The CommonlyUsed Anesthetic Propofol Dramatically Increases Host Susceptibility toMicrobial Infection

BY

Lavanya Visvabharathy
B.S. University of Illinois at Chicago, Chicago, IL 2006

Submitted as partial fulfillment of the requirements
for the degree of Doctor of Philosophy in Microbiology and Immunology
in the Graduate College of the
University of Illinois at Chicago, 2013

Chicago, Illinois

Defense Committee:

Nancy Freitag, Chair and Advisor
Mike Federle, Biopharmaceutical Sciences
Gail Hecht, Digestive Diseases and Nutrition
Guy Weinberg, Anesthesiology
David Ucker, Microbiology/Immunology

ACKNOWLEDGEMENTS

The work in this thesis would not have been possible without the trust extended to me by Dr. Nancy Freitag, my advisor and mentor. I came to her lab initially in a very difficult position, and she not only took me in but allowed me to explore original ideas with no reservations. This was in spite of the fact that the project I proposed had next to nothing relating it to the work historically done in the lab! I was very lucky: my project took off and results came fairly quickly, allowing me to graduate on time. None of this would have been possible without Nancy's openness and guidance. I am sincerely grateful for the opportunities I have gotten in her lab. I don't think I personally could have been this productive or content anywhere else.

I also would like to acknowledge all of the lab members who have been so helpful through the years. I am especially grateful to Bobbi Xayarath for all her help with experiments and general knowledge about the lab and microbiology. With working on such an interdisciplinary project, sometimes the details of individual fields get overlooked. I could always count on Bobbi to explain things to me clearly, and this was hugely valuable to moving my work forward. I would also like to thank David McMullen for all his help with understanding the finer points of pathology and anatomy. He has been a wonderful resource for me when attempting to decipher what the histology slides I got back from the RRC Histology Core actually indicated about organ pathology.

This project also greatly benefited from the diverse knowledge my committee brought to the table. I have two MDs and three Ph.Ds on my committee, and all of their suggestions were thoughtful, practical, and really served to hone this project into something that made sense as a narrative instead of disjointed experiments. I am particularly grateful to Dr. Guy Weinberg; I knew next to nothing about anesthesia until you took me under your wing!

Finally, I would like to thank my family and friends- I couldn't have completed my degree without their support. My sisters, mother and my best friend Maryam Hussain played the biggest roles in my staying the course and obtaining my Ph.D. I have always had a desire to conduct research because I wanted to be able to explain the world around me. Somewhere during the first few years of my graduate work, everything seemed to be working against my obtaining this degree, and I lost that desire. Maryam reminded me constantly why I got into this field in the first place and encouraged me to take a risk in putting all of my efforts back into my work. I couldn't have dreamed of a better result. I am only here because she gave me the fortitude to continue when I was thinking of giving up.

Contents

Summary of dissertation work……...xii

Chapter 1: Introduction to anesthesia and immunity: Hospital-acquired Infections, Anesthetic Mechanism of Action, Anesthesia and Immunity, *Listeria monocyctogenes* immune responses, Goals of thesis project……1

1.1 The burden of nosocomial infections……...2
1.2 Anesthetic mechanisms of action…….3
1.3 Barbiturates and immunomodulation……...6
1.4 Propofol and immunomodulation……10
1.5 Propofol and iNOS suppression……..10
1.6 Propofol and NF-κB modulation…….12
1.7 Anesthetics and immunomodulation *in vivo*……15
1.8 *L. monocytogenes*: model pathogen for the study of host immunity……...15
1.9 Goals of thesis……..21

Chapter 2: Materials and methods…….22

Bacterial strains, media, culture conditions……...…22
Drug types and formulations……..23
Intravenous infections of mice……...25
Oral infections of mice……...27
Cell culture infection assays……..28
Measurement of transepithelial resistance in Caco-2 cell monolayers…….31
Bioplex cytokine and chemokine assays……...32
Histological examination of infected tissues…….33
Flow cytometry……..33
Statistical analysis……..34

Chapter 3: Characterization of the effects of propofol on mouse models of both oral and intravenous listeria infection……...35

3.1 Summary……..35
3.2 Introduction……..36
3.3 Results……..37
Propofol exposure increases host susceptibility to oral infection with LM…….37
Propofol treatment does not affect LM infection of host cells in vitro……47
Mice exposed to propofol fail to clear bacteria from target organs…….47
Propofol increases bacterial translocation to the brain……51
Propofol exposure increases host susceptibility to infection up to 96 hours after recovery from sedation……...56
Alternate anesthetics and drugs targeting the GABA-A receptor do not recapitulate the effects of propofol on host innate immunity…….....61

3.4 Discussion……..67

Chapter 4: Propofol treatment and immunomodulation: effect of drug exposure on host serum cytokine profiles immune cell recruitment and differentiation in target organs……73

4.1 Summary……73
4.2 Introduction……74
4.3 Results……75
Exposure to propofol during LM infection results in pathological alterations in organ structure……75
Propofol alters the expression of host cytokines and chemokines in response to LM infection..……81
Propofol exposure alters splenocyte populations following LM infection……90
4.4 Discussion……..101

Chapter 5: Effects of propofol treatment on infections caused by additional pathogens: *Streptococcus pyogenes, Salmonella enterica* serovar Typhimurium, and *Staphylococcus aureus*……110

5.1 Summary……..110
5.2 Introduction……111
5.3 Results……114
Propofol treatment increases host susceptibility to MRSA infection……...114
Propofol anesthesia increases severity of systemic salmonellosis……118
Anesthetized animals display increased frequency of necrotic lesions in *S. pyogenes* infection……121
5.4 Discussion……124

Chapter 6: Conclusions and plans for future studies……130

6.1 Summary of results……...130
Effects of propofol on intragastric LM infection……..……130
Effects of propofol on systemic LM infection……..……..131
Alternate anesthetics and infection susceptibility……...…131
Modification of immune responses by propofol……132
MCP-1 and inflammatory monocyte recruitment/differentiation……...133
Propofol and infection with other pathogens……..134
6.2 Future studies……137
S. aureus……..137
S. pyogenes……..138
S. Typhimurium……..139
Mechanistic studies: propofol and immunomodulation……149
Does propofol block cell differentiation/induce cell death?.....140
Does propofol decrease other immune cell populations during infection?.....140
If not the GABA-A receptor, what is the immunological target of propofol?...141
6.3 Concluding remarks……142

Cited literature……144

Curriculum Vitae……..161

List of Figures

Chapter 1

1.1: GABA-A receptor schematic…….5

1.2: Chemical structures of pentobarbital and propofol……..9

1.3: NF-κB activation by TNF-α.……13

1.4: LM infection and spread to target organs……18

1.5: Induction of immune cell differentiation by LM-infected macrophages…….20

Chapter 3

3.1: Propofol increases host susceptibility to oral infection with LM……39

3.2: Propofol increases host susceptibility to oral infection with LM- timecourse……40

3.3: Propofol increases host susceptibility to sub-infectious oral doses of LM…….41

3.4: Propofol worsens intestinal organ pathology in LM infection……....42

3.5: Propofol exacerbates oral infection with Δ*inlA/B* strain of LM……..44

3.6: Propofol exacerbates oral infection with $inlA^M$ strain of LM……..……45

3.7: Oral infection with InlAm results in high mortality after 72h…….……46

3.8: Exposure to propofol does not increase invasion of Caco-2 cells……..…..48

3.9: Propofol does not increase invasion of/intracellular growth in J774 cells…..……49

3.10: LM infection and propofol both do not affect tight junction integrity……..….50

3.11: Propofol inhibits immune clearance of LM……52

3.12: Cell-to-cell spread is required for LM infection, regardless of propofol……53

3.13: Propofol does not increase BMM killing of LM……54

3.14: Propofol does not increase killing of LM by peritoneal macrophages……55

3.15: Propofol increases LM brain infection……57

3.16: Propofol increases LM translocation to the brain at low infectious doses……58

3.17: Propofol decreases immune clearance up to four days post-sedation ……...59

3.18: Propofol does not affect immune clearance of LM 7 days post-sedation…….60

3.19: Alternate anesthetics increase host susceptibility to oral infection with LM……63

3.20: Alternate anesthetics do not affect immune clearance of LM……...64

3.21: GABA-A receptor drugs do not increase susceptibility to oral LM infection…...65

3.22: GABA-A receptor drugs do not increase susceptibility to IV LM infection…….66

3.23: Diagram of LM translocation across M cells…….70

Chapter 4

4.1: Propofol exposure increases liver pathology during LM infection…….77

4.2: Propofol treatment increases spleen pathology during LM infection……..78

4.3: Propofol treatment alone does not affect splenic architecture……..79

4.4: Spleens of LM infected animals treated with propofol are smaller.....80

4.5: Schematic of LM-induced cytokine secretion by macrophages……...83

4.6: Propofol alters serum levels of IL-1β, IL-6…….....84

4.7: Propofol alters serum levels of IL-12, TNF-α……85

4.8: Propofol causes IL-10 levels to spike late in LM infection……86

4.9: Propofol increases serum chemokine levels throughout LM infection……...87

4.10: Propofol changes patterns of IFN-γ expression in the serum.....88

4.11: Propofol decreases TGF-β levels late in infection…….89

4.12: Propfol does not affect monocyte recruitment early in LM infection……...91

4.13: Propofol does not affect monocyte recruitment, does decrease the number of mature macrophages ……..93

4.14: Propofol does not decrease neutrophils, DCs in LM-infected spleens……..94

4.15: Propofol reduces TGF-β^+ cells in LM-infected spleens……95

4.16: Propofol decreases TipDCs in the spleen at 48h post-LM infection……97

4.17: Propofol decreases Mac-3^{+}/$Cd11b^{+}$ cells 48h post-LM infection……98

4.18: Propofol significantly decreases differentiated TipDCs during infection……99

4.19: Propofol reduces TipDCs at 48h, 64h post-LM infection……100

4.20: Model of IFN-γ-induced macrophage activation……..105

Chapter 5

5.1: Propofol treatment increases severity of MRSA infection……..115

5.2 Propofol increases kidney pathology during MRSA infection…….116

5.3: Propfol increases kidney inflammation, abscess formation (table)…….117

5.4: Propofol increases susceptibility to oral *S.* Typhimurium infection……119

5.5: Propofol increases susceptibility to systemic *S.* Typhimurium infection…….120

5.6: Propofol increases necrotic lesions in *S. pyogenes* infected mice…….122

5.7: In absence of propofol, necrotic lesions are less frequent…….123

Chapter 6

6.1: Model of propofol's effects on host infection susceptibility……..136

List of Abbreviations

LM- *Listeria monocytogenes*

LOS- length of stay

SSI- surgical site infection

NMDA- N-methyl-D-aspartate receptor (CNS)

GABA-A- γ-aminobutyric acid receptor A (CNS)

