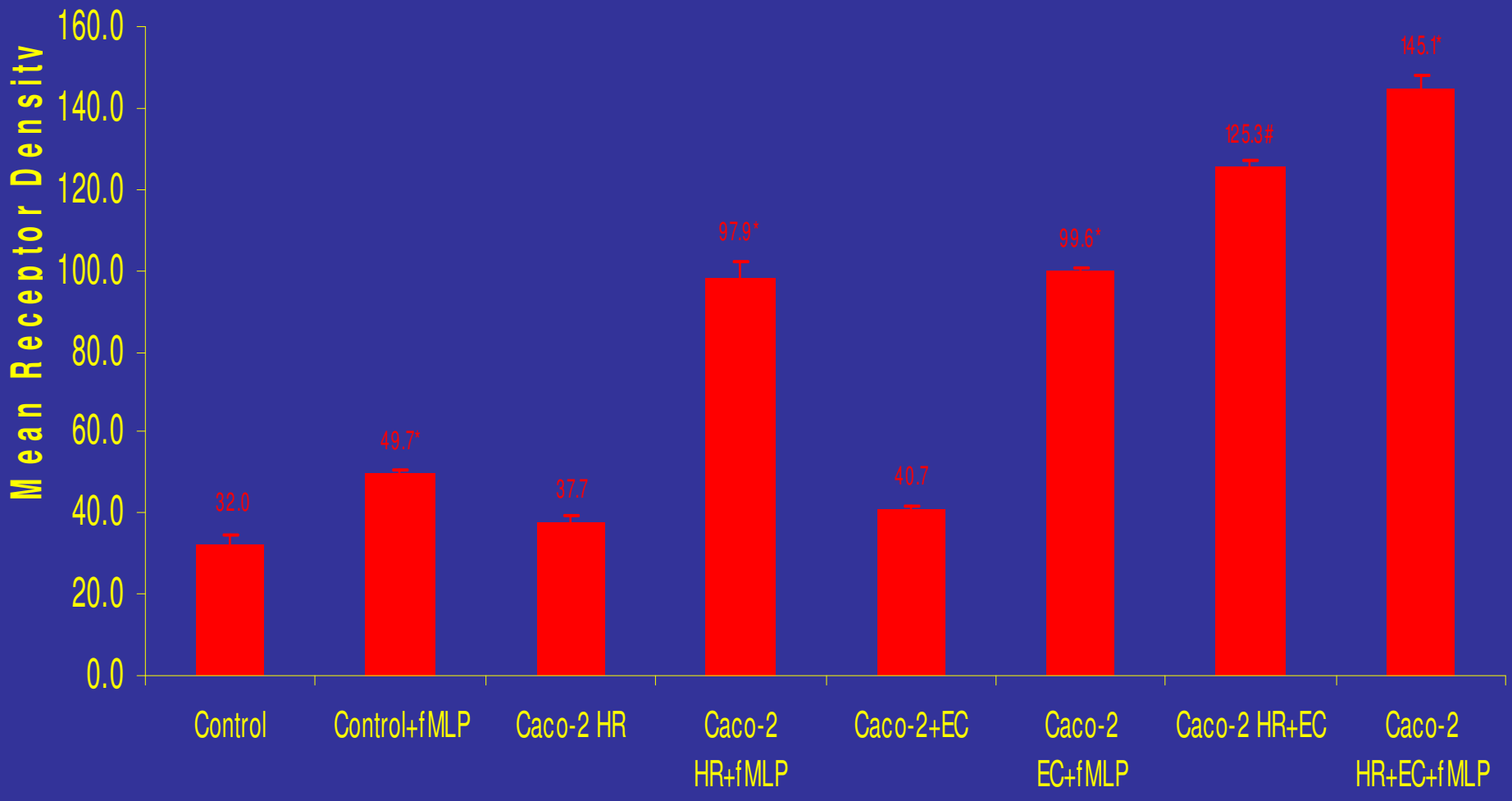
- Control (Caco-2 cells under normoxic conditions)
- Caco-2 cells + H/R
- Caco-2 cells-normoxia + *E.coli* C-25
- Caco-2 cells + H/R + *E.coli* C-25

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



*p<0.001 vs. same group without fMLP

# p<0.001 vs. Control

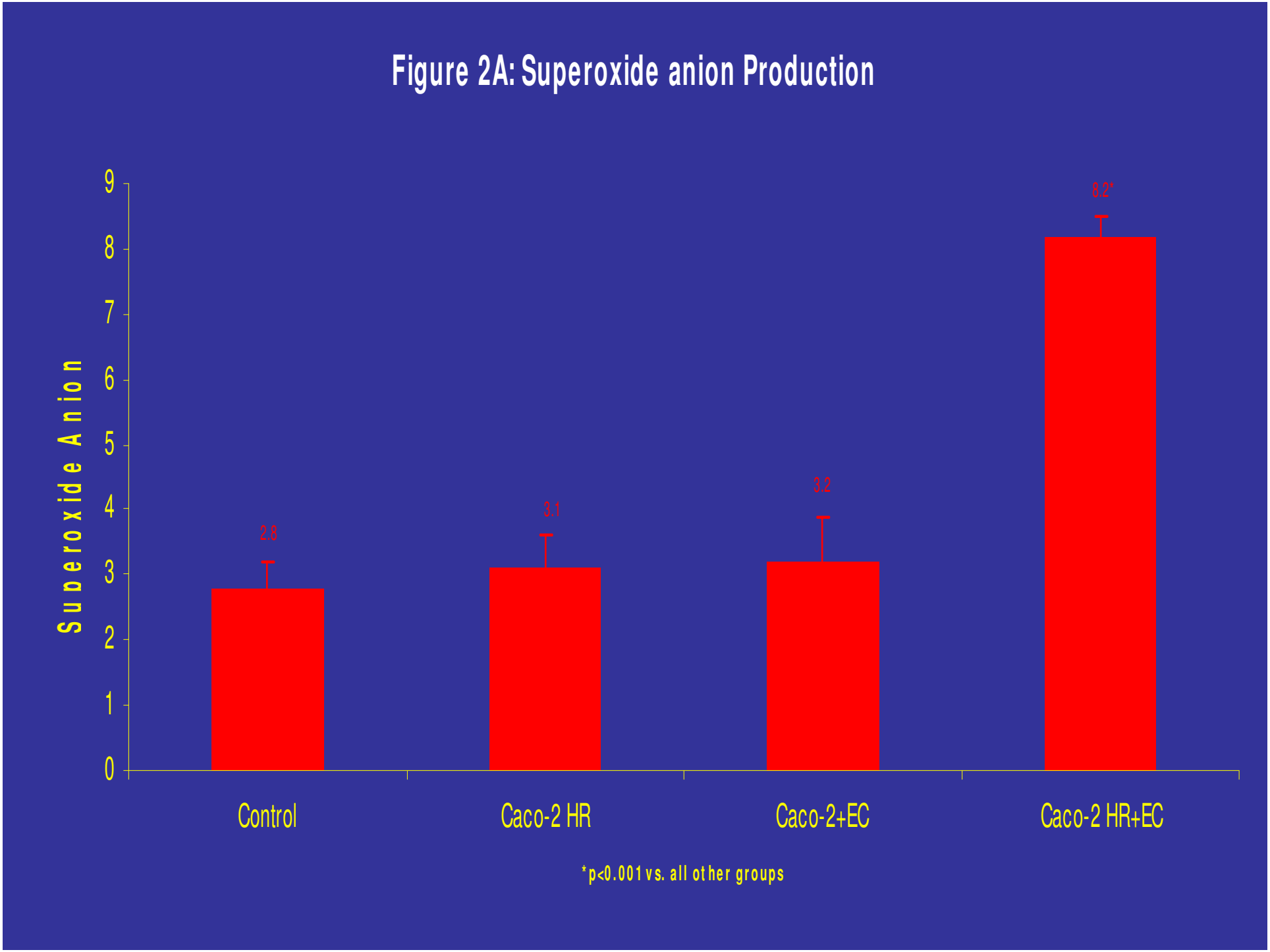
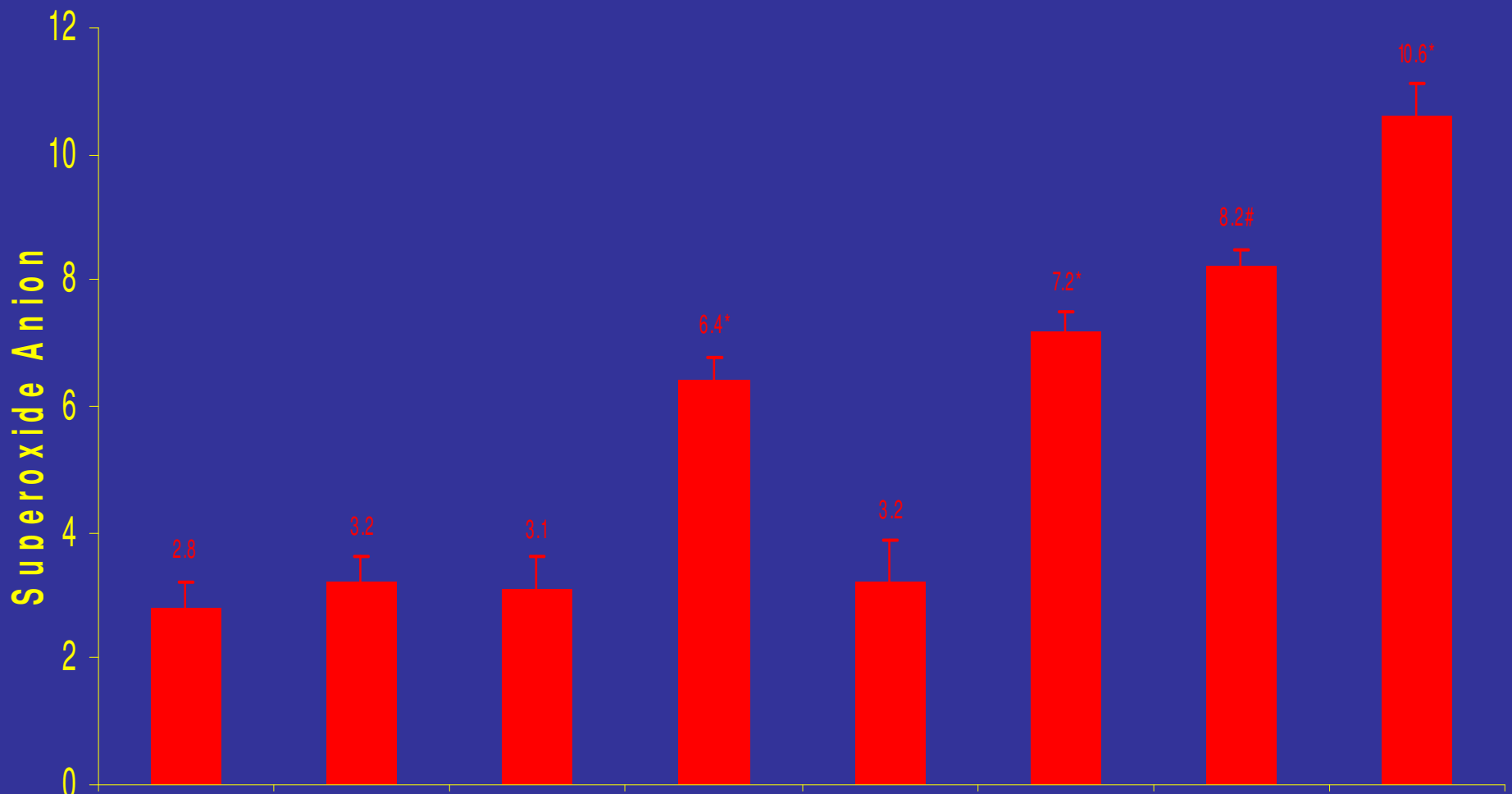


Figure 2A: Superoxide anion Production
Superoxide Anion
0
1
2
3
4
5
6
7
8
9
2.8
3.1
3.2
8.2*
Control
Caco-2 HR
Caco-2+EC
Caco-2 HR+EC
*p<0.001 vs. all other groups

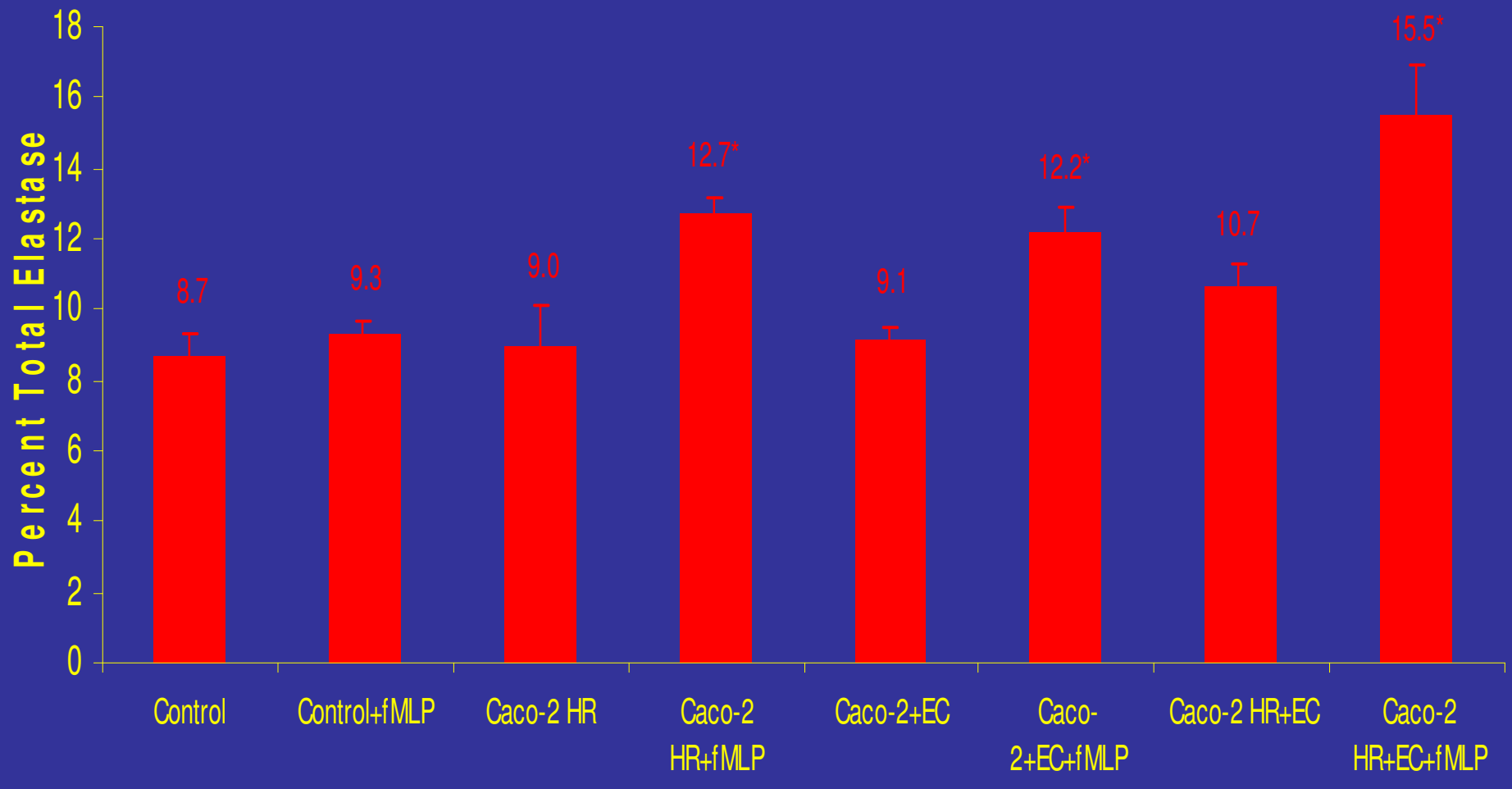
Figure 2B: Superoxide Production in PMNs in the Presence and Absence of fMLP



*p<0.001 vs. Same group without fMLP

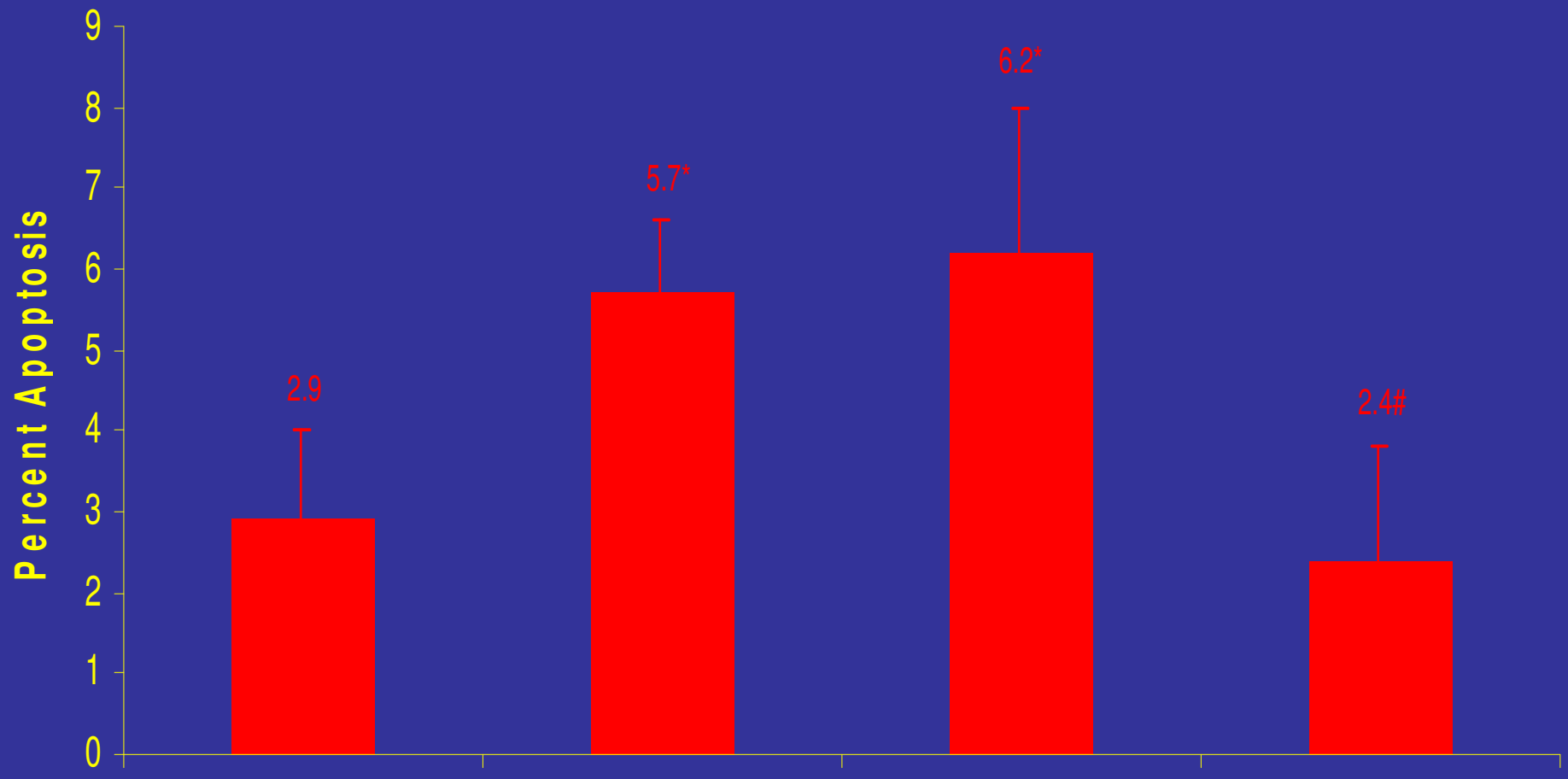
# p<0.001 vs. Control

Figure 3B: Total Elastase Release by PMNs in the Presence and Absence of fMLP



*p<0.001 vs. groups without fMLP

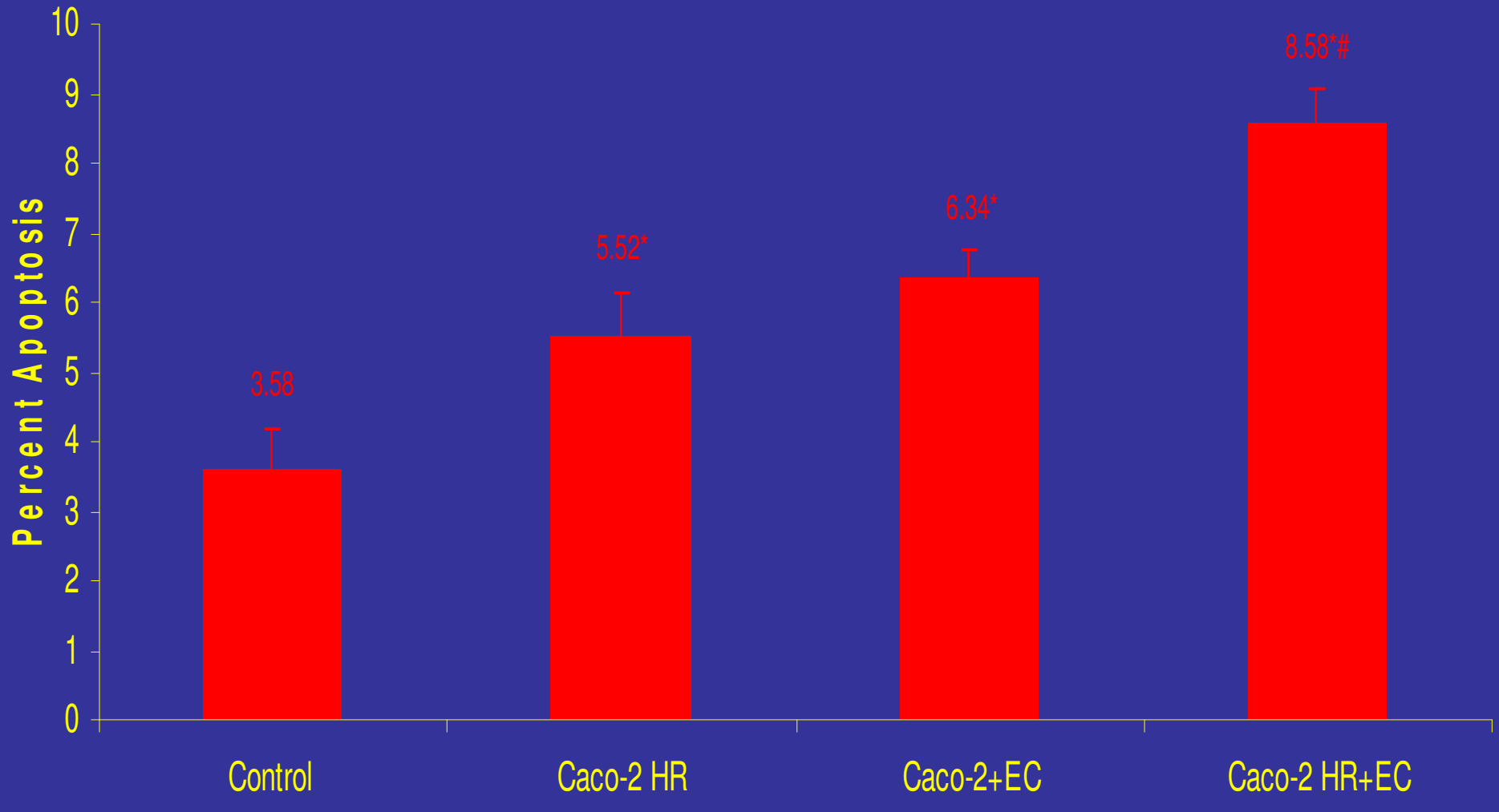
Figure 4: Effect of Supernatants on PMN Apoptosis at 90 minutes



*p<0.001 vs. Control

#p<0.001 vs. Caco-2 HR and Caco-2+EC

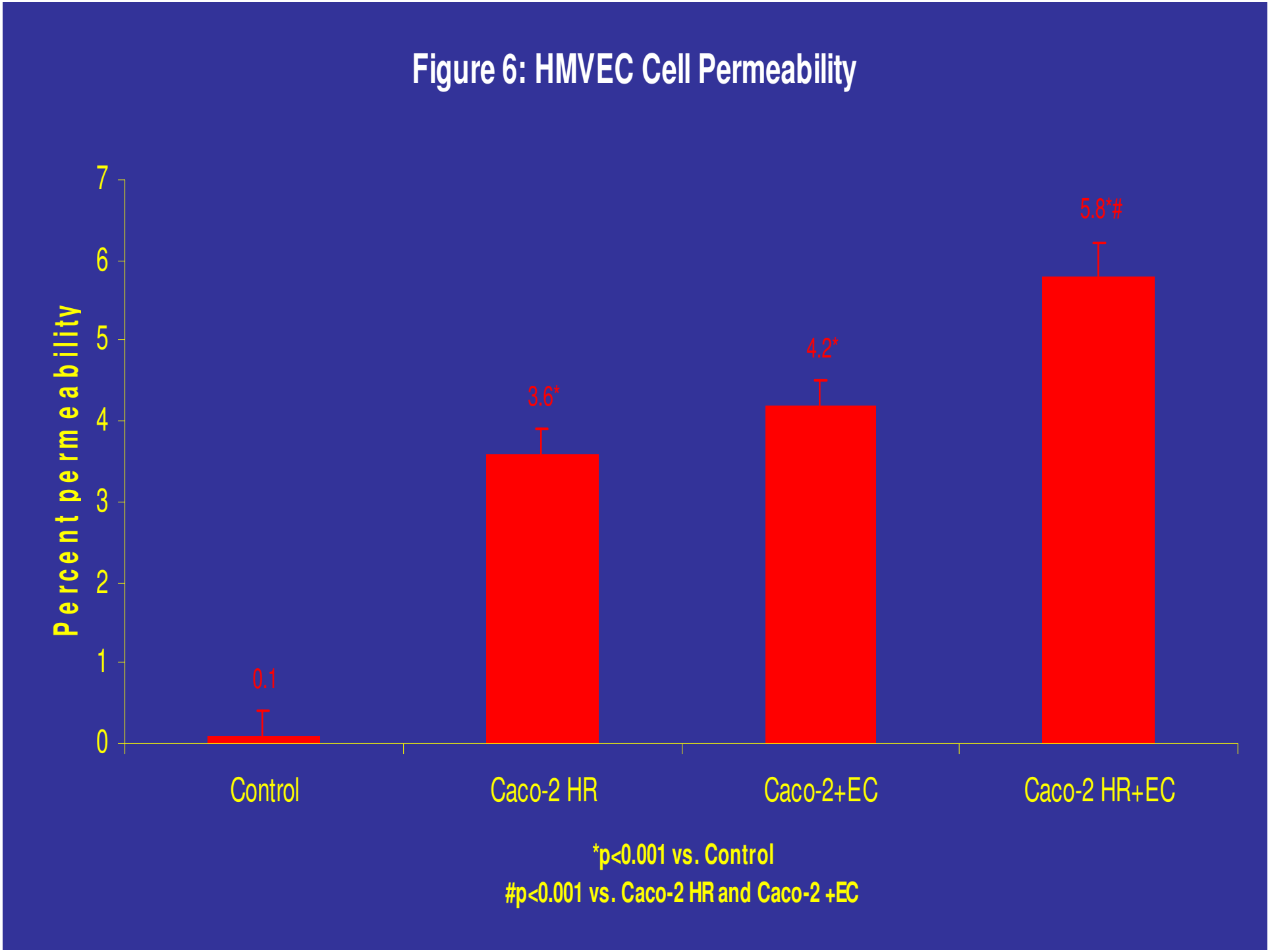
Figure 5: Apoptosis of HMVEC Cells at 90 minutes