MAPK- mitogen activated protein kinase

IFN-γ- interferon γ

LPS- lipopolysaccharide

NK cell- natural killer cell

iNOS- inducible nitric oxide synthase

NO- nitric oxide

NF-κB- nuclear factor kappa B

dsRNA- double-stranded RNA

NLS- nuclear localization signal

IκB- inhibitor of kappa B

TNF-α- tumor necrosis factor alpha

TLR- toll-like receptor

IL-6- interleukin 6

LTA- lipoteichoic acid

GM-CSF- granulocyte-macrophage colony stimulating factor

CLP- cecal ligation and puncture

MCP-1- monocyte chemoattractant protein 1

NADPH oxidase- nicotinamide adenine dinucleotide phosphate oxidase

ROI- reactive oxygen intermediates

RNI- reactive nitrogen intermediates

IL-12- interleukin 12

TipDC- TNF- and iNOS-producing dendritic cell

InlA and InlB- internalin A and internalin B

TER- transepithelial electrical resistance

MALT- mucosa-associated lymphoid tissue

CCR2- chemokine receptor 2

H&E- hematoxylin and eosin staining

IL-1β- interleukin 1β

KC- CXCL-1 neutrophil chemoattractant

IL-10- interleukin 10

TGF-β- transforming growth factor beta

DC- dendritic cell

MHC- major histocompatibility complex

MRSA- methicillin resistant *Staphylococcus aureus*

CFU- colony forming unit

TSS- toxic shock syndrome

CA-MRSA- community acquired MRSA

PBS- phosphate buffered saline

NET- neutrophil extracellular trap

SCV- Salmonella-containing vacuole

T_{reg}- T regulatory cell

SUMMARY

Hospital peri-operative infections remain a major health concern, with surgery representing a leading cause of nosocomial infections. Anesthetics modulate host immune responses, but it has been difficult to separate the variable of surgery from anesthesia administration when analyzing infection rates. Here, the well-studied bacterial pathogen *Listeria monocytogenes* (LM) was used to assess the impact of a surgical anesthetic on host infection susceptibility. Brief sedation with propofol was sufficient to increase the bacterial burdens of LM in mouse target organs by 10,000-fold following both oral and intravenous routes of infection. Increased host susceptibility to oral infection with LM was dependent on heightened bacterial translocation across the intestinal barrier, but not through intestinal epithelial cells. This indicated that propofol increases LM translocation through alternate portals of entry. Propofol treatment did not alter LM invasion or replication within host cells in culture, disrupt tight junction integrity in Caco-2 intestinal epithelial cells, or decrease the efficacy of LM killing in primary murine macrophages. Though sedation with propofol is brief due to its short half-life, animals remained highly susceptible to infection even 96 hours after recovery from sedation. Though the alternate anesthetics sodium pentobarbital and ketamine increased the susceptibility of mice to oral infection with LM, they did not affect susceptibility to intravenous systemic infection with LM, unlike propofol. Additionally, anesthetized animals infected with LM displayed more severe organ pathology in livers, spleens, and intestines. Propofol treatment altered serum cytokine and chemokine levels throughout infection, with particularly striking effects on IFN-γ, MCP-1, IL-10 and TNF-α. Concurrently, fewer differentiated macrophages and TNF and iNOS producing dendritic cells, both important in clearing *LM*, were evident in animals treated with propofol. Finally, animals sedated with propofol showed heightened susceptibility to

methicillin-resistant *Staphylococcus aureus*, *Salmonella enterica* serovar Typhimurium, and *Streptococcus pyogenes* as evidenced by increased bacterial burdens in target organs. These data indicate that anesthetization with propofol severely compromises host resistance to infection, an observation that has potentially profound implications for surgical outcomes and, ultimately, patient survival.

CHAPTER ONE

Introduction to anesthesia and immunity: Hospital-acquired Infections, Anesthetic Mechanism of Action, Anesthesia and Immunity, *Listeria monocyctogenes* immune responses, Goals of thesis project

Summary

Patients are exposed to a wide variety of pathogenic organisms in hospital settings. As patients undergoing surgery or requiring intensive care often stay at the hospital for days, they have a higher likelihood of being exposed to nosocomial pathogens. Anesthesia is a critical component in successful surgeries as well as long-term stays in the intensive care units (ICU) of hospitals. Though much care is taken to maintain sterility around patients in operating rooms and ICUs, anesthesia represents an understudied variable that may predispose patients to contracting nosocomial infections. The work in this thesis aims to characterize the mechanisms by which one commonly used anesthetic, propofol, increases host susceptibility to infection with bacterial pathogens. To provide perspective on the challenges associated with patient acquisition of microbial infections in hospital settings, background information will be included on nosocomial infections, the mechanisms by which anesthetics induce sedation, links between anesthesia and immunity, and how one human pathogen, LM, can be used as a model pathogen to elucidate the effects of anesthesia on immunity.

1.1 The burden of nosocomial infections

Peri-operative and ICU-acquired infections are major complications in US hospitals, resulting in significant morbidity and mortality in patient populations. Hospital-acquired infections rank amongst the top 10 leading causes of death in the US, with around 1.7 million people affected and 99,000 deaths in 2002[1]. Among these, surgical site infections (SSI) occur after ~2% of all surgeries[1-3]. While over 99% of surgery patients receive prophylactic antibiotics, the incidence of post-operative infections remains high, negatively impacting patient health outcomes and increasing health care costs by $1-$10 billion dollars per year[4-6]. Post-operative infections also significantly increase the length of stay (LOS) for patients, thus inflating healthcare costs. In SSIs resulting from neurologic, cardiovascular, or gastrointestinal surgical procedures, the LOS was increased by at least 10 days[1]. Additionally, the costs incurred by the increased LOS averaged about $20,000 extra per patient in 2009, with the largest increases in patients recovering from cardiovascular surgery[1]. Some of the greatest disparities in cost burdens and LOS pertaining to post-surgical infections result from *Staphylococcus aureus* and *Enterococcus faecalis* infections[7,8].

In order to prevent incidence of SSIs, a variety of techniques are commonly used. There is a strict policy of asepsis, where healthcare providers shave the patient's surgical site and may even bathe the patient in chlorhexidine[9], commonly used as an antimicrobial mouthwash. Additionally, healthcare providers themselves take precautions to be as sterile as possible prior to entering the surgical area, as well as during the procedure itself. Some other factors that surgeons and hospital staff try to control prior to performing surgery are the following: sterility

and ventilation of operating room air, surgical attire and drapes, and optimizing the use of antibiotic prophylaxis prior to starting surgery[5,6,9]. Antimicrobial prophylaxis, in particular, has been shown to be important in decreasing SSI and sepsis risk[10], though this may depend on the type of surgery as some studies have shown that SSI risk and bacterial colonization of the surgical site remain the same regardless of antimicrobial prophylaxis[11,12].

In the case of ICU infections, patients most commonly contract pneumonia associated with ventilator intubation [13-15] One third of patients worldwide are intubated[16], and the majority of the patients are concurrently sedated, often with propofol[17]. There were 3,525 cases of ventilator acquired pneumonia reported to the CDC in 2010, resulting in up to 5.8 cases per 1000 ventilator days[18]. Most infections result from *S. aureus* and *Pseudomonas aeruginosa* contamination of the lung and intubation apparatus[19]. Prophylactic measures include elevation of the head, precautions against internal bleeding, deep vein thrombosis, and interestingly, a daily assessment of sedation as pertaining to readiness to extubate patients[10], however, there were few if any studies looking at how sedation directly affects infection rates.

1.2 Anesthetic mechanisms of action

Whereas significant effort has focused on preventing hospital-acquired infections by reducing patient exposure to infectious agents during surgery as well as time spent in the ICU, relatively little attention has been directed toward understanding how anesthetics may negatively impact patient immunity to infection. Anesthetics generally used in a hospital setting consist of two groups: inhalational anesthetics and intravenous anesthetics. Inhalational anesthetic drugs include halothane, sevoflurane, and isoflurane, and these are commonly used to maintain anesthesia in patients undergoing surgery[20,21]. The intravenous anesthetics include ketamine,

thiopental/pentobarbital, and propofol, and are mainly used to induce anesthesia prior to surgery. Propofol is unique, however, in that it is administered to patients in the ICU as a sedative as well, and patients are often under anesthesia for days when intubated[17,22,23].

Though all anesthetic agents result in a loss of consciousness, the mechanisms by which they mediate anesthesia are distinct. Anesthesia is defined as a physical state characterized by the absence of pain and a loss of awareness[24]. Of the three intravenous anesthetics mentioned above, ketamine acts at the N-methyl-D-aspartate (NMDA) receptor, while thiopental and propofol bind to the γ-aminobutyric acid (GABA) receptor in the CNS[25-27] (Figure 1.1). The NMDA receptor binds the neurotransmitter glutamate and mediates excitatory synaptic transmission in a calcium-dependent manner[28]. Ketamine acts by blocking NMDA receptor activation through inhibiting calcium influx, thereby inducing a state of "dissociative anesthesia". This results in sensory loss, analgesia, and amnesia without actually resulting in a full loss of consciousness, and is accomplished by the dissociation of communication between the thalamus and the cortex[29]. Patients anesthetized with ketamine experience a state of unconsciousness between general anesthesia and deep sedation[29].

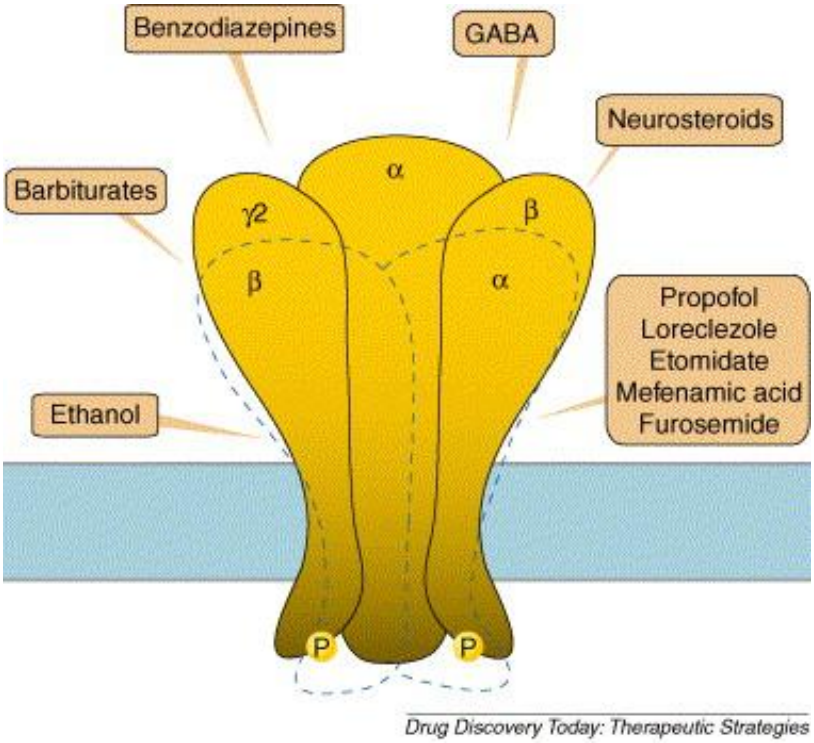


Figure 1.1 Schematic representation of GABA-A receptor with sites indicated for drug binding. *Adapted from Mohler et al, 2004*.

Thiopental and propofol are both GABA-A receptor agonists. GABA is the primary inhibitory neurotransmitter in the CNS, and is responsible for mediating the majority of fast synaptic inhibition of signals between neurons[24]. One study estimated that approximately one-third of all synapses in the CNS are responsive to GABA (GABAergic)[30], and thus GABA receptor agonists and antagonists can have effects on multiple brain systems. Structural studies using surrogate protein targets have shown that barbiturate anesthetics (such as thiopental, pentobarbital, and phenobarbital) and propofol bind to the same site[27]. However, the interactions between barbiturate drug molecules and the receptor binding site are quantitatively different from propofol's interaction with the same site in that barbiturates utilize both polar and nonpolar interactions to bind, whereas propofol's binding relies solely on van der Waals interactions[27]. This may be because propofol is a simpler, strongly hydrophobic molecule compared to barbiturates (Figure 1.2), and might indicate that propofol can be more promiscuous than barbiturates in its activity by virtue of its high lipophilicity allowing it to easily cross cell membranes. Regardless, both anesthetics mediate their sedative effects through potentiation of the GABA-A receptor, allowing Cl^- ion influx into neurons and causing inhibition of action potentials[27,31].

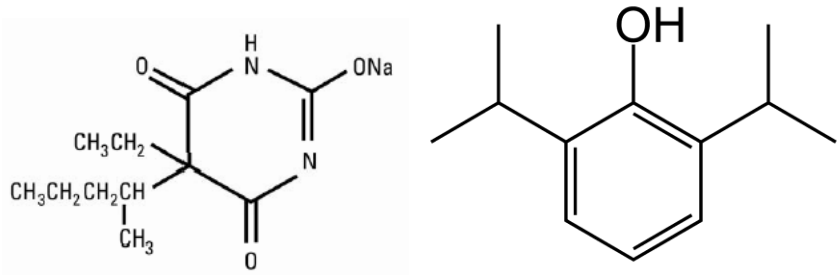
1.3 Barbiturates and immunomodulation

Anesthetic agents have been associated with immunomodulation in a variety of studies[32-52]. Barbiturate anesthetics have been linked to impaired phagocytosis of *S. aureus* by monocytes *in vitro*[49]. This study examined whole blood samples from healthy patients incubated with thiopentone (a barbiturate anesthetic) or propofol and subsequently infected with *S. aureus*. It was found that increasing concentrations of thiopentone in whole blood culture inhibited

phagocytosis of *S. aureus*, though incubation with propofol did not affect the phagocytic capabilities of granulocytes[49]. Barbiturates have also been linked to decreased superoxide generation in macrophages[53], inhibition of neutrophil activity[54], and reduction of T cell synthesis of cytokines, T cell cytotoxicity, and responsiveness to antigens[36]. Regarding neutrophil immunomodulation, neutrophils isolated from human volunteers were assayed for chemotaxis, phagocytosis, and superoxide production in cell culture in the presence of thiopentone. Incubation with thiopentone decreased neutrophil chemotaxis toward the neutrophil chemoattractant FMLP and decreased phagocytosis of opsonized oil droplets in a dose-dependent manner[54]. The study also showed that thiopentone abrogated superoxide production, especially affecting H_2O_2 and OH^- production in primary neutrophil culture[54].

Much work has been done examining how barbiturate anesthesia particularly affects T cell function. In contrast to the studies mentioned above that indicate barbiturates can affect innate immune parameters, one group postulated that the immunosuppressive potential of thiopental was confined to antigen-specific responses[36]. This study examined whole blood from patients anesthetized with thiopentone and sedated for approximately 1 hour. A portion of patients had received the tetanus vaccine 30 days prior to surgery. T cell proliferation and cytokine secretion was measured *ex vivo*. While thiopentone exposure did not alter mitogen-induced T cell proliferation in unvaccinated volunteers, vaccinated volunteers showed decreased tetanus toxoid-specific T cell proliferation[36]. IL-2 production in T cells was similarly abrogated in vaccinated patients exposed to thiopentone, in an antigen-specific manner[36]. Another study found that thiopental treatment of primary $CD3^+$ T cells from whole blood inhibited T cell transcription factor AP-1 in a mitogen activated protein kinase (MAPK)-dependent manner[55], indicating that barbiturate anesthetics can not only affect gene transcription generally, but also

immune-related genes particularly. AP-1 activates the transcription of multiple genes involved in the initiation of both innate and adaptive immune responses[56,57]. The studies outlined above suggest that barbiturate anesthetics are immunosuppressive with regards to T cell function as well as neutrophil and macrophage function in vitro.



Pentobarbital **Propofol**

Figure 1.2 Chemical structures of pentobarbital and propofol showing simplicity of propofol's molecular structure compared to a barbiturate agonist of the GABA-A receptor.

1.4 Propofol and immunomodulation

Propofol is the most commonly used induction agent in surgeries performed in the US[58,59]. Anesthesiologists prefer to use propofol due to its quick induction of anesthesia and relatively low incidence of agitation upon patient recovery from sedation[60-63]. In spite of these benefits, propofol also has been shown to adversely affect immune parameters in vitro. Many studies have linked propofol exposure to impaired phagocytosis by macrophages[34,50,52], as well as decreased iNOS production in macrophage cell lines[35,39,64]. One study conducted in murine macrophages exposed to varying concentrations of propofol found that drug exposure significantly reduced chemotaxis, oxidative burst, and interferon-γ (IFN-γ) production upon contact with lipopolysaccharide (LPS)[34]. However, other *in vitro* studies have indicated that IFN-γ secretion increases when macrophages cocultured with NK cells are exposed to propofol[41]. Various studies also have shown propofol to protect against LPS-induced cell death in culture[65], though the protective effect propofol has on cell survival has been shown to have adverse effects in cancer progression[66]. Interestingly, the same study showed that exposure to propofol in cell culture can alter gene expression patterns[66], suggesting that propofol may play a downstream role in regulating transcription. Propofol is a potent antioxidant due to the presence of a phenol ring in its chemical structure[35,39,67-69], and inhibition of reactive oxygen species is one mechanism by which propofol decreases apoptosis in cell culture[33].

1.5 Propofol and iNOS suppression

Though many reports have indicated the immunomodulatory potential of propofol, studies have been inconclusive in cohesively defining how propofol sequentially affects the immune response by altering first innate then adaptive immunity. One emerging point not in

contention, however, is that propofol blocks inducible nitric oxide synthase (iNOS) expression in cell culture[39,52,64,67]. Initial host defense against invading bacterial pathogens is largely dependent on iNOS for signaling and coordinating the different arms of innate immunity, as well as direct action against intracellular bacteria[70-74]. For example, host clearance of LM depends on iNOS-driven production of reactive nitrogen species to kill bacteria trapped in the phagosomal compartment and decrease intracellular replication, as well as NO-mediated immune signaling[70,75]. Pretreatment with propofol was found to significantly reduce LPS-induced iNOS expression and generation of reactive NO in mouse macrophages[39]. Another study using LPS to induce sterile inflammation in mice similarly found propofol to inhibit free radical generation and thus decrease cell death[67]. As NO is a potent mediator of inflammation[76], propofol treatment was found to be protective to the host when septic shock was induced by a non-infectious inflammatory stimulus.

The mechanism by which propofol suppresses iNOS activity is not fully known, but the decrease in NO production mediated by propofol seems to be dependent on its antioxidant properties[63,77]. Studies using chemiluminescence and electron spin resonance spectroscopy showed that propofol acted as a free radical scavenger of peroxynitrite, a free radical formed by the association of nitric oxide and the superoxide radical[78], and formed phenoxyl radicals[79]. These studies suggest that, by virtue of its lipophilicity and ability to easily cross cell membranes[63], propofol can enter cells and scavenge free radicals such as NO, thus acting as an anti-inflammatory compound. While this phenomenon has been investigated in numerous model systems in vitro and in vivo, the studies did not use infectious agents to induce inflammation in hosts when examining the effects of propofol on immunomodulation. Thus, it is as yet unknown

whether propofol anesthesia protects or increases adverse outcomes in a physiologically relevant model system.

1.6 Propofol and NF-κB modulation

The observations in cell culture and in vivo that propofol can alter cytokine secretion[41,45,63,80] and iNOS expression[35,39,64] indicate that exposure to the drug can affect nuclear factor kappa B (NF-κB) activation and/or signaling, perhaps through direct modulation of NF-κB. NF-κB consists of a family of seven related transcription factor subunits that can operate in various combinations to activate gene transcription[81] (Figure 1.3). NF-κB activation is induced by a variety of environmental signals, including ultraviolet light, dsRNA, cytokines, vasoactive peptides, and viral oncogenes[81-83]. NF-κB in its inactive form is normally localized in the cytoplasm as a homo- or heterodimer[83,84]. DNA-binding and dimerization domains as well as a nuclear localization signal (NLS) are located at the N-terminal region of NF-κB[83,85]. Inactive NF-κB is retained in the cytoplasm through interaction with inhibitor of kappa-B (IκB) family proteins[84,85]. These proteins interfere with the NLS present on NF-κB proteins and prevent their translocation to the nucleus, which is necessary for activation. Binding of tumor necrosis factor alpha (TNF-α) to its cell-surface receptor and bacterial endotoxin or byproduct interaction with some toll-like receptors (TLRs) are two signals that result in downstream activation of NF-κB through degradation of IκB and dimerization of NF-κB[86].

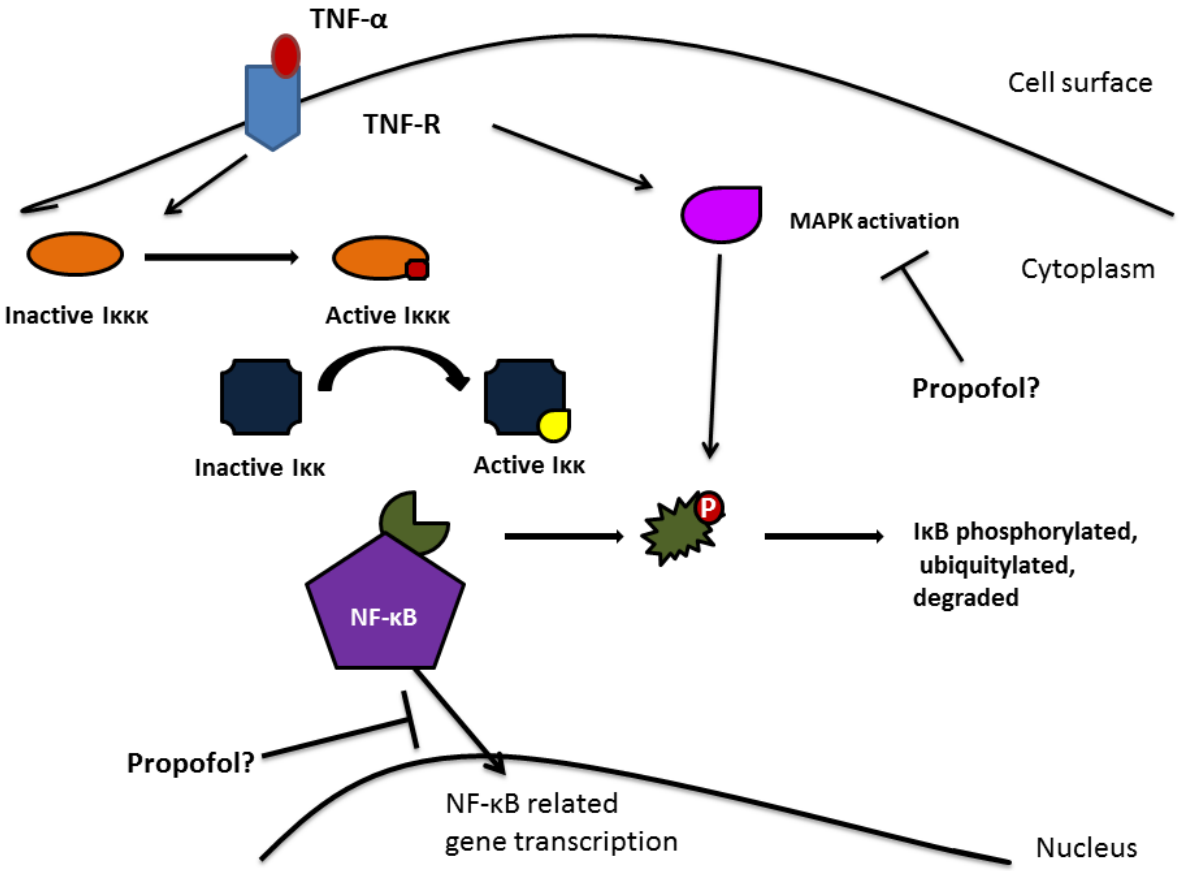


Figure 1.3 NF-κB pathway activation by binding of TNF-α to its cell surface receptor, and modulation by propfol. *Adapted from Motifolio Biomedical Toolkit, 2012.*

Propofol has been shown to decrease TNF-α secretion and LPS-related proinflammatory signals in vivo and in vitro[38,64,67,80,87], indicating that exposure to the drug could decrease NF-κB activation and thus be immunosuppressive. Studies conducted in murine macrophages showed that when cells were stimulated with LPS, propofol reduced TNF-α and IL-6 secretion, and concurrently inhibited NF-κB translocation to the nucleus[39]. The same pattern was seen in an in vivo polymicrobial sepsis model, where rats exposed to infectious stimuli via cecal ligation and puncture in the presence of propofol displayed a decrease in serum levels of TNF-α and IL-6, as well as lower NF-κB activation as determined by Western Blot[87]. Propofol treatment also reduced NF-κB activation in breast cancer cells[88], downregulated NF-κB translocation to the nucleus in macrophages stimulated with the TLR2 agonist lipoteichoic acid (LTA)[64] and the TLR4 agonist LPS[89], and attenuated granulocyte-macrophage colony stimulating factor (GM-CSF) production in hepatocyte cell culture through preventing NF-κB translocation to the nucleus[90]. Propofol also seemed to block NF-κB translocation through modulation of the pro-survival MEK/ERK pathway, according to a study conducted in cardiomyocytes[91]. The above studies indicate that propofol treatment suppresses inflammatory signaling from cytokines and microbial byproducts at the cell surface, and subsequently interferes with the transcription of pro-inflammatory genes regulated by NF-κB by preventing its translocation from the cytosol to the nucleus. Propofol seems to have promiscuous activity in modifying cellular processes relating to inflammation in that it not only acts as an antioxidant, but also as an agent that blocks signaling and represses transcription.