*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

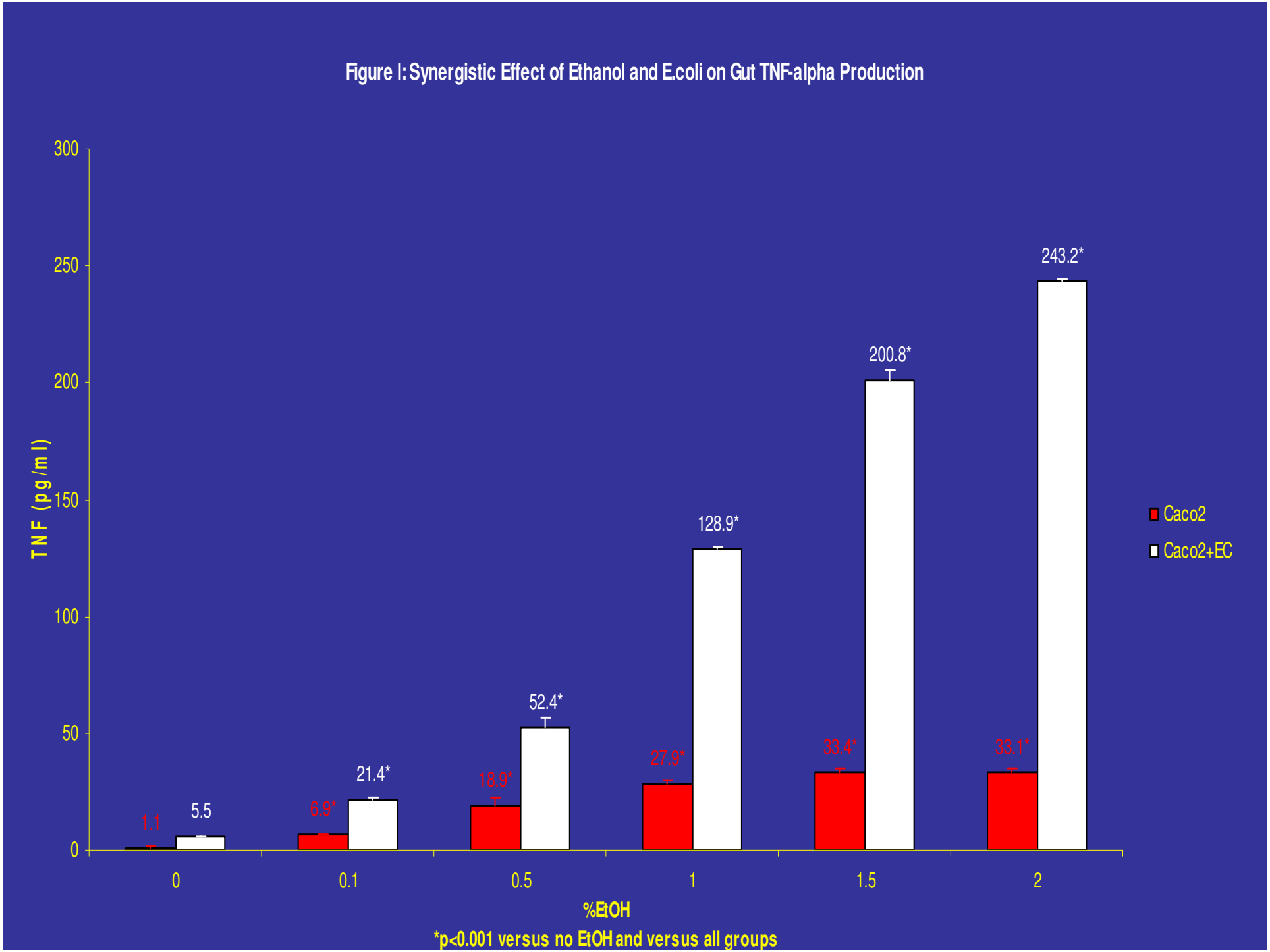


Figure I: Synergistic Effect of Ethanol and E.coli on Gut TNF-alpha Production
TNF (pg/ml)
300
250
200
150
100
50
0
1.1
5.5
6.9*
21.4*
18.9*
52.4*
27.9*
128.9*
33.4*
200.8*
33.1*
243.2*
0
0.1
0.5
1
1.5
2
%EtOH
Caco2
Caco2+EC
*p<0.001 versus no EtOH and versus all groups

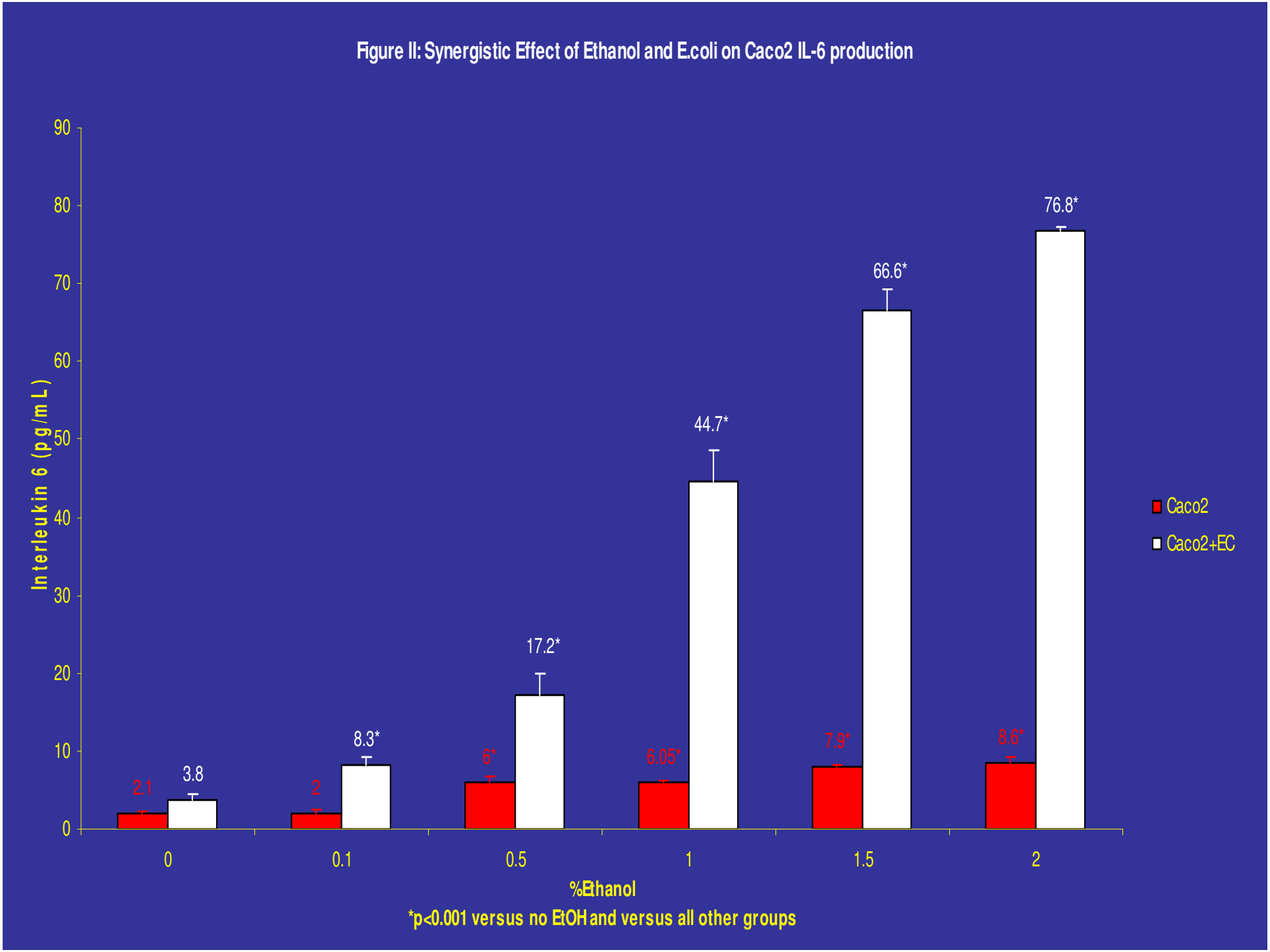


Figure II: Synergistic Effect of Ethanol and E.coli on Caco2 IL-6 production
Interleukin 6 (pg/mL)
90
80
70
60
50
40
30
20
10
0
2.1
3.8
2
8.3*
6*
17.2*
6.05*
44.7*
7.9*
66.6*
8.6*
76.8*
0
0.1
0.5
1
1.5
2
%Ethanol
Caco2
Caco2+EC
*p<0.001 versus no EtOH and versus all other groups

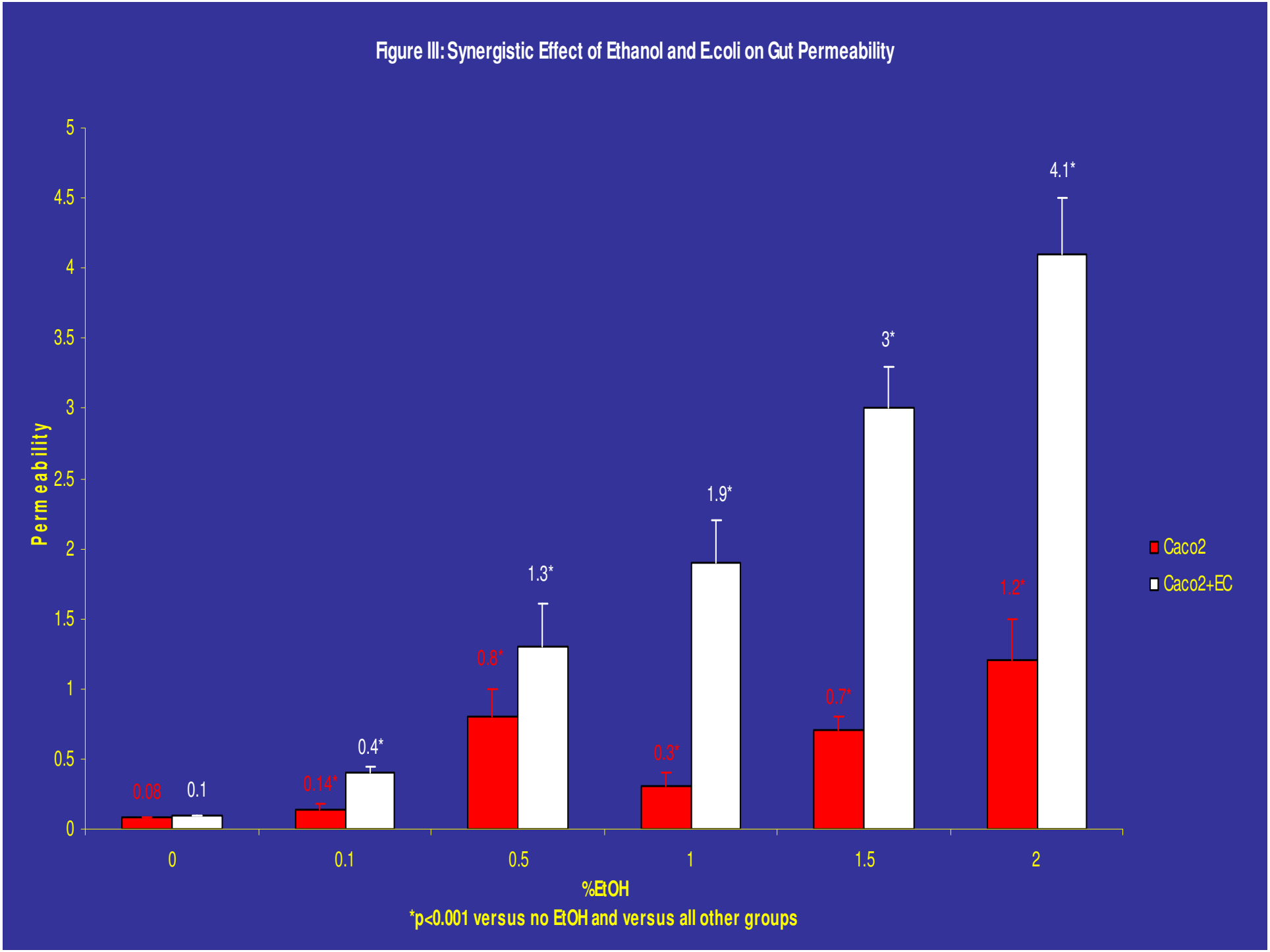
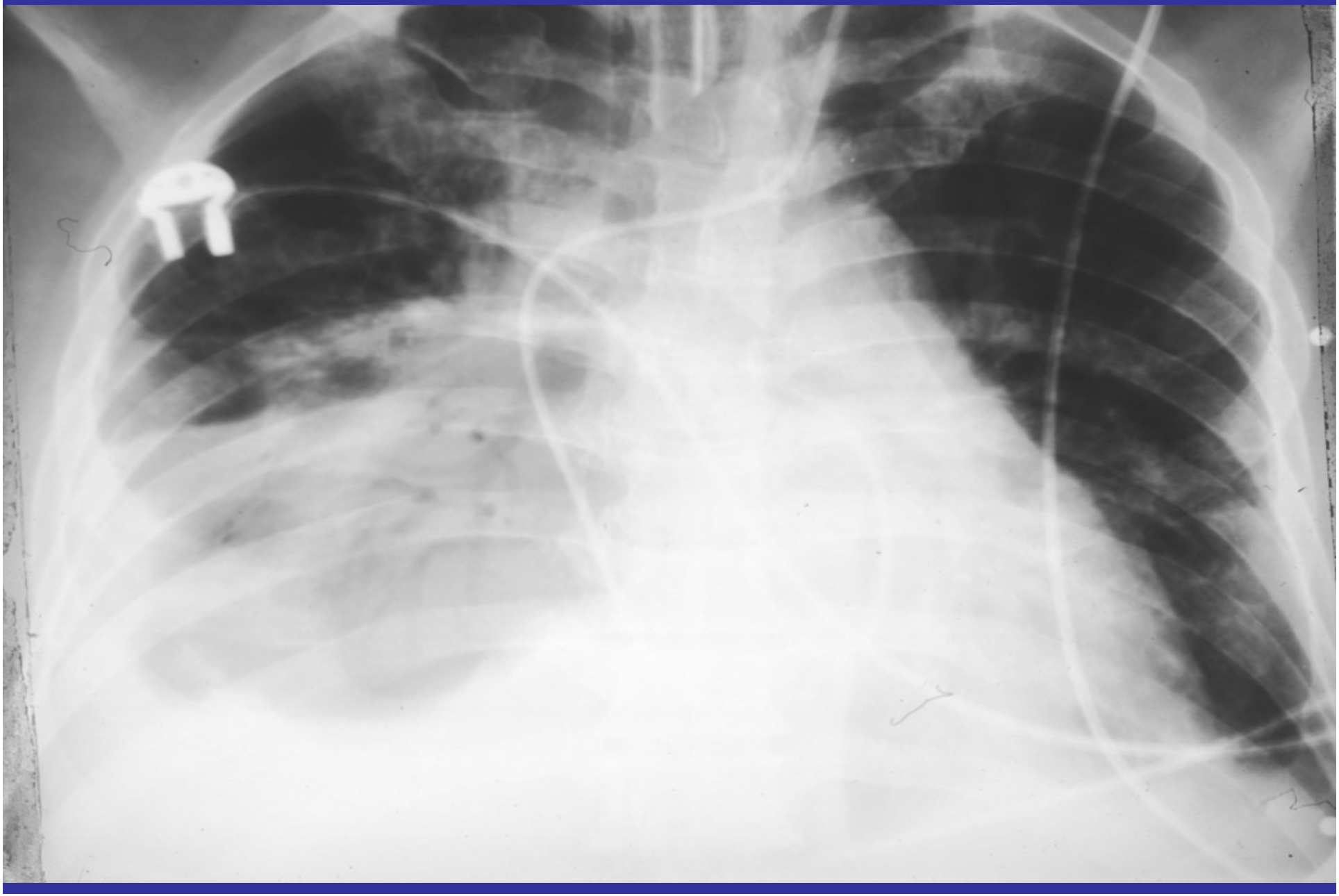