1.7 Anesthetics and immunomodulation in vivo

In spite of the multitude of reports indicating the immunomodulatory potential of anesthetics, only a small number have been conducted in vivo[38,49,92,93]. One study to address the effects of anesthesia in mice has indicated that mice anesthetized intraperitoneally with the barbiturate anesthetic pentobarbital displayed increased susceptibility to oral but not intravenous infection with LM[37], but the reported studies did not address mechanism. Studies have also shown that colonization of the small intestine of mice by *Vibrio cholera* was greatly enhanced by intravenous ketamine anesthesia[94]. In contrast, another study indicated propofol anesthesia was protective against polymicrobial sepsis in a cecal ligation and puncture (CLP) model of sepsis in rats through suppression of NF-κB[87]. These in vivo studies raise many questions about how anesthetics modulate immunity mechanistically, and in what context they are immunosuppressive. There are numerous conflicting reports in the literature describing propofol as both proinflammatory and anti-inflammatory. In vivo data has shown intriguing correlates between anesthesia and increasing infection susceptibility[37,94,95], but studies have mostly been phenomenological without delving into underlying mechanism. In order to fully understand how anesthetics may impact immunity, further studies need to be conducted in animal models in the context of infection. The work in this thesis attempts to more fully understand the role of anesthesia in the progression of infection and immunity by examining how propofol alters the host response to infection by a well-characterized model pathogen, *Listeria monocytogenes*.

1.8 *L. monocytogenes*: model pathogen for the study of host immunity.

LM is a gram-positive intracellular pathogen commonly found in the environment in soil and decaying plant matter[96]. Infection in human hosts is commonly contracted through the

ingestion of contaminated food products[96,97], and in immunocompetent people is limited to mild gastroenteritis[98]. Susceptible individuals can contract systemic listeriosis which can manifest in meningoencephalitis and septicemia, in its most serious form[98,99] (Figure 1.4).

LM has been used as a model pathogen to study the host immune response to intracellular microbes for decades, and both innate and adaptive immune responses to LM have been extensively characterized. In order to establish infection, LM first invades or is phagocytosed by host cells (often macrophages) and is temporarily trapped in the phagosome[100]. Upon escape to the cytosol, the bacteria replicate and spread from cell to cell using actin-based motility[97,101]. Intracellular bacteria are rapidly transported to the liver and spleen, where innate immune defenses are activated.

Innate immunity against LM is comprised of a series of complex but interrelated signaling and effector phenomena. Phagocytosis of LM and degradation of microbial products activates NF-κB (as well as AP-1) and causes the release of inflammatory stimuli, such as the chemokine MCP-1, which induces inflammatory monocyte recruitment to the spleen[102-105]. Though bacteria are taken up by multiple types of immune cells, their fate following engulfment can vary.

Some immune cells directly kill LM (NK cells)[106], while others are involved in antigen presentation to T cells (conventional dendritic cells, CDCs)[107,108]. Still other cell types are involved in both immune signaling/coordination and direct killing of intracellular LM. Macrophages are crucial to host defense against LM and perform a number of microbicidal and signaling functions (Figure 1.5). They phagocytose and digest invading bacteria, produce and secrete cytokines important for immune signaling, and occasionally present antigens to T cells[103,109]. Once LM is taken up by macrophages into the phagosome, the nicotinamide adenine

dinucleotide phosphate (NADPH) oxidase complex is recruited and begins to produce superoxide radicals[110]. This luminal superoxide decays to form reactive oxygen intermediates, or ROI, that act to break down intraphagosomal components and facilitate microbial killing[109]. In order to trigger NF-κB or produce superoxide, infected macrophages must first become activated by exposure to IFN-γ[109]. Resting macrophages containing phagocytosed bacteria secrete IL-12 and stimulate T cells to produce IFN-γ, which then goes on to activate macrophages and other immune cells at sites of infection and inflammation[111].

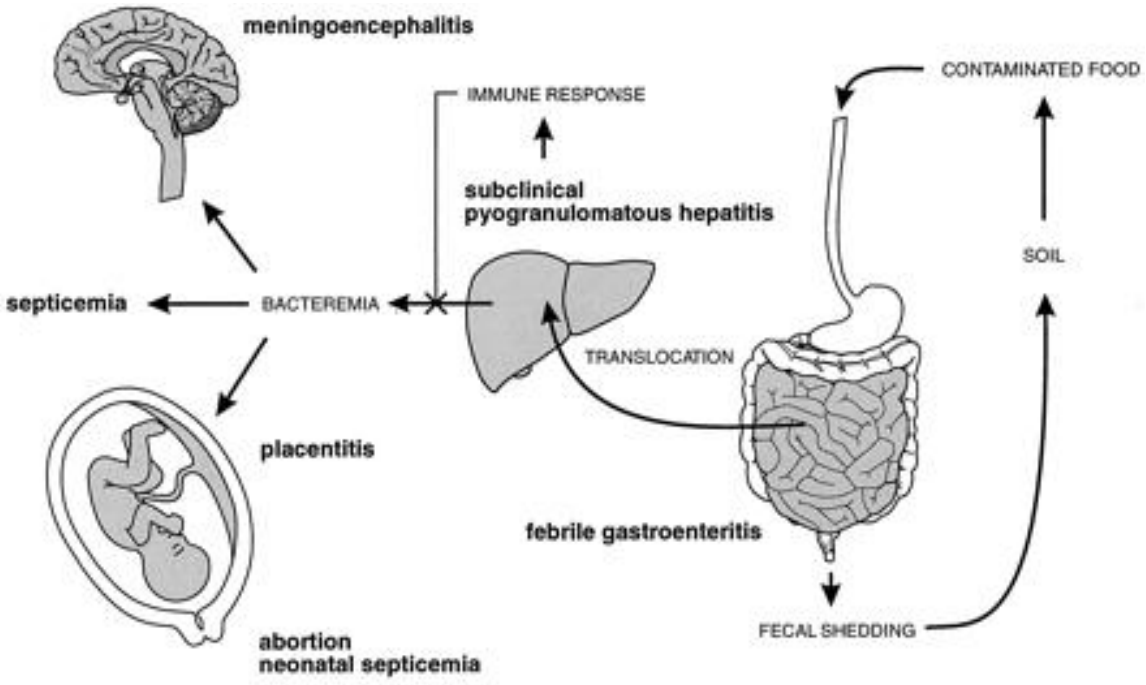


Figure 1.4 LM infection and spread to target organs. *From Vazquez-Boland et al, Clin. Microbiol. Rev., 2001*.

Circulating myelomonocytic cell populations are also crucial to clearance of systemic LM infection. In mice, a number of distinct monocytic subsets exist that have been defined by chemokine receptor expression[112]. Monocytes that traffic to sites of infection and inflammation are termed "inflammatory monocytes" and are characterized by high expression of Ly-6C, a GPI-anchored monocyte differentiation marker[107] and expression of chemokine receptor 2 (CCR2) at the cell surface[113] (Figure 1.5). Monocyte recruitment depends on the secretion of the chemokines CCL2 and CCL7, or MCP-1 and MCP-3, from activated macrophages at sites of LM infection[114,115]. Once they reach sites of infection, Ly-6C^{hi} CCR2^{+} inflammatory monocytes differentiate into discrete effector populations, including mature F4/80^{+} macrophages and TNF-α and iNOS-producing dendritic cells (TipDCs) (Figure 1.5)[112]. TipDCs are crucial for LM clearance as they produce and secrete cytokines as signaling molecules to coordinate immune responses. Once they reach foci of infection, inflammatory monocytes differentiate into TipDCs and migrate to lymph nodes where they promote T cell expansion[112], thus giving them the name "dendritic cell". TipDCs are dendritic cells of myeloid origin that can bridge the innate and adaptive immune response to LM infection.

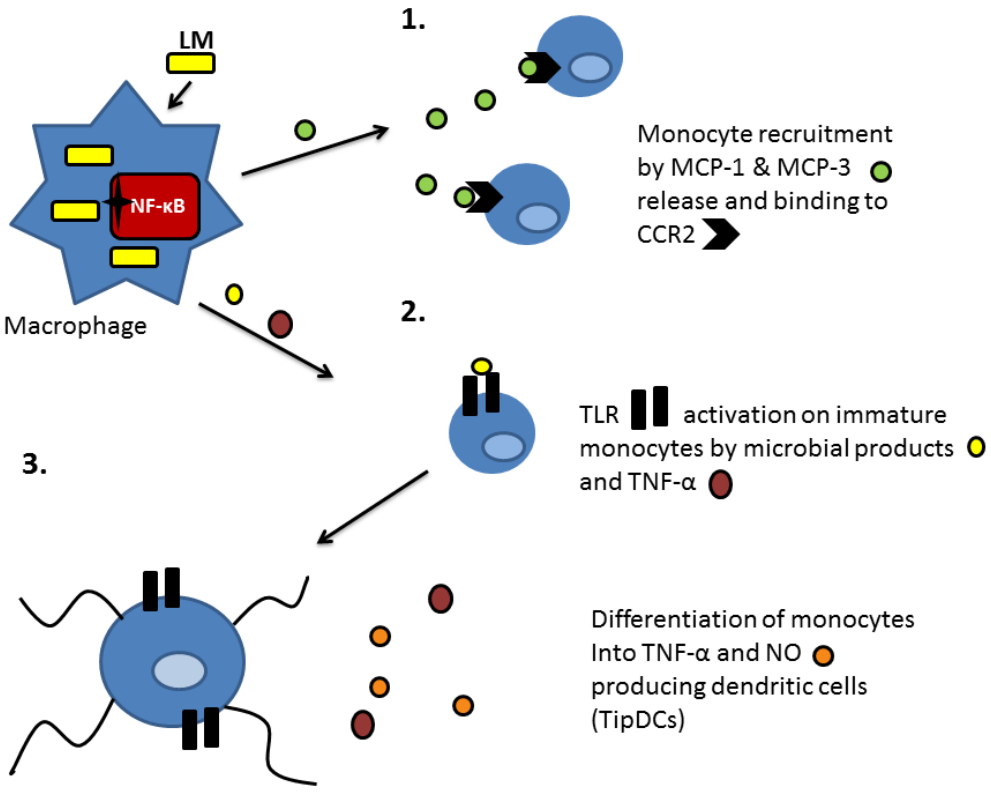


Figure 1.5 Phagocytosis and degradation of LM by macrophages induces a number of innate immune responses, including NF-κB-dependent gene transcription, recruitment of inflammatory monocytes, and activation/differentiation of monocytes into effector cells. *Adapted from Pamer et al, 2004.*

1.9 Goals of thesis.

The work in this thesis aims to characterize and understand the mechanisms by which propofol anesthesia modifies the host immune response to infection. Studies conducted by Czuprynski et al in 2003 indicated that anesthesia with the GABA-A receptor barbiturate anesthetic sodium pentobarbital increased murine susceptibility to oral infection with LM by up to 100,000-fold[37]. As pentobarbital is not commonly used in adult surgeries or in the ICU, most of our studies were performed using propofol. A number of questions were proposed: Does brief anesthesia with propofol increase host susceptibility to infection? If so, does the route of infection matter? How much of the decrease in host resistance to infection is due to barrier disruption vs. direct modulation of the immune response by anesthesia? Does immunomodulation by propofol persist after sedation has worn off? Is immune suppression specific to propofol, or is it a side effect of anesthesia in general? How does propofol act to specifically disrupt the innate immune response in the context of listeriosis? And finally, is the immunomodulatory effect of propofol specific to LM infection, or does it globally regulate immunity and increase host susceptibility to infection with other pathogens?

The work contained in this thesis serves as a foundation upon which to build a larger narrative on how propofol anesthesia may modify immune responses to make hosts more susceptible to infection. By elucidating the mechanisms behind a previously unknown but serious side effect to propofol sedation, better practice can be implemented at hospitals, and further attention paid to unforeseen side effects of drugs previously thought to be safe.

CHAPTER TWO

Materials and Methods

Bacterial strains, media, and culture conditions.

All LM strains in this study were derived from the 1/2a serotype 10403S strain, a streptomycin resistant derivative of strain 10403[116]. Mouse intravenous infections were carried out with wild type 10403S (WT) or an in-frame *actA* deletion mutant (*ΔactA*)[117]. Mouse intragastric infections were carried out with a derivative of strain 10403S containing a copy of *inlA* (*inlA*^{M})[118] that has been optimized for the binding of the murine receptor E-cadherin, and with strains containing in-frame deletions of *inlA* and *inlB* (*ΔinlAB*). All strains were grown in brain heart infusion (BHI) medium (Difco Laboratories, Detroit, MI) overnight with agitation at 37°C prior to both *in vitro* and *in vivo* assays. BHI medium consists of 7.7g calf brains, 9.8g beef heart, 10g protease peptone, 5g NaCl, and 2.5g disodium phosphate per liter.

All intravenous *Salmonella enterica* serovar Typhimurium infections were carried out using the DW273 strain containing a kanamycin resistance cassette (courtesy of Dr. Linda Kenney). *S.* Typhimurium was struck out onto LB agar plates containing 20μg/mL kanamycin two days prior to infection. One colony from this plate was used to inoculate LB broth (Invitrogen, Carlsbad, CA) and was grown overnight at 37°C with agitation prior to infection. LB broth consists of 10g SELECT Peptone 140, 5g SELECT yeast extract, and 5g NaCl.

All intravenous *Staphylococcus aureus* infections were performed using the USA300 strain of methicillin resistant *S. aureus* (courtesy of Dr. Victor Torres). USA300 was derived from the parent strain USA500, also a methicillin resistant strain[119]. USA300 was grown overnight in

TSA broth (MP Biomedicals, Solon, OH) at 37°C with agitation prior to infection. TSA broth consists of 17g casein digest peptone, 3g papaic digest of soybean meal, 2.5g disodium phosphate, 2.5g dextrose, and 5g NaCl.

Streptococcus pyogenes was also used in IV infections of mice. *S. pyogenes* strain MGAS5005 (a gift from Dr. Michael Federle), a strain shown to be virulent in animals[120], was struck out on Todd-Hewitt Yeast agar (THY- Oxoid, Hampshire, England) plates two nights prior to infection. One colony was then inoculated into THY broth and grown statically at 37°C, 5% CO_2 overnight. THY broth consists of 10g minced meat, 20g tryptone, 2g glucose, 2g sodium bicarbonate, 2g NaCl, 0.4g disodium phosphate, and 6.25% yeast extract.

Drug types and formulations.

Purified muscimol, topiramate, and picrotoxin (Sigma-Aldrich, St. Louis, MO) were diluted in sterile PBS prior to intravenous injection of mice at the following concentrations: muscimol (1.141mg/kg), topiramate (30mg/kg), and picrotoxin (2.0mg/kg). The empirical formula for muscimol is $C_4H_6N_2O_2$ with molecular weight 114.1 g/mol. Its active ingredient is 3-Hydroxy-5-aminomethyl-isoxazole, and is in alcohol hydrate form. Muscimol is hydrophilic and formulated as a white powder, and acts as GABA-A receptor specific agonist. Topiramate has the empirical formula $C_{12}H_{21}NO_8S$ with a molecular weight of 339.36 g/mol. The active chemical compound in topiramate is 2,3:4,5-Bis-O-(1-methylethylidene)-36-D-fructo-pyranose sulfamate. Topiramate comes as a white solid hydrophilic powder, and acts as a GABA-A receptor agonist. Topiramate is also commercially available as Topamax®, a drug used to treat epilepsy. The commercial formulation was not used in experimentation. Picrotoxin has the empirical formula $C_{15}H_{18}O_7 \cdot C_{15}H_{16}O_6$ and a molecular weight of 602.58 g/mol. Picrotoxin is supplied as a white crystalline powder, and is a GABA-A receptor-specific antagonist. Propofol

(propofol suspension, 10 mg/mL, Abbot Labs, North Chicago, IL) was diluted in 5% dextrose and used at a concentration of 18.75 mg/kg for intravenous tail vein injections. Propofol's active chemical compound is 2,6-Diisopropylphenol. Propofol (Propoflo®, Abbot Laboratories, North Chicago, IL) is supplied as a lipophilic suspension consisting of 2,6-Diisopropylphenol 1%, H_2O 85-86%, soybean oil 10%, glycerol 2-3%, and egg phosphatide 1-2%. Propofol is a GABA-A receptor agonist used to induce anesthesia in humans and animals[121]. For *in vitro* experiments, propofol was diluted in dimethylsulfoxide (DMSO, molecular biology grade: Sigma Aldrich, St. Louis, MO) to a concentration of 50 mM and a final concentration of 50 uM was used for tissue culture experiments. For *in vivo* experiments, propofol suspension was diluted in a filter sterilized solution of 5% dextrose dissolved in sterile H_2O. Vehicle solutions for non-drug-treated animals consisted of Intralipid® (Sigma Aldrich, St. Louis, MO), which is comprised of the same components as Propoflo® suspension, without the active chemical compound 2,6 diisopropyl phenol. Ketamine (brand name Ketalar; NDC 42023-113-10) is supplied as Ketamine HCl, 10mg/mL (JHP pharmaceuticals, Parsippany, NJ). The empirical formula of ketamine is $C_{13}H_{16}ClNO \bullet HCl$. Ketamine is dissolved in aqueous solution and is hydrophilic with a final pH of 3.5-5.5. Ketamine is a N-methyl-D-aspartate receptor antagonist used to anesthetize humans and animals[122]. Xylazine (brand name AnaSed, Lloyd Laboratories, Shenandoah, Iowa) is supplied as a sterile solution containing 20mg/mL xylazine, methylparaben 0.9mg, propylparaben 0.1mg, and H_2O. Xylazine is an α_2-adrenergic receptor agonist, and is a central nervous system depressant used for anesthesia in dogs[123]. Ketamine and xylazine are used in combination to induce anesthesia in animals[124]. Sodium pentobarbital (brand name Nembutal, Akorn Pharmaceuticals, Lake Forest, IL) is supplied as a sterile solution containing pentobarbital sodium 50mg/mL, propylene glycol 40%, alcohol, and 10% H_2O. Sodium

pentobarbital is a short-acting barbiturate anesthetic that is a GABA-A receptor specific agonist[125]. Ketamine/xylazine and sodium pentobarbital were used at concentrations of 25 mg/kg ketamine and 4 mg/kg xylazine, and 50 mg/kg pentobarbital and administered by intravenous tail vein injection. Both ketamine/xylazine and pentobarbital were diluted in sterile PBS to reach desired concentrations.

Intravenous infections of mice.

Animal procedures were IACUC approved by the UIC Animal Care Committee and performed in the Biological Resources Laboratory (BRL) at the University of Illinois at Chicago. *LM* 10403s grown overnight in BHI broth at 37°C with agitation was diluted 1:20 into fresh BHI broth and grown to an OD_{600} of 0.6 (~3-4 hours). Bacteria recovered by centrifugation (3 min @ 13,200 rpm on a tabletop centrifuge) were washed, suspended, and diluted in PBS to reach a final concentration of $2x10^3$ CFU/100µL or $2x10^4$ CFU/100µL. Immediately prior to infection, 100 uL of bacterial suspension was mixed with 100 uL of vehicle solution [Intralipid [Sigma Aldrich, St. Louis, MO) + 5% dextrose] or the indicated drug. 6–8 week old female ND4 Swiss Webster mice (Charles River Laboratories, Chicago, IL or Harlan Laboratories, Madison, WI) were used for all experiments because they are outbred mice with dissimilar genetic backgrounds. This allowed us to use an *in vivo* model that most closely mimicked human infection. Swiss Webster mice were infected via tail vein injection with 200 uL of the bacterial suspensions for an infectious dose (ID) of $2x10^3$ CFU or $2x10^4$ CFU as indicated. For IV infections using ketamine/xylazine as the sedative, mice were tail vein injected with ketamine/xylazine 24h prior to tail vein infection with $2x10^3$ CFU *LM*, as the low pH of ketamine solution (pH 3.5-5.5) proved to be bactericidal when bacteria were in contact with the solution for even brief periods (data not shown). 6, 24, 72, and/or 96 hours after infection, the

livers, spleens, and occasionally brains of infected animals were harvested. Each organ was placed in 5 mL of sterile Milli-Q water and homogenized with a Tissue Master-125 Watt Lab Homogenizer (Omni International, Marietta, GA). Homogenized tissues were diluted and plated on BHI agar containing streptomycin 200 ug/mL to determine bacterial burdens as CFU/organ. Dilutions were determined experimentally, and 20uL of homogenate was diluted into 180uL of H_2O in 96 well plates to achieve the initial 10x dilution. Homogenates were then serially diluted up to 10^4-fold. In total, dilutions plated ranged from neat (50uL of straight homogenate) to 10^6-fold (50uL of 10^4-fold dilution). All dilutions were plated on BHI-agar plates (37g BHI + 15g granulated agar/liter [Difco Laboratories, Detroit, MI]) containing 200ug/mL streptomycin.

For experiments examining whether propofol influenced host immunity following infection at increasing time periods post-sedation, mice were injected IV via the tail vein with 18.75mg/kg propofol suspension (approximately 10 minutes of sedation) or Intralipid suspension, allowed to recover, and then intravenously infected with $2x10^3$ CFU LM at 24h, 96h, or 7 days post-anesthesia. At 96h post-infection, animals were euthanized and livers and spleens were isolated, homogenized, and plated for viable CFU as described in the preceding paragraph.

Intravenous experiments with *Salmonella enterica* serovar Typhimurium were performed as follows. *S.* Typhimurium overnight cultures were diluted 1:50 into fresh LB broth and were grown for ~2 hours to OD 0.4-0.6 at 37°C with shaking. Bacteria were washed once in sterile PBS and resuspended in fresh PBS to a concentration of $1x10^4$ CFU/mL, of which 100uL was injected into the animal. Immediately prior to infection, bacteria were mixed 1:1 with vehicle or propofol solution. 6 days post-infection, livers and spleens were harvested and organs were homogenized and plated for bacterial burden enumeration, as described above for *LM* infections.

S. aureus USA300 overnight cultures were diluted 1:100 into 2 tubes containing 5 mL each of fresh TSA broth and grown for ~3 hours at 37°C with agitation. Cultures were combined and centrifuged for 6 minutes at 4000 rpm in a tabletop centrifuge. They were then washed 1x with sterile PBS, and resuspended in fresh PBS. 2mL of the combined culture was added to 8mL of sterile PBS and the OD600 was measured using 1mL of culture. 1mL of PBS was again added to the original culture and the OD was again measured, this cycle repeated until the measured OD was near 0.32±0.02, corresponding to $1x10^7$ CFU/100uL. This was then diluted 1:10 to reach the infectious dose of $1x10^6$ CFU/100uL. Immediately prior to infection, bacteria were mixed 1:1 with vehicle or propofol solution, as above. 7 days post-infection, livers, spleens, hearts, and kidneys were harvested, homogenized, and plated for viable bacterial CFU. Spot plating was used to enumerate bacterial burdens in target organs of *S. aureus* infected animals. Dilutions were made in sterile 96-well plates and 10uL of each dilution was plated in a spot on TSA agar plates containing 2μg/mL erythromycin. Dilutions ranged from plating straight homogenate (liver, spleen, heart samples) to up to 10^4-fold dilutions (kidney).

S. pyogenes intravenous infections were carried out as follows. Overnight cultures were diluted 1:15 into 10 mL of THY broth. Bacteria were grown statically at 37°C, 5% CO_2 in a 14mL round-bottom sterile tube for 4.5 hours, at which point they were at an OD600 of 0.386. 1.295mL of the culture was centrifuged for 3 minutes at 13,200 rpm in a tabletop centrifuge, resuspended in sterile PBS, spun down again, and resuspended in 5mL of 5% dextrose (the same solution used to dilute propofol and Intralipid suspensions). This corresponded to 10^7 CFU/100μL, which was the target infectious dose used in IV experiments. Bacteria were plated prior to and after returning from mouse infections. Mice were sacrificed 16h post-infection, and

livers, spleens, and lungs were isolated. Organs were homogenized and plated for viable CFU. Dilutions ranged from straight homogenate to 100-fold dilutions.