Figure III: Synergistic Effect of Ethanol and E.coli on Gut Permeability
Permeability
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
0.08
0.1
0.14*
0.4*
0.8*
1.3*
0.3*
1.9*
0.7*
3*
1.2*
4.1*
0
0.1
0.5
1
1.5
2
%EtOH
Caco2
Caco2+EC
*p<0.001 versus no EtOH and versus all other groups

Intestinal Epithelial Cells: orchestrating the immunoinflammatory response

BOOK / VOLUME 3: IgA AND RESPITORY PATHOGENS IN THE LUNG

Post-Op Pneumonia-Not Just an Epiphenomena



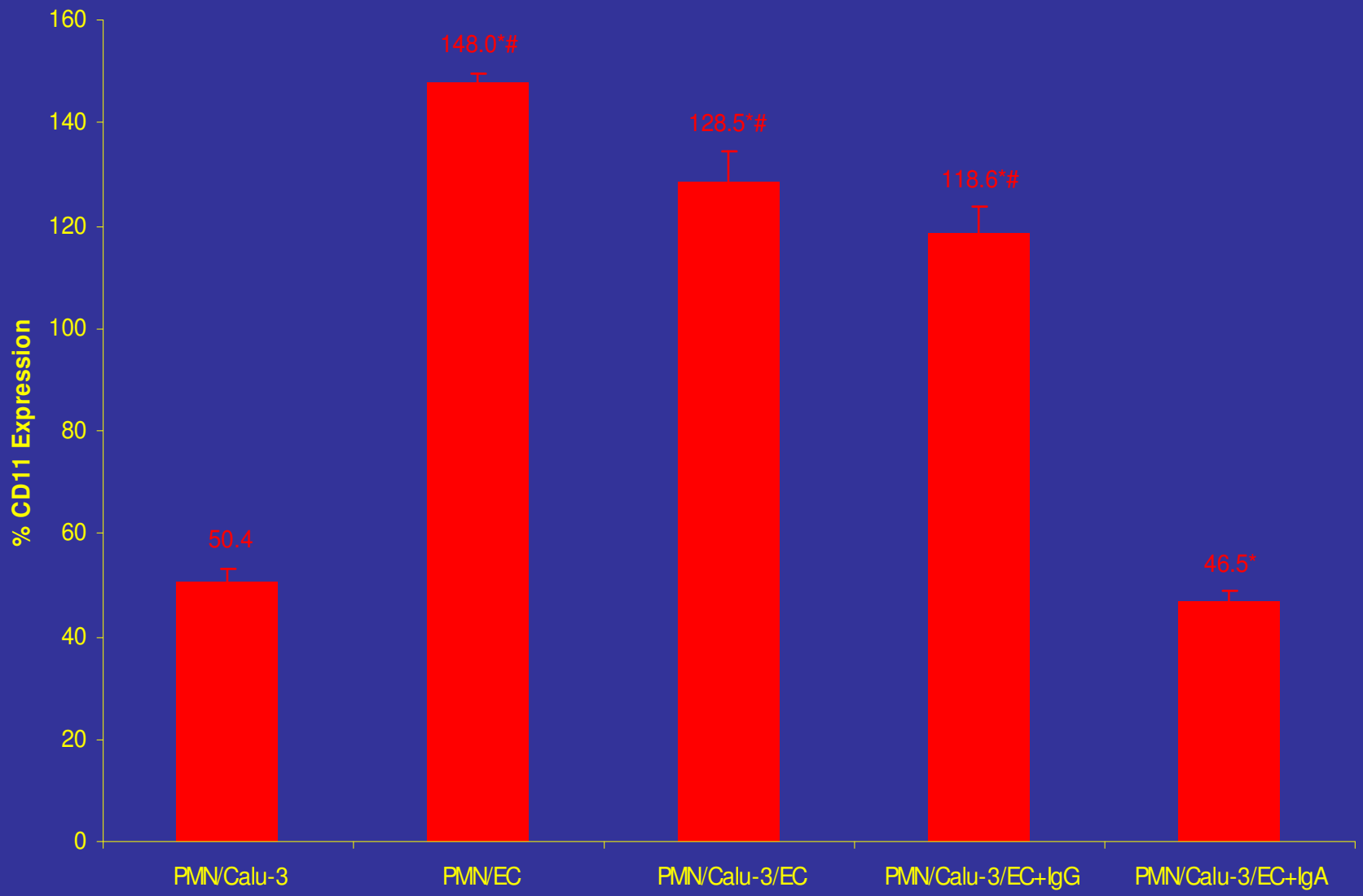
HOST DEFENSE OF THE LUNG IN ICU PNEUMONIA

Humoral Immunity

- Secretory IgA
  - 10% of total protein in BAL fluid
  - Produced locally, pIgR 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

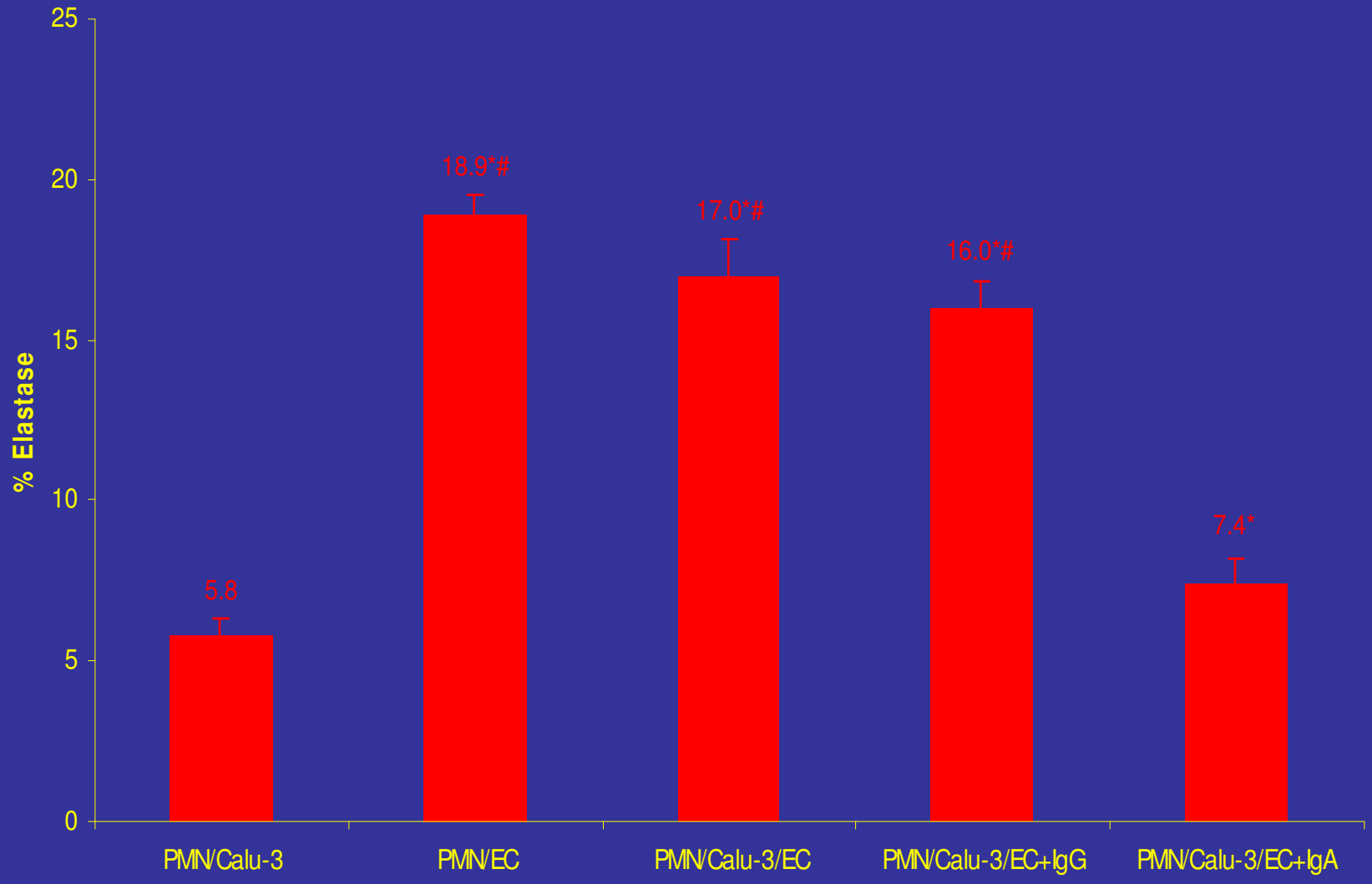
IgA Modulates Inflammatory Responses in an In Vitro Model of Pneumonia

Figure 1: Comparison of CD11 in PMNs Cocultured with Calu-3 Cells



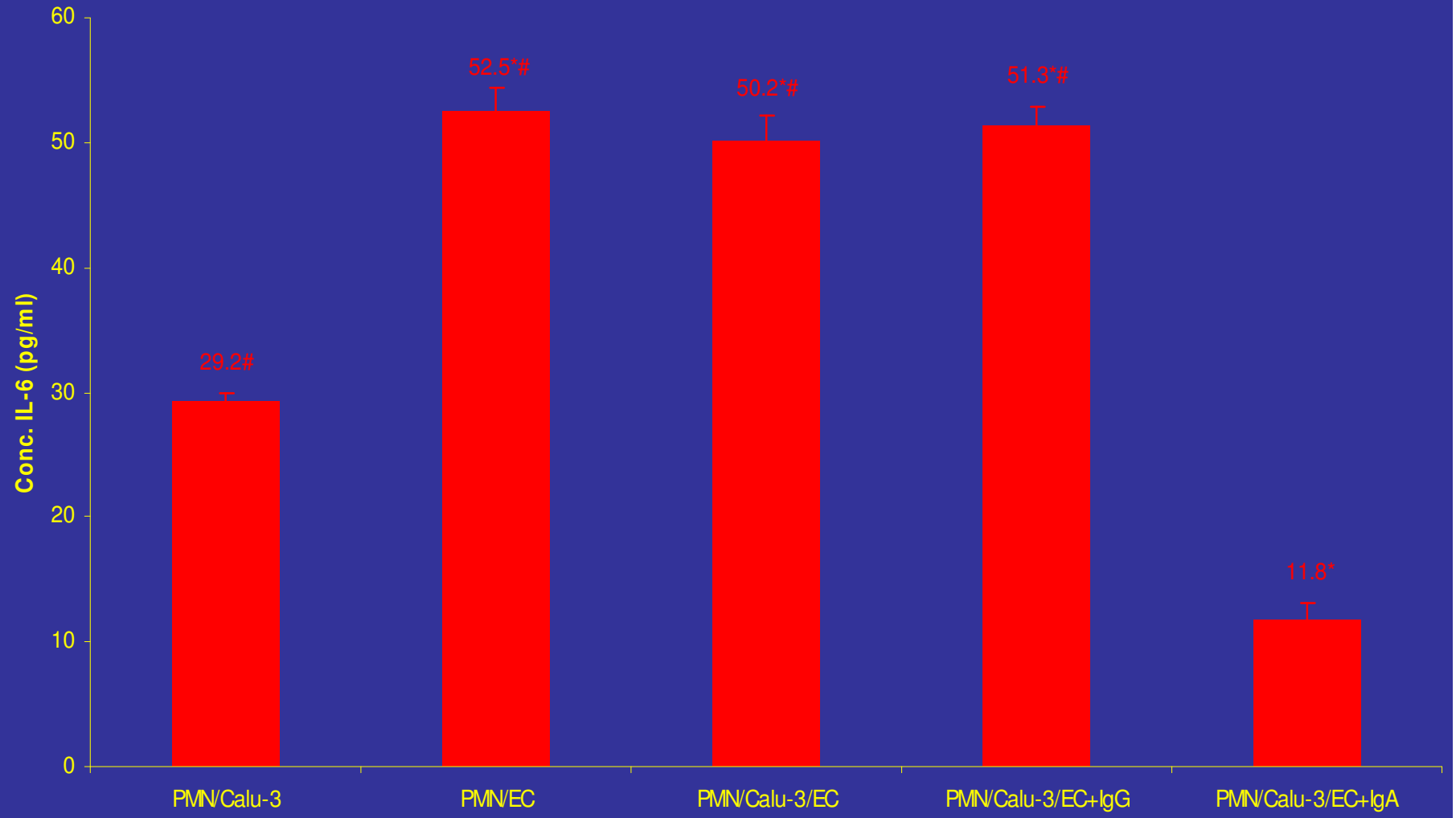
*p<0.001 vs. PMN/Calu-3
#p<0.001 vs. PMN/EC
N=5 for each group

Figure 3: Comparison of Elastase in PMNs Cocultured with Calu-3 Cells



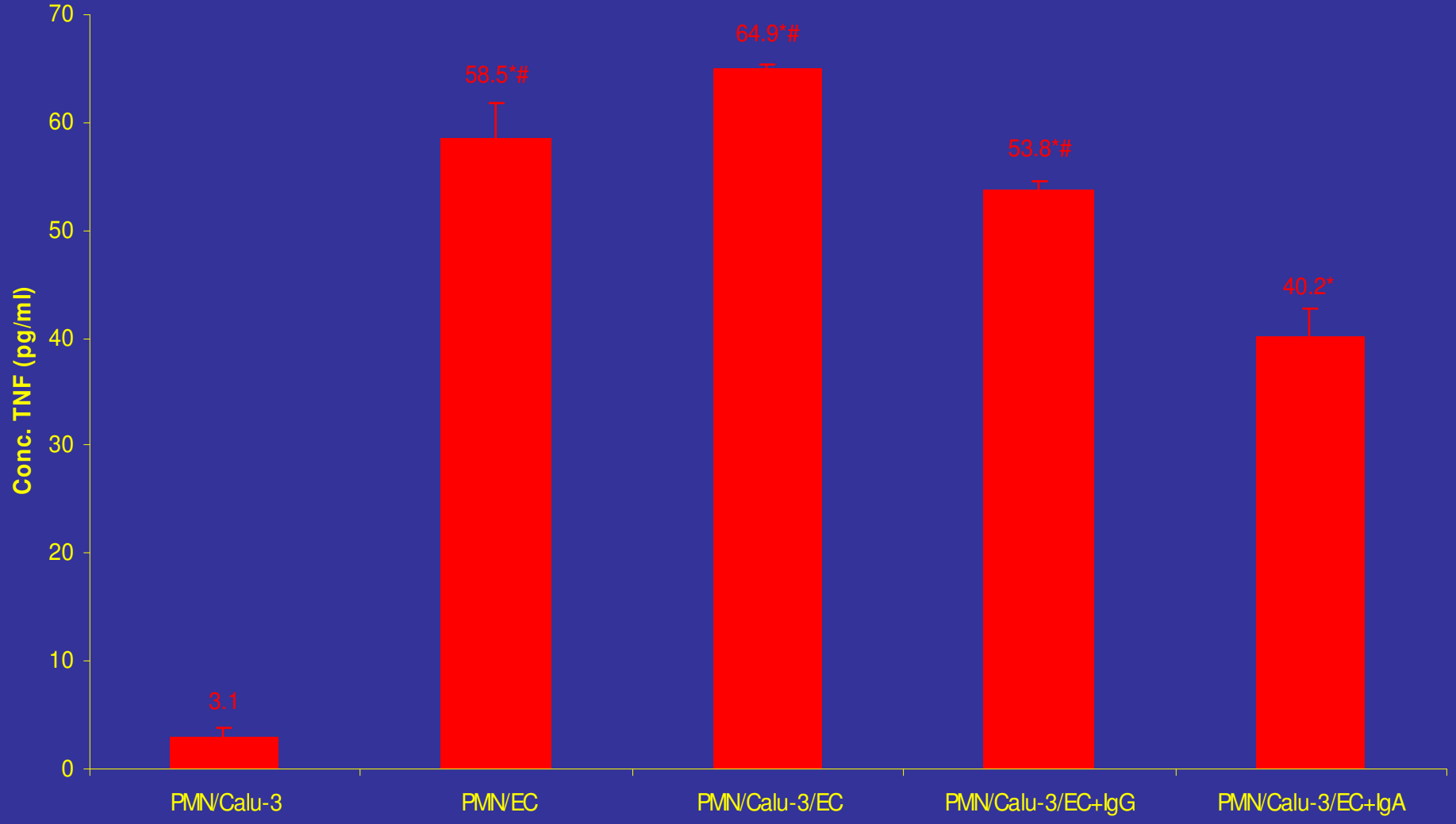
*p<0.001 vs. PMN/Calu-3
#p<0.001 vs. PMN/Calu-3/EC+IgA
N=5 for each group

Figure 5: Basal Compartment IL-6 Levels



*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 effecter 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

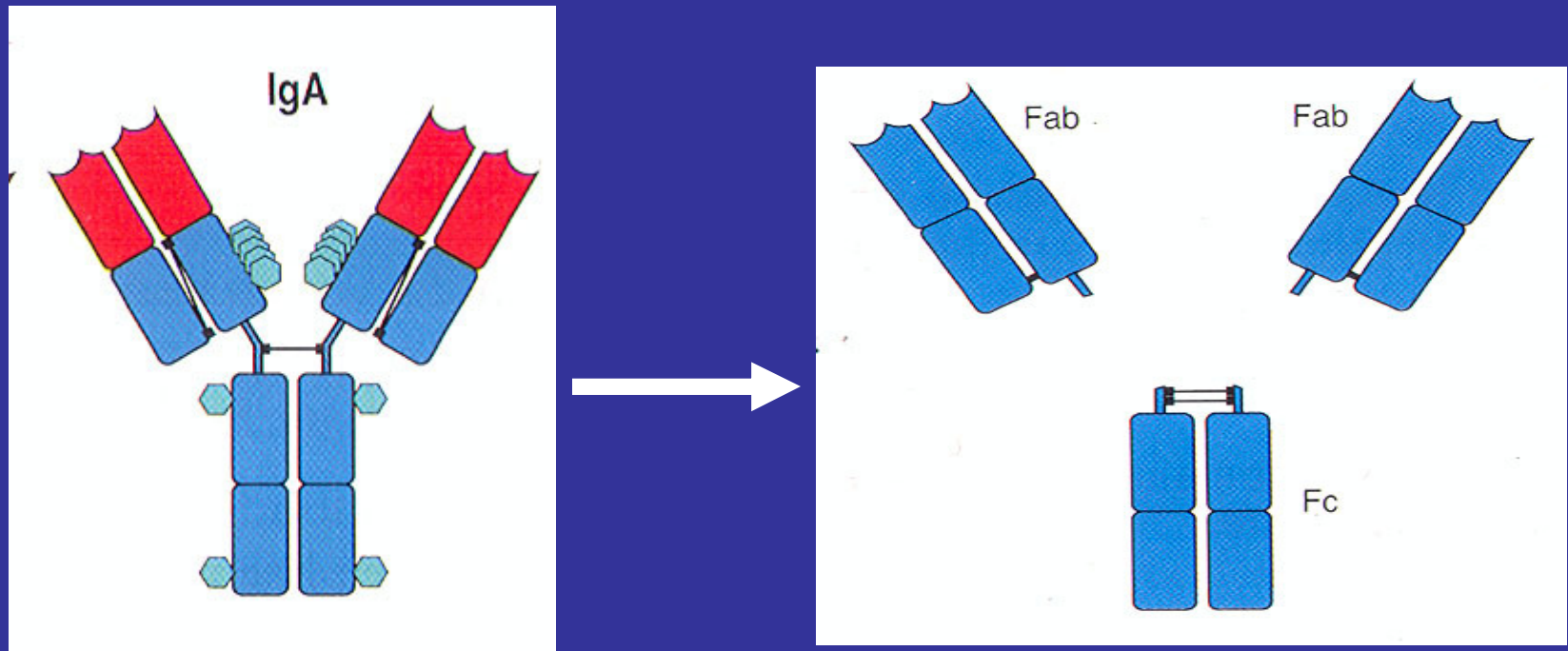
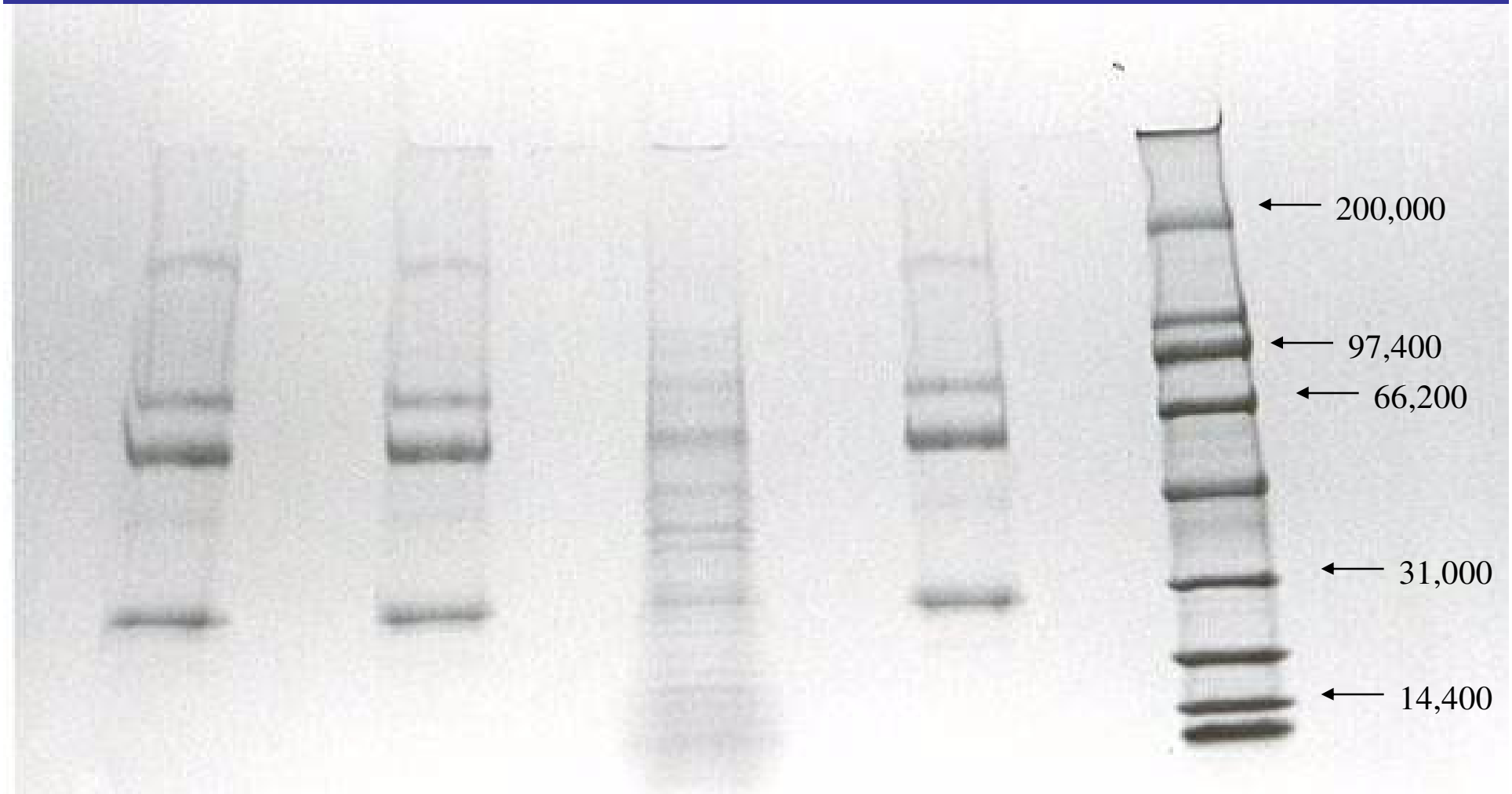


Figure 6: SDS-PAGE of Bacterial Isolates



Gram pos. resp. isolates

Non- resp. gram neg. isolates

Gram neg. resp. isolates

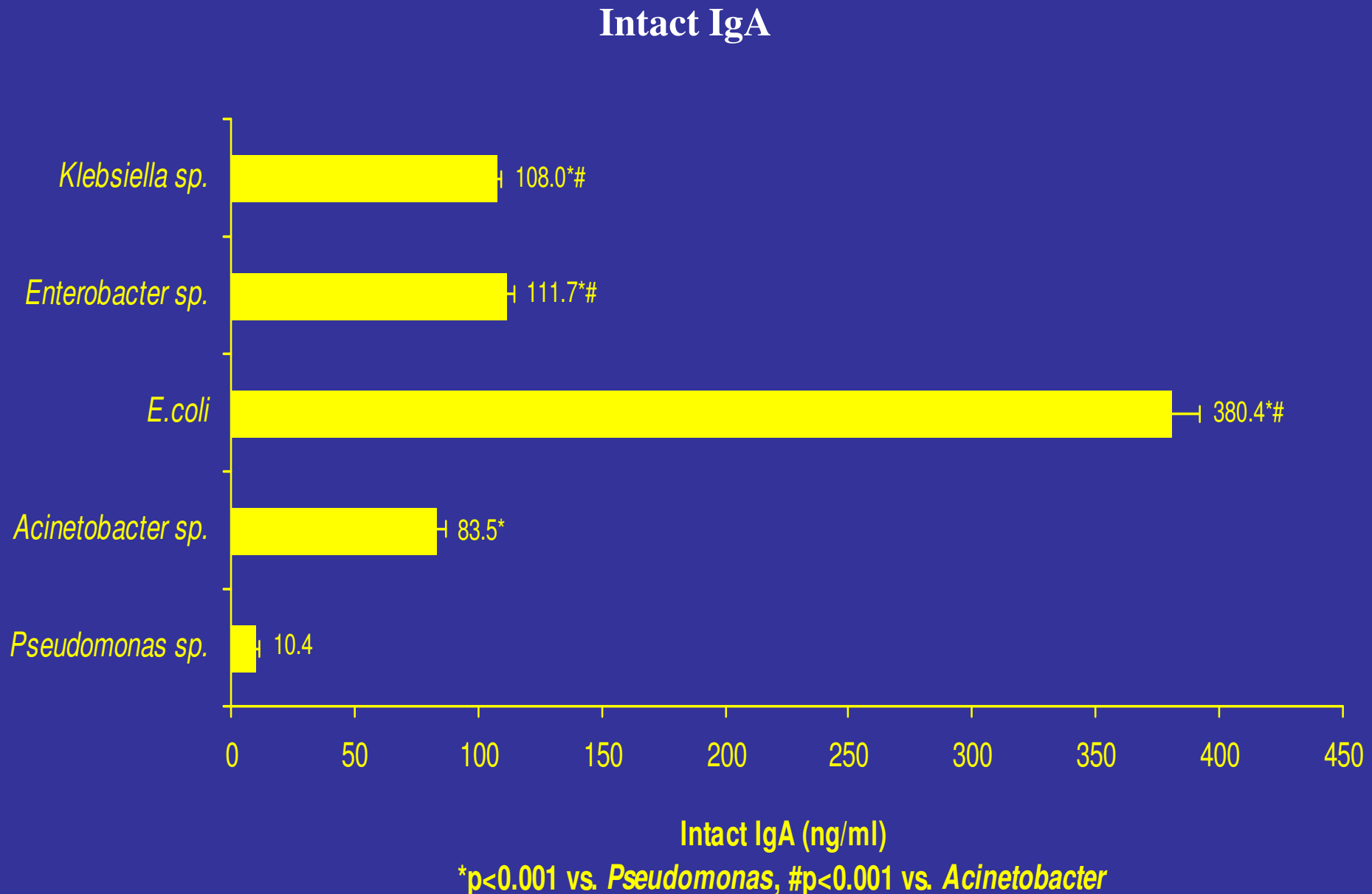
Control

VAP: Summary from 24 Studies (1,689 episodes and 2,490 pathogens

	Frequency (%)
P. aeruginosa	24.4
Acinetobacter sp.	7.9
Stenotrophomonas	1.7
Enterobacteriacae	14.1
Haemophilus sp.	9.8

Other species all less than 8%

Figure 1: IgA protease activity among respiratory isolates (10^4 organisms) at 12 hrs.