Oral infections of mice.

Overnight cultures of LM 10403S, *inlA*^{M}, or Δ*inlAB* grown in BHI broth were diluted 1:20 in BHI broth and grown to mid-log phase (OD_{600} ~0.6). Bacteria were washed and resuspended in sterile PBS + 100 mg/mL $CaCO_3$ (Sigma-Aldrich, St Louis, MO) to reach a final concentration of $5x10^8$ CFU/mL. 6-8 week old female ND4 Swiss Webster mice were given 200 uL of vehicle solution (Intralipid + 5% dextrose) or drug solutions (propofol or GABA-A receptor agonists/ antagonists) via tail vein injection 5-10 minutes prior to infection. They were then infected via oral gavage with 200 uL of the bacterial suspensions for an infectious dose of $1x10^8$ CFU/mouse. Animals were euthanized at the indicated time points post-infection and livers, spleens, and intestines of infected animals were harvested, homogenized and bacterial CFUs were determined via plating with glass beads.

Cell culture infection assays.

Caco-2 intestinal epithelial cells (ATCC HTB-37) were maintained following ATCC guidelines in DMEM high glucose medium supplemented with 10% fetal calf serum, 5000U of penicillin/ streptomycin, and 2.5g HEPES. Caco-2 medium was adjusted to pH 7.4. $2x10^6$ Caco-2 cells were seeded onto glass coverslips in tissue culture dishes on the night prior to infection. Overnight cultures of LM statically grown in BHI broth at 37°C were used to infect cells at a multiplicity of infection of 30:1 bacteria to Caco-2 cells. After 1 hour, the cells were washed with warm PBS and fresh 37°C media containing gentamicin (10 ug/mL) to kill extracellular bacteria was added. At the indicated time points, coverslips were removed and

lysed in 5 ml sterile H2O with vigorous vortexing and bacterial CFU were determined through plating with glass beads on LB agar plates.

In order to differentiate into bone marrow-derived macrophages, bone marrow cells must first be exposed to L-cell conditioned medium, a potent source of macrophage colony stimulating factor (M-CSF)[126]. This is derived from the supernatant of L-cells, a fibroblast cell line. One 1mL aliquot of L-cells was thawed and cultured in a sterile 25mm^2 tissue culture flask in L-cell conditioned medium containing MEM (Minimal Essential Medium, Cellgro, Manassas, VA), 10% FBS, 1% glutamine, 1% nonessential amino acids, 10,000U pen/strep, and 1% pyruvate (all from Cellgro, Manassas, VA). Cells were grown to confluency, and diluted back to 1.25x10^5 cells/25mL to seed 7 larger, 75mm^2 flask. Supernatants were collected, filter sterilized, and frozen at -20°C. Bone marrow-derived macrophages (BMMs) and peritoneal macrophages were obtained from female Swiss Webster mice as previously described[127,128]. Briefly, 8-10 week old mice were sacrificed via CO_2 asphyxiation from a bottled source and cervical dislocation. Femurs were dissected out and placed in dishes of cold DMEM cell culture medium containing 10,000U penicillin/streptomycin and 10% FBS (Hyclone, Logan, UT). Bone marrow was eluted first by filling a 10mL sterile syringe attached to a 26G sterile needle with DMEM medium. Marrow was eluted by slowly passing 5mL of DMEM through the open end of the bone. The femur was then flipped over and the same procedure was applied. This was repeated for both femurs isolated. Cells were counted using a hemacytometer, and then centrifuged for 1 minute at 500rpm on a tabletop centrifuge. The supernatant was transferred to a 15mL sterile conical tube. This supernatant was further centrifuged for 10 minutes at 1000 rpm, after which the supernatant was aspirated and the resultant cell pellet suspended in 10mL of fresh DMEM. After one more centrifugation step (10 minutes, 1000 rpm), the cell pellet was resuspended in 10mL of Bone

Marrow Medium (BMM) containing 50mL DMEM, 20mL FBS, 30mL L-cell colony stimulating factor (obtained as described above), 1mL 0.2M glutamine, 1mL 0.1M pyruvate (both from Cellgro, Manassas,VA). Cells were counted and 10^7 cells were added into 50mL of BMM in a 125x50 sterilized Pyrex glass petri dish. The dish was placed at 37°C, 5% CO_2, and the remaining BMM was refrigerated. At day 3, cells were fed by the addition of 20mL of BMM directly into the dish. At day 6, a semi-confluent monolayer was observed with adherent cells exhibiting membranous processes characteristic of mature macrophages[129]. The medium was aspirated from the dish, and 20mL of cold PBS carefully added, the dish rocked gently, and the PBS aspirated off. Another 20mL of cold PBS was added and the dish was refrigerated for 10 minutes on a completely flat surface. The cells were then scraped from the bottom of the dish with a cell scraper and pipetted up and down to dislodge any remaining adherent cells. Cells were then transferred to a 50mL conical tube and counted. Cells were then centrifuged for 10 minutes at 1000rpm, 4°C, and resuspended in DMEM + 10% FBS without antibiotics at a concentration of $2x10^6$ cells per 6mL medium. $2x10^6$ macrophage cells were placed onto glass coverslips in a sterile 60 mm x15 mm sterile petri dish on the night before infection. For propofol, lipopolysaccharide (LPS), and gamma interferon (IFN-γ)-treated BMMs, the medium was supplemented with 50uM propofol and/or 25 ng/mL of LPS for 2 hours and/or 1 ng/ml of IFN-γ (Biosource, Carlsbad, CA) for 18 hours prior to infection. Infections and bacterial CFU quantifications were performed as described[130]. 1mL of a statically grown overnight culture of LM (OD_{600} of 0.8) was centrifuged for 3 minutes at 13,200 rpm, washed with PBS and spun again, and resuspended in DMEM without antibiotics. Macrophages were infected at an MOI of 0.1 for 30 minutes, after which medium was aspirated off and coverslips were washed 4x with 5mL of warm, sterile PBS. DMEM + 10μg/mL of gentamicin was added to kill extracellular

bacteria. At the indicated time points, 3 coverslips were taken out and put into 3 individual 15mL conical tubes containing 5mL each of sterile H_2O. Tubes were vortexed to lyse macrophages and release bacteria, and dilutions were plated on LB agar to enumerate CFU at various time points.

Measurement of transepithelial resistance in Caco-2 cell monolayers.

Caco-2 cells were grown to confluency in DMEM with 2.5g/500 mL HEPES (Fisher Chemical, Pittsburgh, PA), 5000U penicillin/streptomycin (Cellgro, Herndon, VA), and 20% FBS (Hyclone, Logan, UT) (Caco-2 medium) in a 75mm^2 tissue culture flask. For the electrophysiological studies cells were grown to confluence on 33 cm^2 collagen coated permeable supports (Transwell; Costar/Corning, Wilkes Barre, PA) and allowed to differentiate for 1 week to allow tight junction formation prior to conducting experiments; the procedure is outlined as follows. Collagen solution was made as 4% Type I collagen (from rat tail, Sigma-Aldrich, St. Louis, MO) in 95% EtOH and stored at 4°C. On the night prior to seeding cells in transwells, 50µL of the collagen solution was used to coat each transwell in its apical chamber and allowed to dry overnight in the tissue culture hood. To seed cells on the next day, 700µL of warm Caco-2 medium without antibiotics was added to the basal chamber of each transwell. Caco-2 cells were trypsinized with 3mL Trypsin/EDTA (Cellgro, Herndon, VA). Once cells were dissociated, the total volume was brought up to 25mL with warm Caco-2 medium without antibiotics. 280µL of the 25mL cell suspension was added to the apical layer of each transwell, and cells were allowed to polarize and form tight junctions for 1 week in 37°C, 5% CO_2. Medium was changed weekly for up to 3 weeks if not performing experiments within one week of seeding cells. For LM infection, cell medium was removed and all transwell monolayers were washed 4x with warm PBS by pipetting 1mL gently onto cells in the apical layer and allowing

the solution to overflow. PBS was pipetted out first from the basal chamber and then from the apical chamber, with the pipette tip never touching the cell monolayer at the apical chamber's bottom. LM 10403S was struck out onto BHI agar containing 200μg/mL streptomycin 2 days prior to infection. On the night before infection, one colony was used to inoculate 4mL of sterile BHI broth and allowed to statically incubate overnight at 37°C. Bacteria were prepared for infection as described in *Cell culture infection assays*. Caco-2 cells were infected with an MOI of 50:1 *LM* per Caco-2 cell. The indicated monolayers were exposed to a propofol concentration of 50uM for the duration of the experiment, which is the approximate blood serum level reached by the drug at steady state concentrations[64]. Transepithelial electrical resistance (TER) was measured before infection and at 2 h intervals during infection using an epithelial Voltohmmeter (World Precision Instruments) by inserting the dual pronged electrode into the apical and basal chambers simultaneously. The electrode was sterilized between measurements by dipping in 70% EtOH followed by submersion in sterile PBS. Values were calculated to reflect Ohms x cm^2. All experiments were done in 12-well transwell plates with 3 wells per treatment group per experiment.

Bioplex cytokine and chemokine assays.

3-5 mice were used per treatment group per time point for all assays in two independent experiments. Serum cytokine levels were measured in uninfected mice or in mice infected intravenously with LM in the presence and absence of drug treatment. Mice were sacrificed at 0, 24, 48, and 72h post-infection and whole blood was drawn via cardiac puncture. Serum was isolated by allowing whole blood to clot for between 15-20 minutes and spun down for 15 minutes at 1000*g* at 4°C in a tabletop centrifuge. Serum was isolated as supernatant and stored at -80°C. Thawed serum was diluted 1:4 (12.5μL into 37.5μL of the provided Bio-plex sample

buffer) and used in custom 96-well plate Bioplex assays (Bio-Rad Laboratories, Hercules, CA) containing the following targets: IL-1β, IL-6, IL-10, IL-12p40, MCP-1, TNF-α, IFN-γ, TGF-β, KC, TGF-β and Eotaxin. Assays were performed according to instructions provided (Bio-Rad Laboratories). Plates were read using the Bioplex 200 plate reader and analyzed with Bio-Plex Manager 5.0 software.

Histological examination of infected tissues.

Mice infected via the tail vein with $2x10^4$ CFU or orally with $1x10^8$ CFU of LM in the presence of propofol or Intralipid were euthanized at 0, 24, 48, and 72h post-infection and spleens were isolated. Spleens were resuspended in 10% PBS-buffered formalin (Sigma-Aldrich, St. Louis, MO) overnight, then prepared for histological analysis by slicing each organ into smaller portions using a sterile scalpel. Organ blocks were placed in plastic histology cassettes and labeled with pencil. The organ blocks were then prepared and processed by the UIC Research Resource Center Histology Core and stained with hematoxylin and eosin (H&E).

Flow cytometry

Mice were infected via tail vein with $2x10^4$ CFU of LM and euthanized at the indicated time points. Spleens were isolated and processed for flow cytometry as described in Pamer et al[131] and as follows. Dissected spleens were placed in 5mL of sterile PBS. They were then disaggregated into single cell suspensions by mashing each spleen between the frosted ends of 2 microscope slides, followed by straining through a 70μM sterile cell strainer (BD Biosciences, San Jose, CA). Single cell suspensions were then incubated for 5 minutes in ice cold 0.16M NH_4Cl (9 mL, sterile filtered, pH 7.4 in sterile H_2O) and 0.17M Tris (1 mL, sterile filtered, pH 7.4 in sterile H_2O) in order to lyse erythrocytes. The solutions were added individually to each tube. The reaction was quenched by adding 10mL of ice cold DMEM without antibiotics to each

tube after the 5 minute incubation. Cells were then spun down at 1000rpm for 5 minutes at 4°C in a tabletop centrifuge, and supernatant was aspirated off. Cells were washed 2x with FACS wash buffer (1% FBS, 0.09% NaN_3 in sterile PBS) and resuspended in FACS buffer. The remaining cells were then counted and $2x10^6$ cells were seeded per well in 96-well round-bottom plates. There were 3 treatment groups (naïve, Intralipid/LM infected, and propofol/LM infected), and splenocytes from one mouse from each treatment group was used for single color stains and isotype controls. The following fluorescent and non-fluorescent primary antibodies were used: anti-Ly-6C (AL-21), TGF-β1, Mac-3, CD11c (BD Pharmingen, San Diego, CA); anti-CCR2 (Abcam, Cambridge, MA), anti-NOS2 (C-11 epitope) (Santa Cruz Biotechnology, Santa Cruz, CA), anti-F4/80, 33D1, Ly-6G, CD-11b (Ebioscience, San Diego, CA), anti-TNF-α (clone MP6-XT22) (Biolegend, San Diego, CA). FACS was performed with a Cyan ADP flow cytometer, and data were analyzed with Summit software.