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 SIgA Degradation

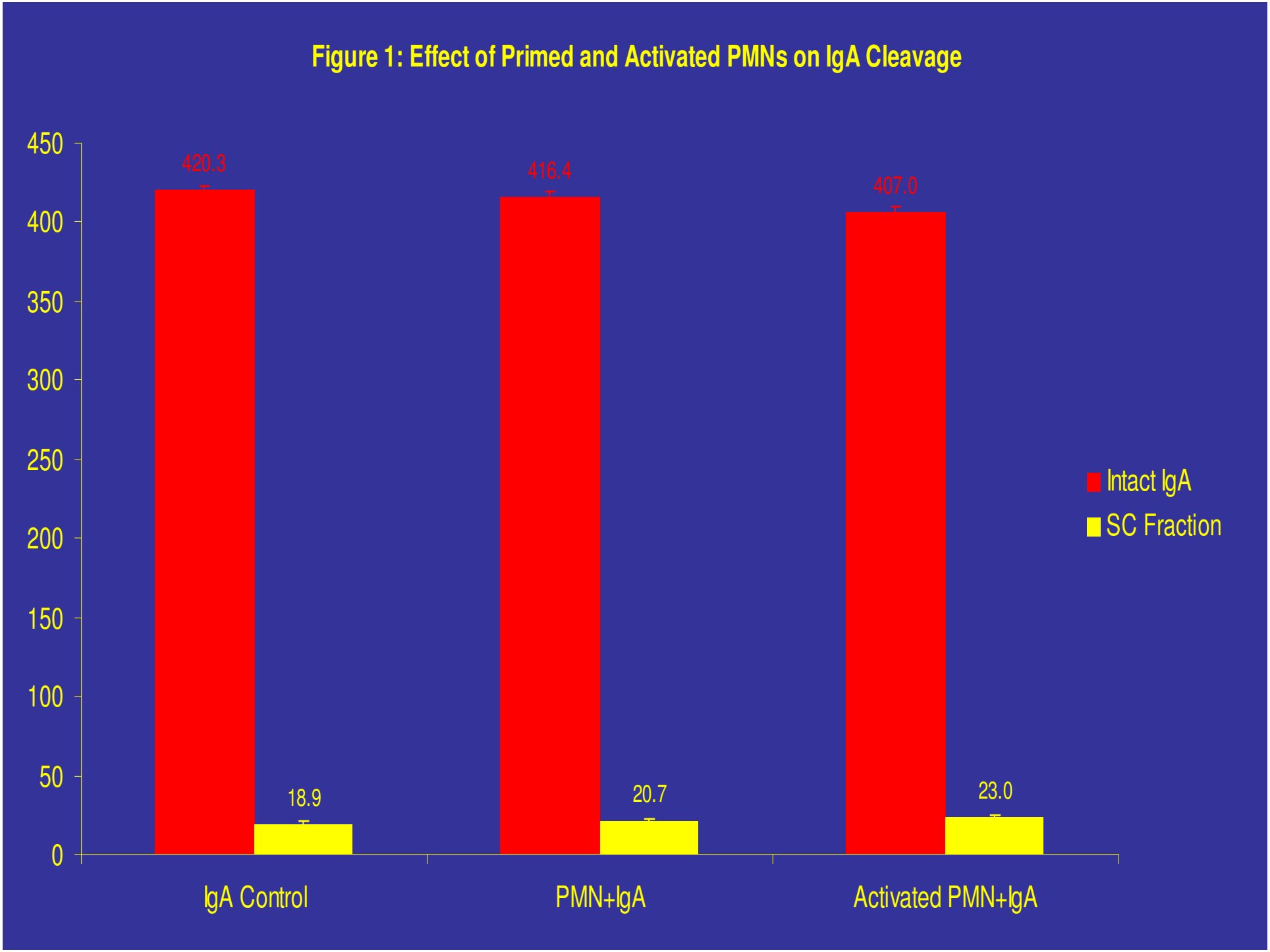
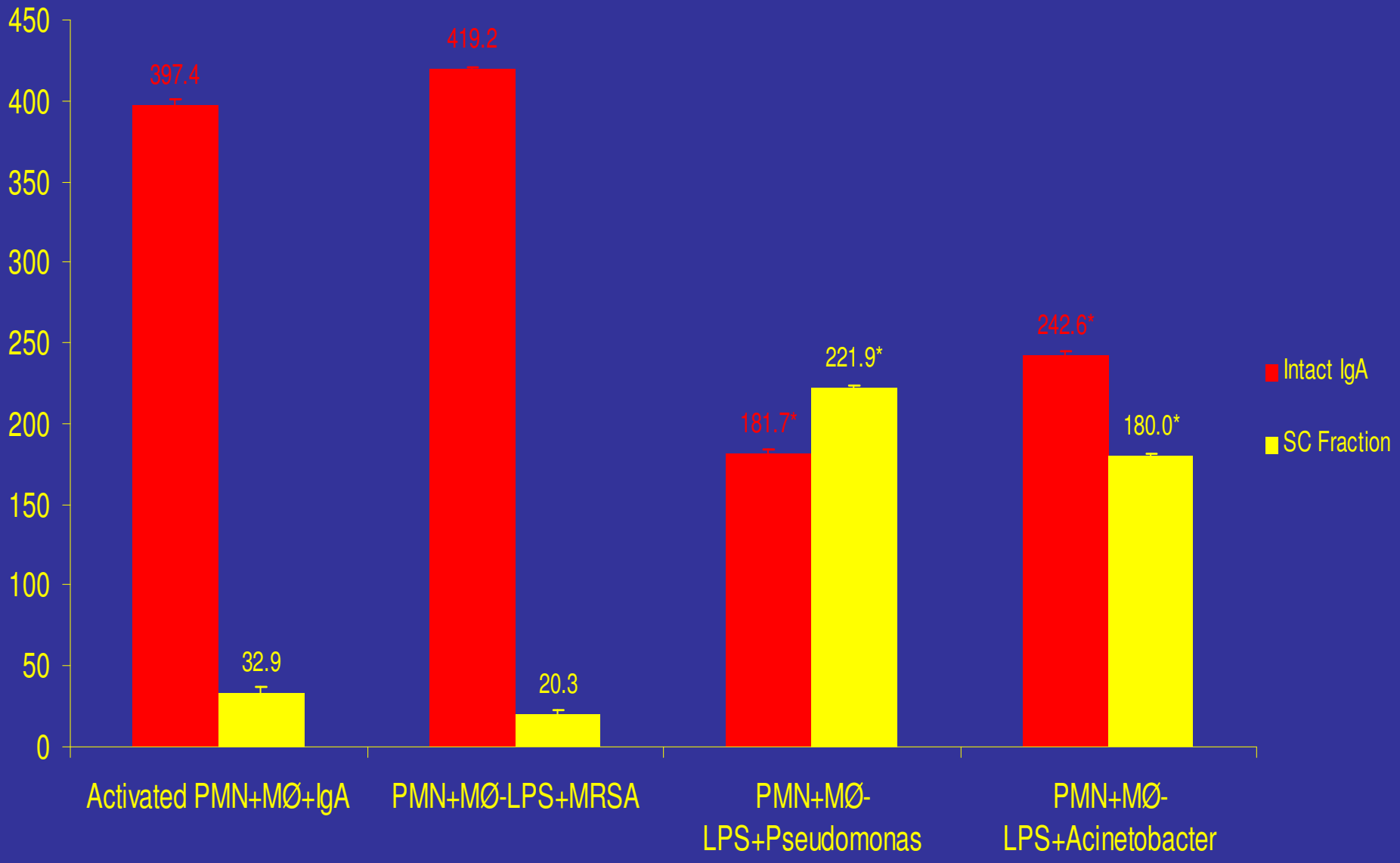


Figure 1: Effect of Primed and Activated PMNs on IgA Cleavage
450
400
350
300
250
200
150
100
50
0
420.3
416.4
407.0
18.9
20.7
23.0
IgA Control
PMN+IgA
Activated PMN+IgA
Intact IgA
SC Fraction

Figure 4: Effect of Bacterial Isolates on IgA Cleavage



*p<0.001 vs. all other groups

Purpose

- To compare the ability of SIgA vs. IgG to modulate PMN production of proinflammatory cytokines and chemotactic potential.
- To compare the sequence of addition of Ig isotypes SIgA 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

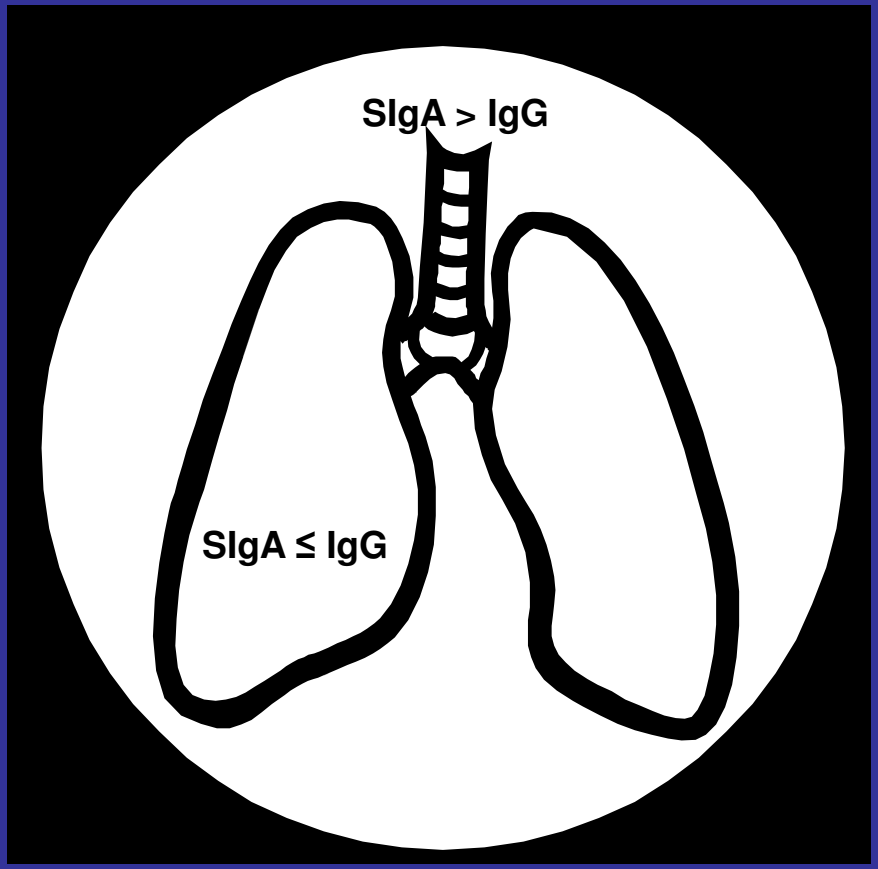
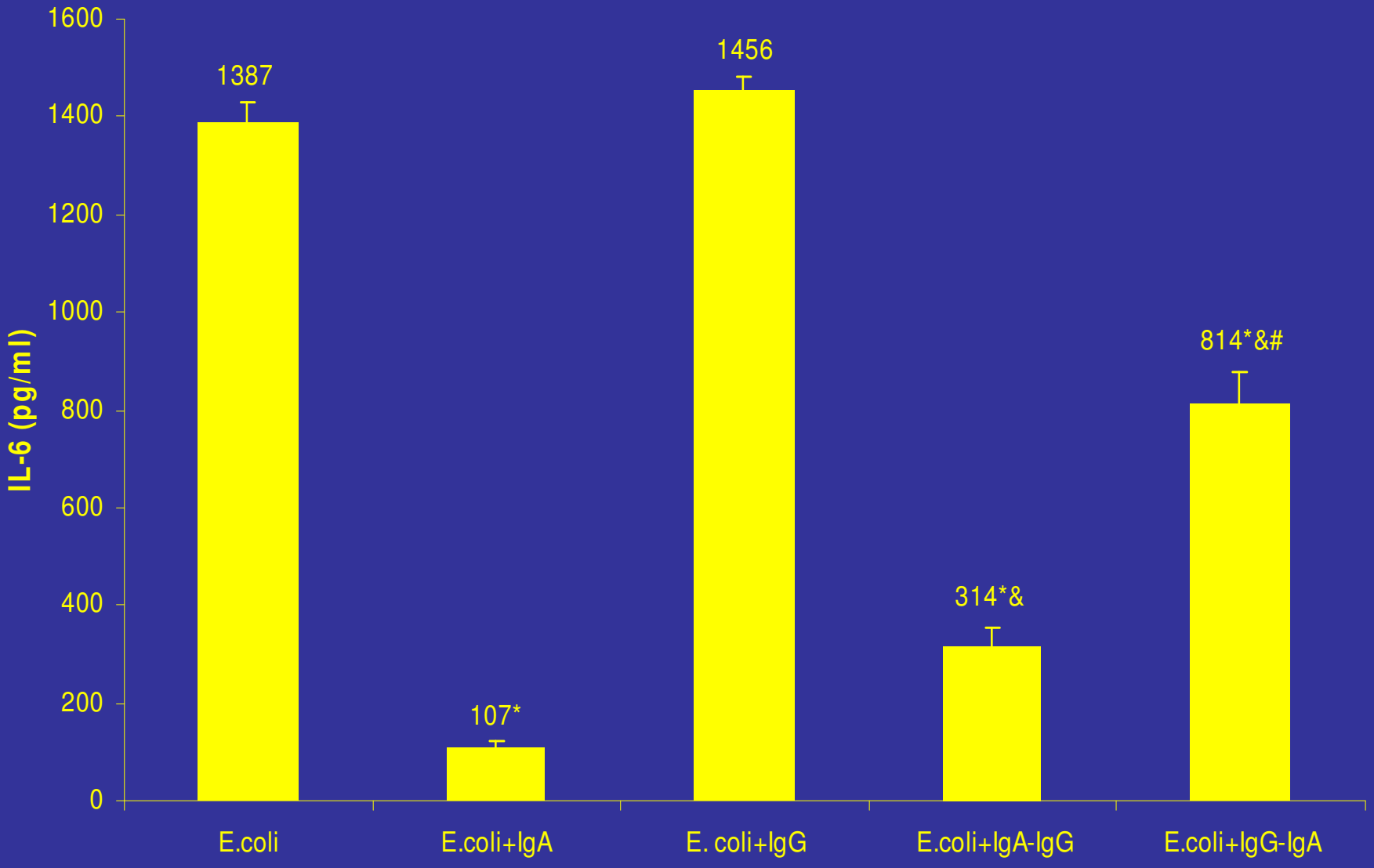


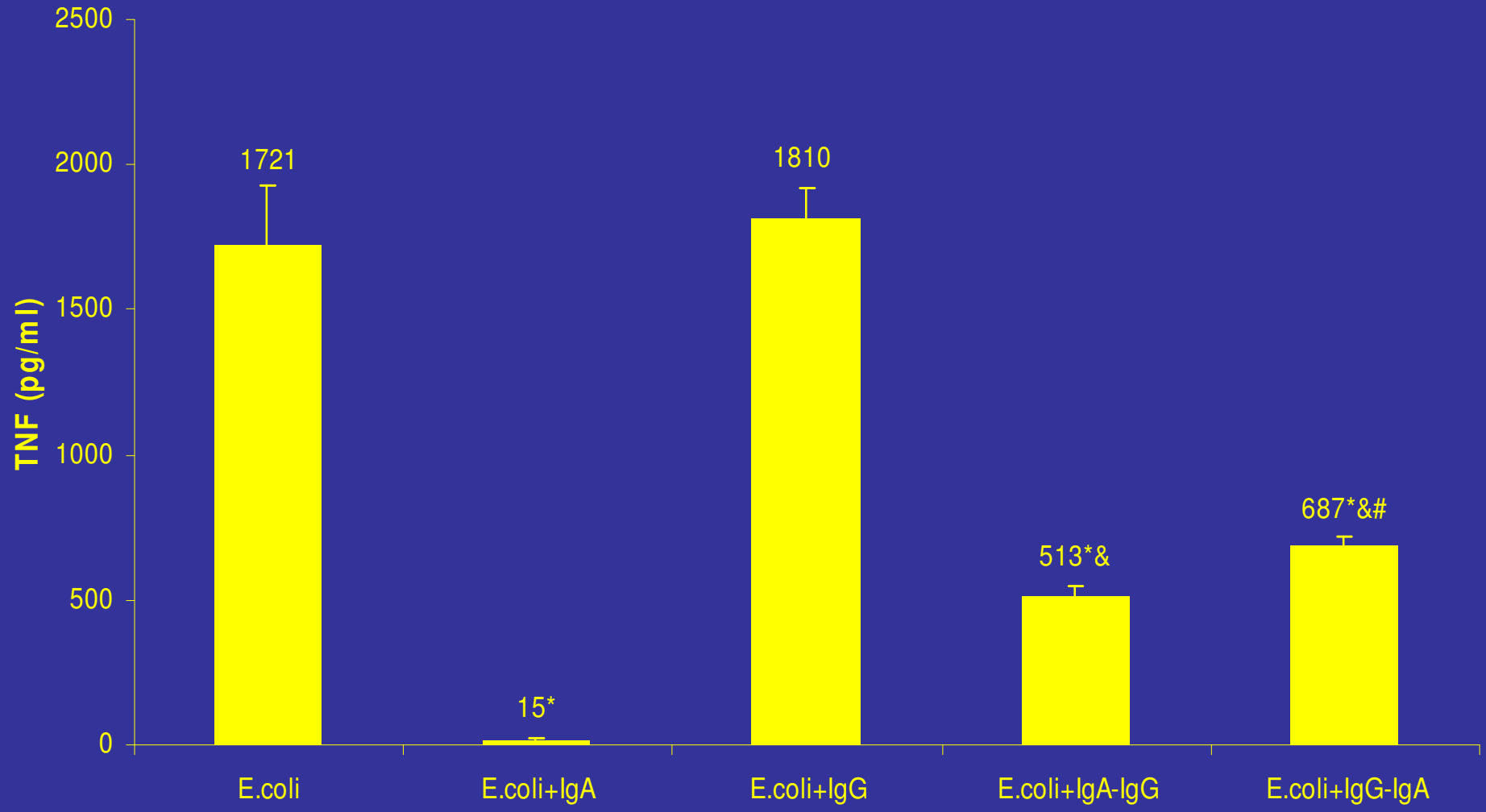
Figure 1: Effect of the sequence of exposure by IgA and IgG to E. coli mediated IL-6 production by monocyte-PMN cultures



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

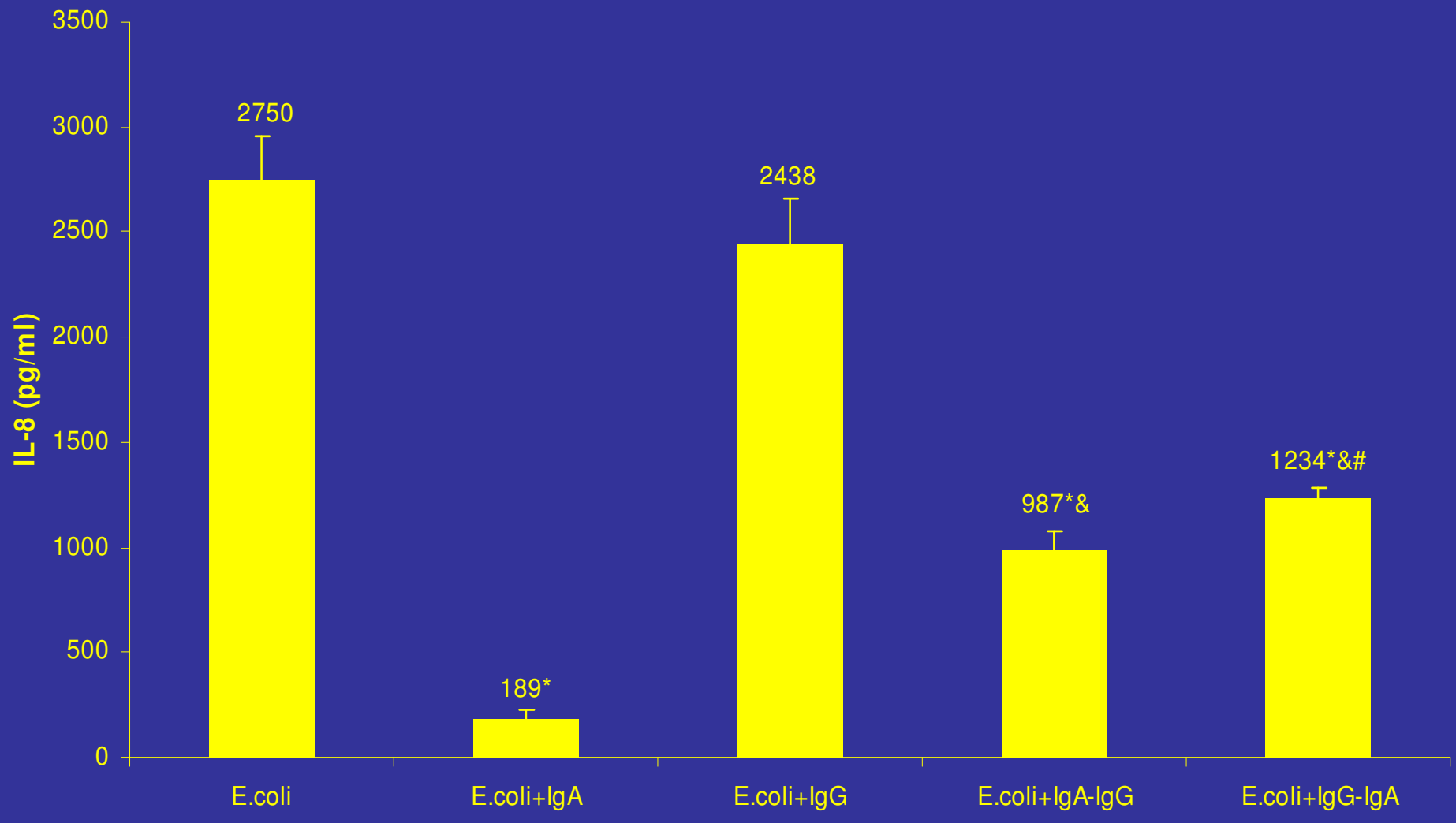
N = 4 for all groups

Figure 3: Effect of the sequence of exposure by IgA and IgG to E. coli mediated TNFα production by monocyte-PMN cultures



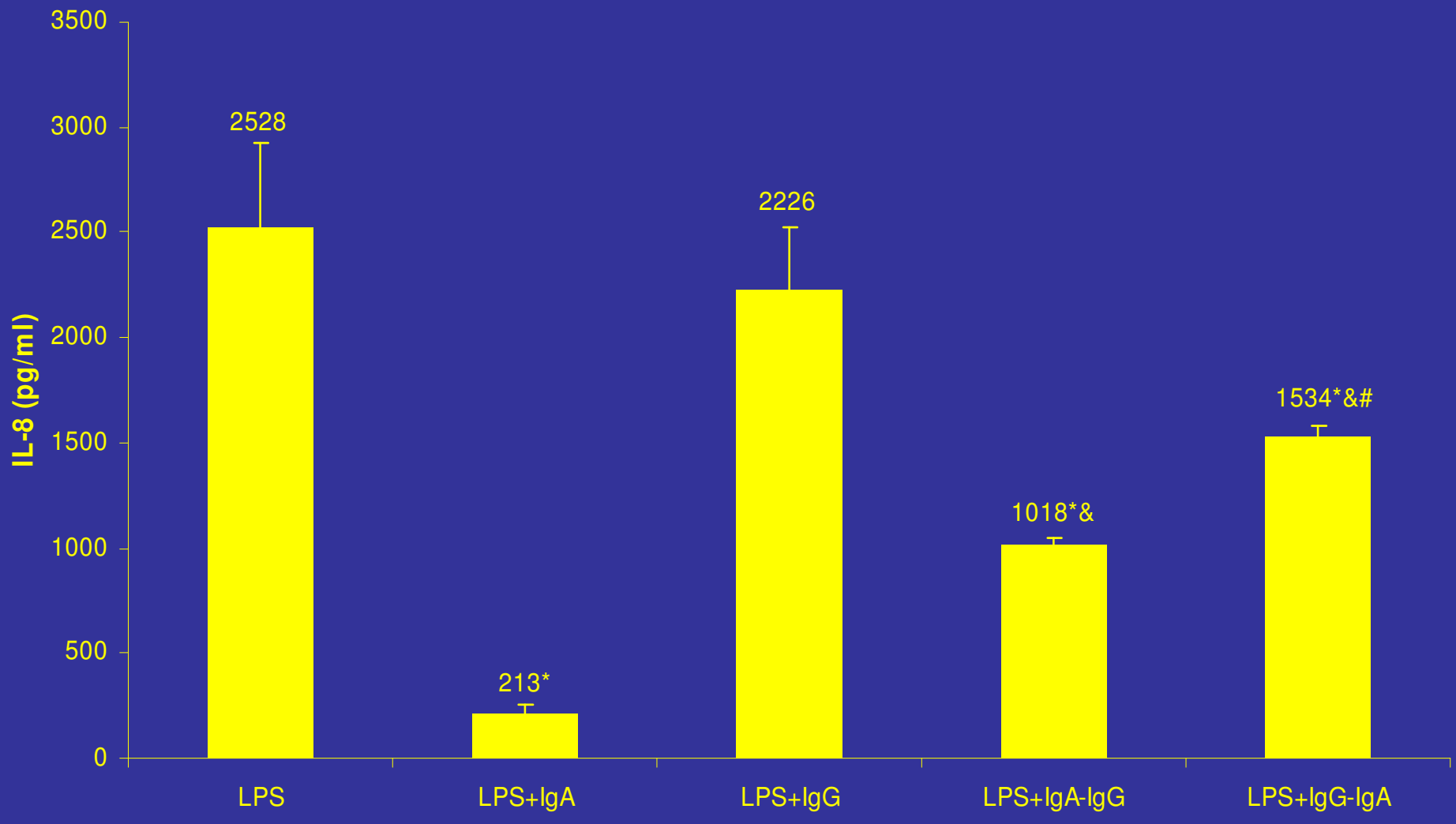
***p<0.001 vs. E. coli, &p<0.001 vs. E. coli+IgA, #p<0.001 vs. IgA-IgG sequence**
N = 4 for each group

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

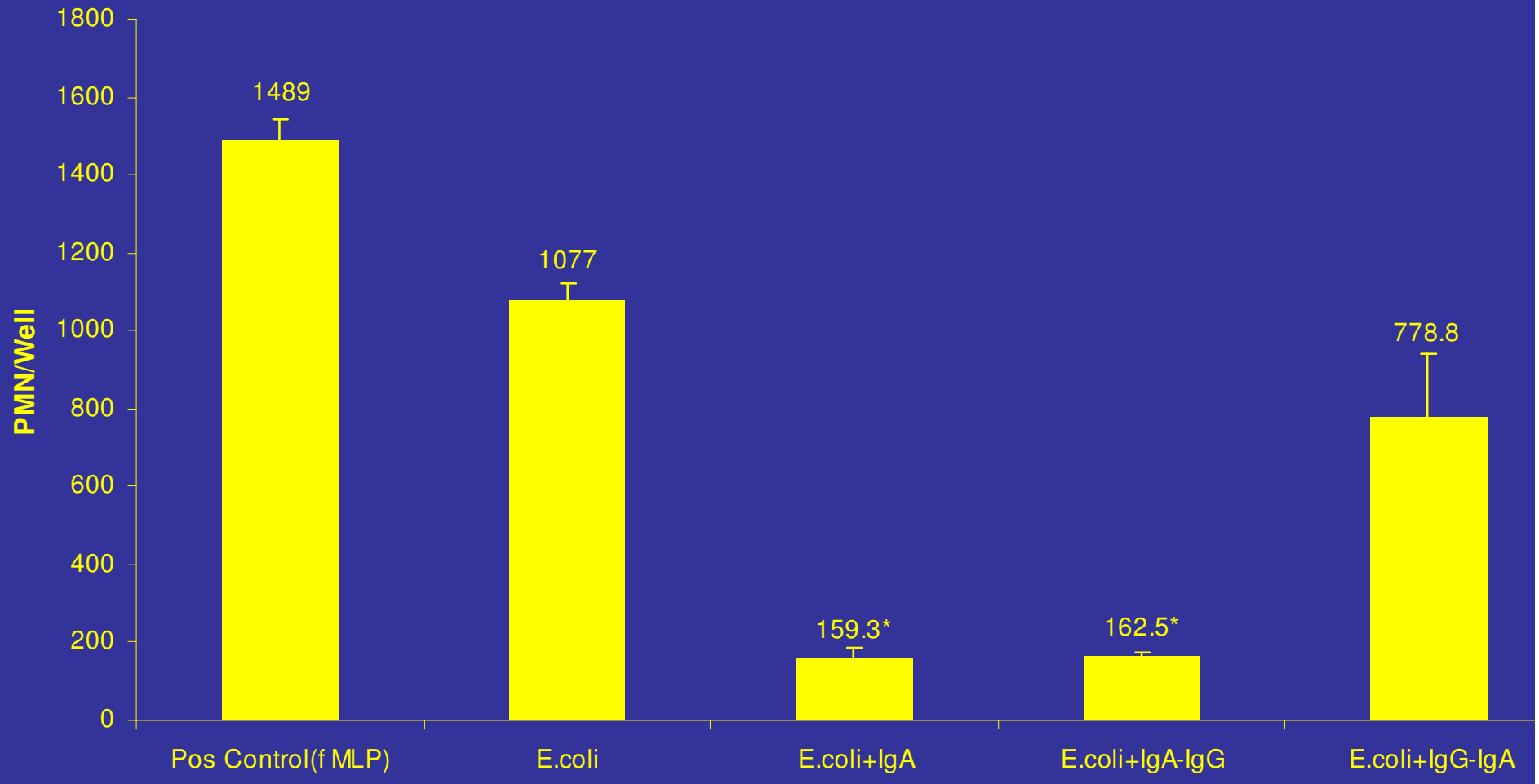
Figure 6: Effect of the sequence of exposure by IgA and IgG to LPS mediated IL-8 production by monocyte-PMN cultures