Statistical analysis. All statistical analyses for in vivo infection assays were performed using an F test to measure variance. All statistical analyses for flow cytometry were performed using two-way ANOVA with a Bonferroni posttest.

CHAPTER THREE

Characterization of the effects of propofol on mouse models of both oral and intravenous listeria infection

3.1 Summary

Microbial infection is a major complication for surgical patients in the US, occurring in the aftermath of approximately 2% of all surgeries. Propofol is the most commonly used general anesthetic in surgery and has been demonstrated to have immunomodulatory effects *in vitro*. Propofol exposure has been linked with decreased secretion of pro-inflammatory immune signaling molecules such as TNF-α with decreased expression of inducible nitric oxide synthetase (iNOS) gene expression (a potent mediator of antimicrobial defenses), and with decreased macrophage phagocytosis in cell culture. However, the *in vivo* mechanisms by which anesthetic agents influence host susceptibility to microbial infection remain poorly defined. In this study, we show that propofol treatment increases host susceptibility to infection with *LM* (LM) in both oral and intravenous infection models by up to 100,000-fold. Propofol treatment increased persistence of LM in intestines of mice after oral infection and inhibited host clearance of bacteria from target organs following intravenous challenge. While treatment with other anesthetics (ketamine, pentobarbital) also decreased host resistance to LM during oral infection, only propofol treatment resulted in the reduced ability of the host to clear the bacteria following intravenous (IV) challenge. Histological analyses indicated propofol treatment in conjunction with LM infection resulted in gross loss of organ structure in the intestines of mice. In contrast

to the *in vivo* data indicating propofol's effects on barrier function and immunity in the context of LM infection, propofol had no effect on LM invasion, intracellular growth, or barrier disruption in tissue culture models of infection.

3.2 Introduction

Microbial infection is a major complication for surgical patients in the US, occurring in the aftermath of approximately 2% of all surgeries[1,132]. While over 99% of surgery patients receive prophylactic antibiotics, the incidence of post-operative infections remains high, negatively impacting patient health outcomes and increasing health care costs by $1-$10 billion dollars per year[4-6]. Whereas significant effort has focused on preventing surgical infection by reducing patient exposure to infectious agents during surgery, relatively little attention has been directed towards understanding how anesthetics may negatively impact patient immunity to infection.

Propofol is the most commonly used general anesthetic in surgery and has been demonstrated to have immunomodulatory effects *in vitro*[46,50,52]. Propofol exposure has been linked with decreased secretion of pro-inflammatory immune signaling molecules such as TNF-α, with decreased expression of inducible nitric oxide synthetase (iNOS) gene expression (a potent mediator of antimicrobial defenses), and with decreased macrophage phagocytosis in cell culture[35,39,51]. While high concentrations of propofol caused cell death of human macrophages in culture, clinically relevant doses reduced macrophage chemotaxis and oxidative burst[34]; these effects were reversed 24 hours after removal of propofol. Propofol has been reported to decrease the membrane potential of mitochondria[34], as well as abrogate cell death induced by the production of large amounts of iNOS[33] in murine Raw 264.7 macrophages. This inhibition of iNOS production has been linked to propofol treatment inhibiting NFκB translocation from the

cytosol to the nucleus in murine macrophage culture[47,64]. Propofol also has been shown to decrease phagocytosis via binding to its cognate GABA-A receptor on human primary macrophages[50], but this has been refuted in other studies[49]. All of these *in vitro* studies have attempted to understand how propofol administration affects immunity, however these approaches carry important caveats. For example, by definition all of the *in vitro* studies were conducted in the absence of an integrated, multicellular immune response normally found within an animal, and none of the studies were performed in the actual context of pathogen infection. The studies outlined in this section seek to characterize at the whole organism level how propofol affects immunity in the presence of a pathogenic challenge to the host.

Using the facultative intracellular bacterium *Listeria monocytogenes* as a model pathogen, we here provide evidence that even brief periods of anesthetization with propofol dramatically increase host susceptibility to microbial infection. Sub-lethal infections in non-drug treated animals were observed to become lethal following anesthetic exposure. Propofol enhanced bacterial translocation across both the intestinal and blood-brain barriers while impeding host immune clearance of LM from infected tissues. In spite of the increased susceptibility of the host to infection, propofol treatment did not affect LM entry into host cells in cell culture assays, nor did it affect tight junction integrity in trans-epithelial electrical resistance assays. These results demonstrate that propofol treatment increases host susceptibility to infection through increasing bacterial translocation across physiological barriers as well as preventing host clearance of LM from infected tissues.

3.3 Results

Propofol exposure increases host susceptibility to oral infection with LM

Czuprynski et al previously reported that exposure of mice to the anesthetic sodium pentobarbital prior to oral inoculation with LM increased host susceptibility to infection by up to 100,000-fold via an unknown mechanism[37]. This effect was observed only following oral inoculation with LM, not following intravenous injection, and only when sodium pentobarbital was injected intraperitoneally, not intravenously. Sodium pentobarbital targets gamma amino-butyric acid A (GABA-A) receptors[133], to which many anesthetics bind[134], but the drug is not commonly used in adult surgeries[58]. We therefore investigated whether propofol, a commonly used anesthetic that also targets the GABA-A receptor[58,59,135], similarly increases host susceptibility to microbial infection following intravenous drug delivery, thereby mimicking anesthetic induction in surgery. Female Swiss-Webster mice were orally infected with 10^8 CFU of wild type LM in the presence or absence of brief (approximately 5 minutes) intravenous propofol sedation. The amount of propofol given (18.75 mg/kg body weight) was comparable to the clinical doses used for the induction of anesthesia in human surgery, wherein patients become sedated quickly and recover quickly in the absence of continuous anesthetic infusion[136]. At 6 hours post-infection, propofol-treated animals exhibited on average a 100-fold increase in the numbers of bacteria recovered from the intestine in comparison to vehicle treated controls (Figure 3.1), and these higher intestinal numbers were maintained across all time points examined in comparison to the intestines of control animals. By 72 hours post-infection, propofol-treated animals had 100 to 10,000-fold more bacteria recovered from the livers and spleens in comparison to non-drug treated animals (Figure 3.2). The propofol-dependent increase in host susceptibility to infection was strikingly apparent in animals intragastrically infected with a low dose (10^6 CFU) of LM, in which nearly 90% of the propofol treated animals had significant bacterial burdens in the liver and spleen in comparison to only 50% of control animals (Figure 3.3). Persistence of LM in the

intestine also led to increased intestinal pathology, as evidenced by the large areas of necrosis and dissolution of organ structure in propofol-treated mice at 72h post infection (Figure 3.4).

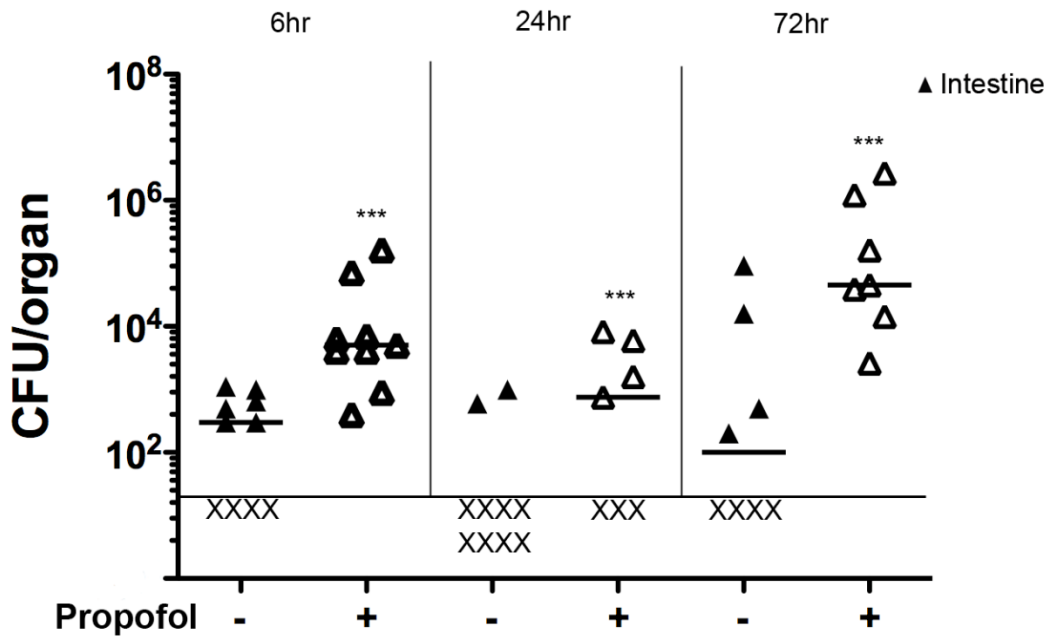


Figure 3.1. Propofol increases host susceptibility to oral infection with 10^8 CFU of LM across 72h of infection. 6-8 week old female Swiss Webster mice (Charles River Laboratories) were infected intragastrically with 10^8 CFU of WT LM in the presence and absence of propofol anesthesia. Intestines were harvested, homogenized, and plated at 6, 24, and 72h post-infection. "X" below line of detection represents undetectable bacterial burdens, closed triangles: intestines from infected controls, open triangles: intestines from animals exposed to propofol. *** $p<0.0005$.

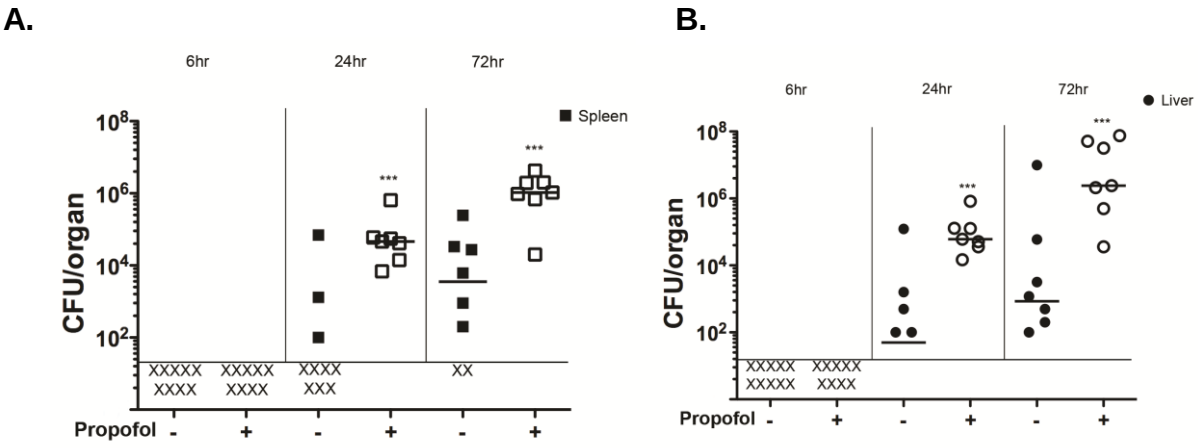


Figure 3.2. Propofol increases host susceptibility to oral infection with 10^8 CFU of LM across 72h of infection. Mice were infected and organs assessed for bacterial burden as described in Figure 1. Propofol treatment resulted in increased bacterial burdens in livers and spleens at early timepoints during infection (24h), indicating barrier disruption. *** $p<0.0005$.