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

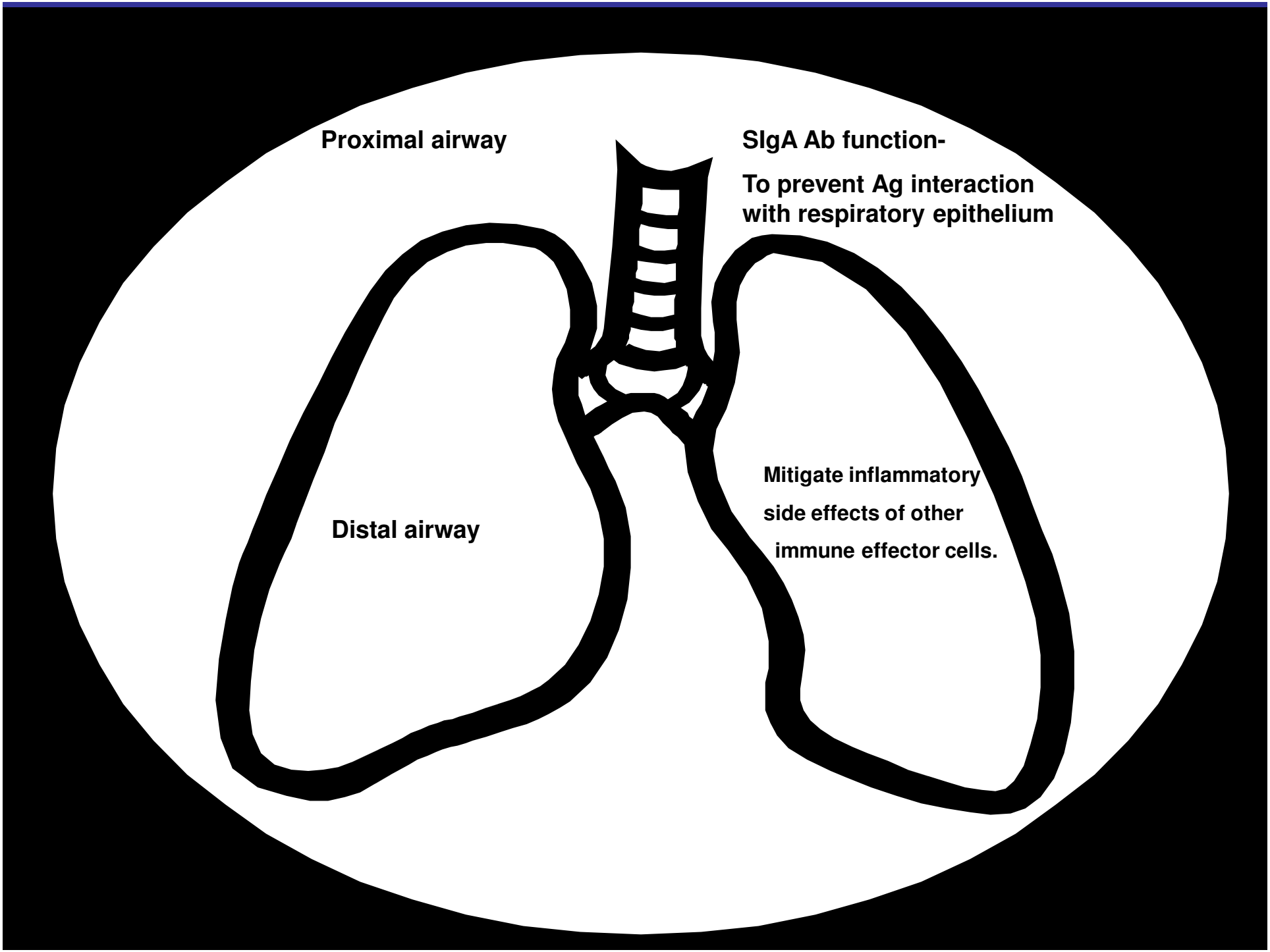
N = 4 for each group

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
SIgA Ab function-
To prevent Ag interaction with respiratory epithelium
Distal airway
Mitigate inflammatory side effects of other immune effector cells.

RECENT STUDIES

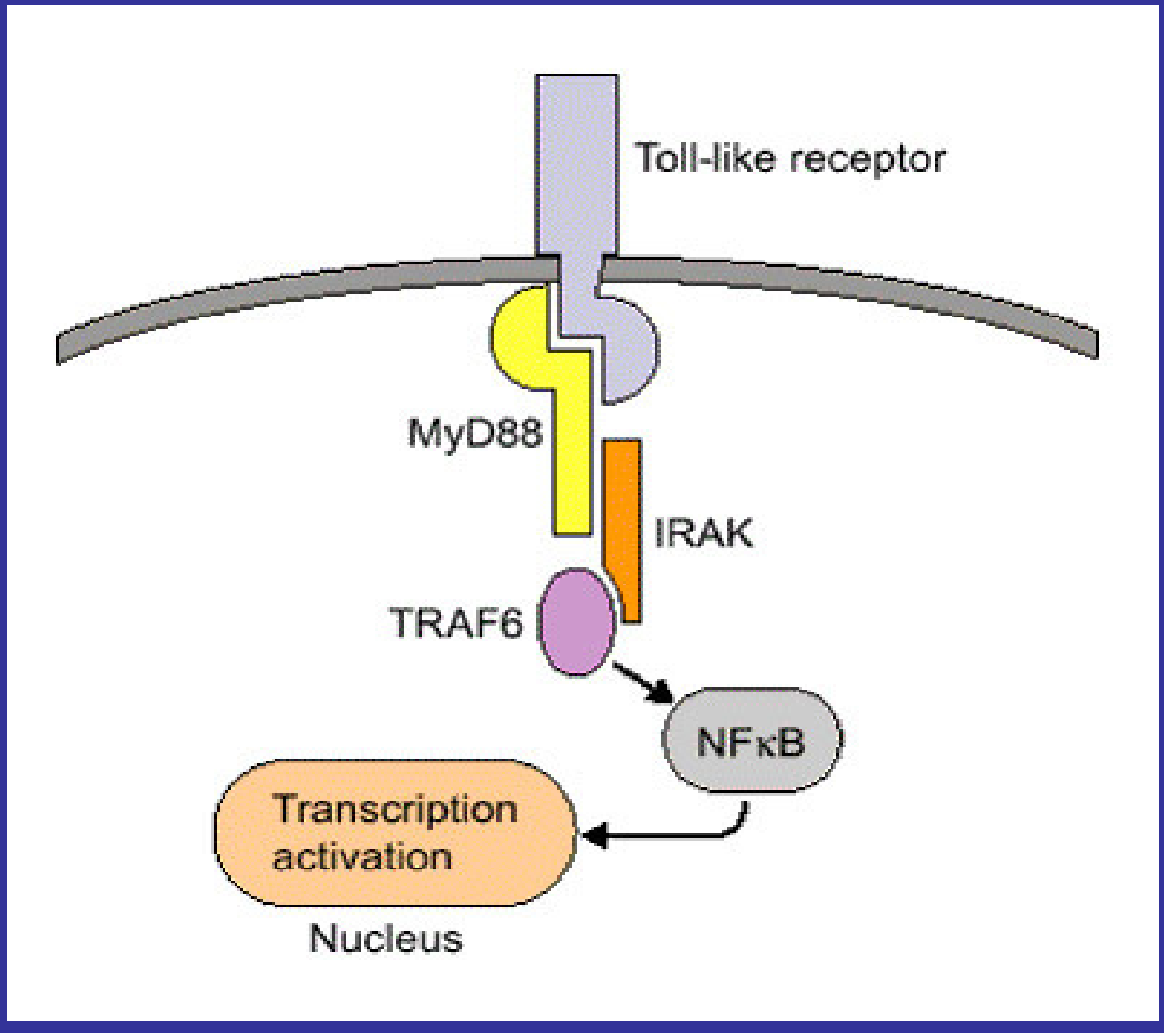
- WTA : 2009 Crested Butte

  Decreased survival and greater lung inflammation in survivors: PIgR 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

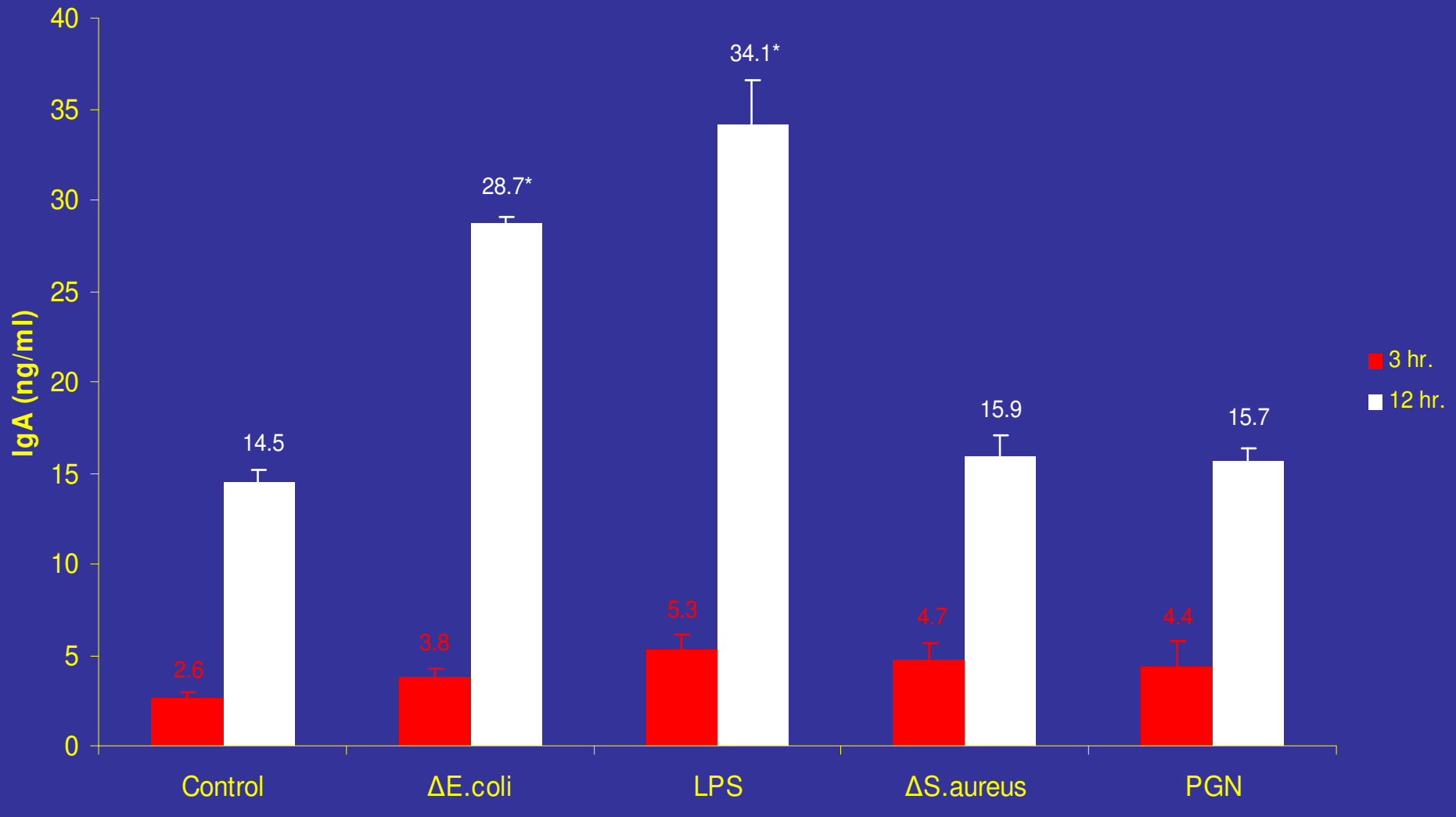


Disparate Effects of Bacteria and Toll-Like Receptor Dependant Bacterial Ligand Stimulation on IgA Transcytosis

Purpose

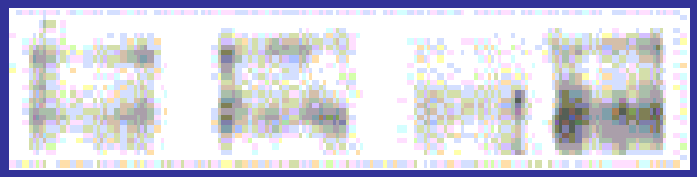
To study the effect of gram negative or gram positive bacteria and Toll-Like receptor bacterial ligand pathways on IgA transcytosis.

Figure 1: IgA Transcytosis in HT-29 Cells Following Stimulation with G- or G+ Bacteria and TLR ligands



***p<0.001 vs. Control**
N = 4 for each group

Figure 8: Densitometry Determination of plgR Expression at Timed Intervals Following Stimulation with LPS

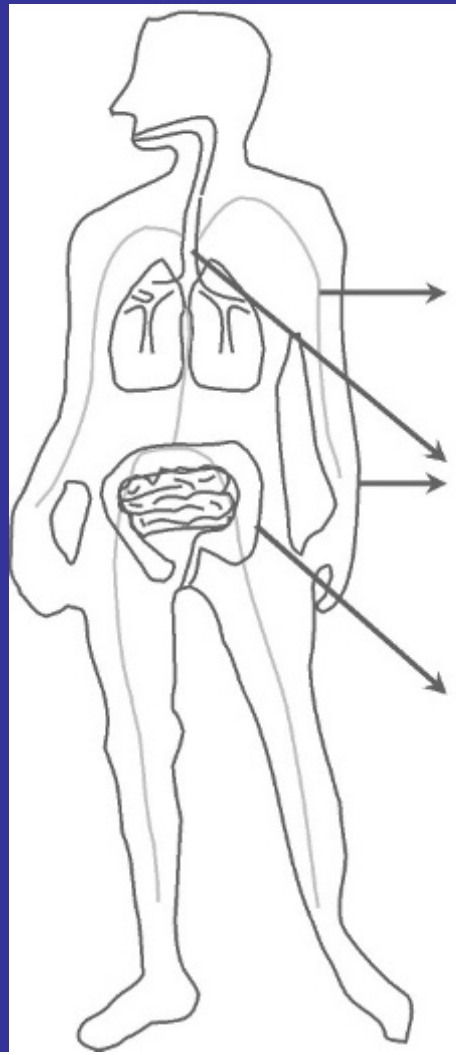


***p<0.001 vs. control and other timepoints**
N = 4 for each group

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?



compartment	microbial contact	PRR sensitivity
blood	none	high every microbial contact indicates danger
airways, skin	frequent	regulated certain microbial load might be tolerated
gut	permanent	suppressed tolerance is dominating

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


IGA
Market


SECRETORY
IgA