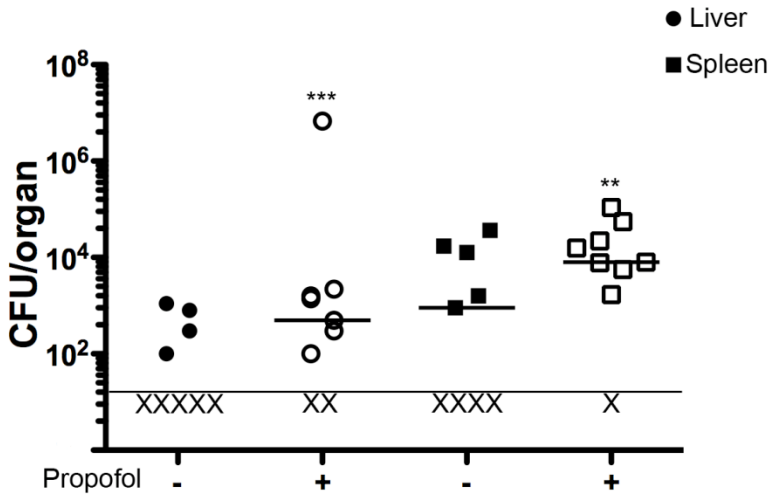


Figure 3.3: Propofol increases host susceptibility to sub-infectious intragastric doses of LM. Mice were intravenously injected with either propofol or vehicle solution followed by intragastric infection with 10^6 CFU of LM. At three days post-infection the animals were euthanized and bacterial burdens were determined in liver and spleen. Data shown is from two independent experiments. ** $p<0.005$, *** $p<0.0005$.

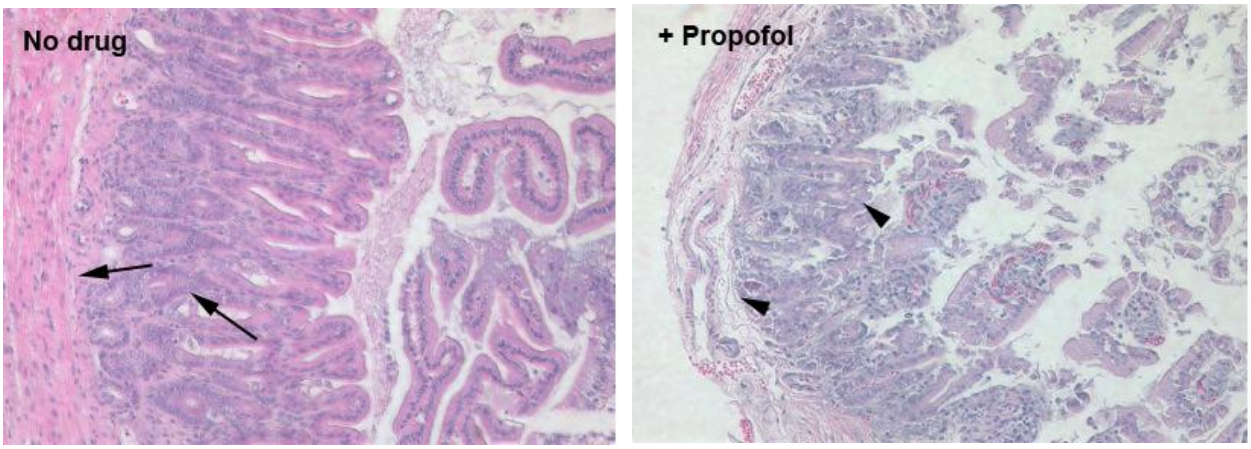


Figure 3.4: Propofol anesthesia increases intestinal necrosis and dissolution of organ structure in an oral model of listeriosis. Animals were infected intragastrically with 10^8 CFU of LM and sacrificed 72h post-infection. Intestines were isolated, fixed, sectioned, paraffin-embedded, and stained with hematoxylin and eosin (H&E). Images representative of 5 animals per group. Arrows: immune cell infiltrates to intestinal lamina and villi. Arrowheads: necrosis and dissolution of laminar and villus structure.

With respect to bacterial invasion of host tissues, the LM surface protein InlA contributes to bacterial translocation across the intestinal epithelium whereas the surface protein InlB mediates bacterial invasion into hepatocytes and other cell types[137,138]. InlA mediates *LM* crossing of the intestinal epithelium through binding to the mammalian intestinal epithelial cell surface adhesion protein E-cadherin[139]. To determine if propofol-enhanced bacterial replication within the liver and spleen was dependent upon InlA or InlB-mediated host cell invasion of intestinal epithelial tissue, mice were orally infected with 10^8 CFU of a LM Δ*inlA* Δ*inlB* mutant strain in which both surface proteins have been deleted. Although overall bacterial burdens in the livers and spleens were slightly reduced at 72 hours post-infection in comparison to mice infected with wild type LM, animals exposed to propofol still exhibited increased bacterial burdens in all organs examined in comparison to controls (Figure 3.5). It has been shown that wild type LM InlA proteins do not bind with high affinity to murine E-cadherin; to correct for this, a "murinized" version of InlA ($InlA^m$) was engineered that binds with higher efficiency to mouse E-cadherin[118]. Mice were infected IV with the $inlA^m$ strain of LM with or without propofol anesthesia, and bacterial burdens were assessed at 24h and 72h post infection. In comparison to infection with the *ΔinlAB* strain, the infection of mice with the $inlA^m$ mutant strain of LM resulted in greater bacterial burdens of LM in infected organs after 24h (Figure 3.6) and 72h post-infection and increased mortality in animals exposed to propofol (Figure 3.7). However, the relative increase in bacterial burdens in drug-treated versus non-drug treated animals remained similar for animals infected with either wild type LM or the $inlA^m$ mutant (Figures 3.6 and 3.7). These results indicate that propofol exposure enhances bacterial infection through a mechanism that is largely independent of either InlA or InlB-mediated bacterial invasion.

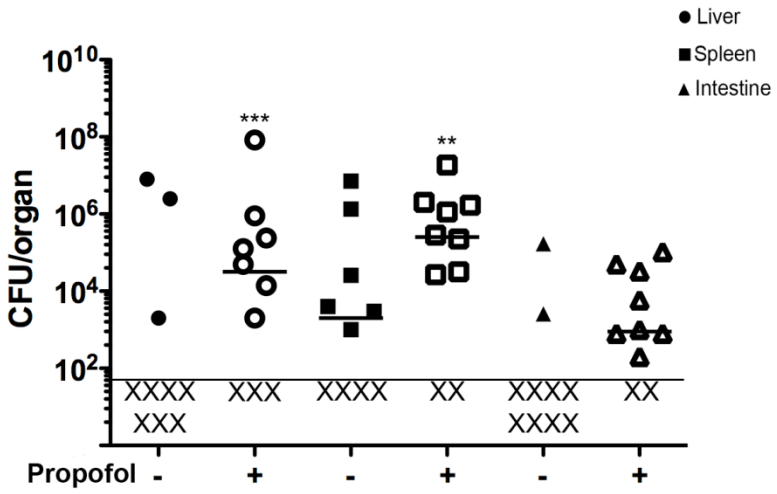


Figure 3.5: Propofol increases host susceptibility to oral infection with LM strain unable to translocate across intestinal epithelial cells. Oral infection with 10^8 CFU of LM lacking host cell invasion proteins InlA and InlB (Δ*inlA/B*) results in increased bacterial burdens at 72h post-infection in animals treated with propofol. Data shown is from two independent experiments. ** $p<0.005$, *** $p<0.0005$.

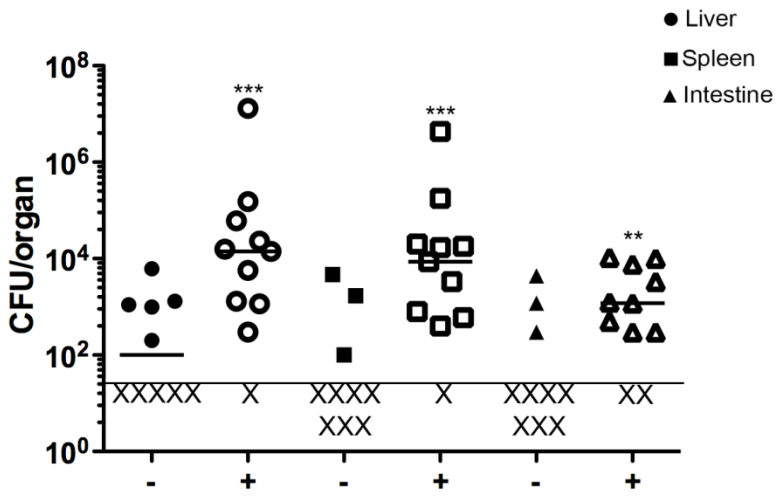


Figure 3.6: Oral infection with the InlA^m strain of LM results in increased bacterial burdens after 24h. Propofol treatment exacerbates bacterial burdens in mice orally infected with 10^8 CFU of LM strains containing the *inlA*^{M} mutation that enhances bacterial translocation across the intestinal epithelium. Data shown is from two independent experiments. ** $p<0.005$, *** $p<0.0005$.

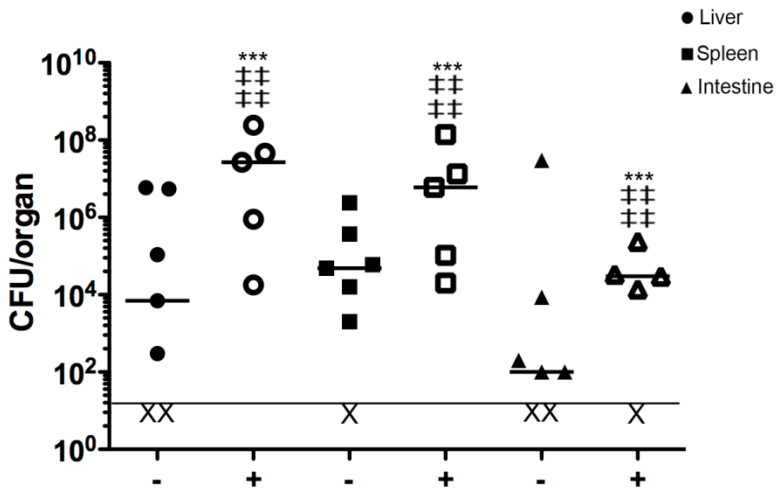


Figure 3.7: Oral infection with InlA^{m} results in high mortality after 72h. Animals were infected with 10^{8} CFU of LM and sacrificed after 72h. Propofol treated animals exhibited higher bacterial burdens in all target organs, as well as a significantly higher mortality rate.
*** $p<0.0005$; ‡ indicates dead animal.

Propofol treatment does not affect LM infection of host cells in vitro.

Based on the studies indicating propofol treatment significantly increased the number of LM in the intestine at early time points after oral infection (Figure 3.1), *in vitro* assays were performed to determine if propofol treatment affected LM invasion and/or intracellular growth within host cells. Additional experiments examined the effect of LM infection and propofol exposure on tight junction integrity in cell culture. Initial experiments investigated the efficacy of LM invasion into Caco-2 human intestinal epithelial cells or J774 murine macrophage-like cells plated in a monolayer on glass coverslips. Propofol did not appear to influence bacterial invasion of or replication within monolayers of Caco-2 (Figure 3.8) or J774 tissue culture cells (Figure 3.9). Transepithelial electrical resistance (TER) measurements were also performed to assess tight junction integrity. TER uses a voltmeter to assess changes in resistance due to infection, drug treatment, or both. Caco-2 cells were plated in the inner chambers of transwell plates, with the outer chambers containing medium alone. After ~1 week, the cells differentiated into a monolayer containing cell-to-cell contacts in the form of tight junctions, effectively forming a model of the intestinal epithelial barrier in vivo. Cells were infected in the presence or absence of both wild-type LM and/or propofol solution, and resistance measurements were taken. Neither propofol treatment nor LM infection resulted in appreciable changes in tight junction integrity (Figure 3.10).

Mice exposed to propofol fail to clear bacteria from target organs.

The increased bacterial burdens in target organs observed for orally infected mice sedated with propofol could reflect enhanced rates of bacterial translocation across the intestinal epithelium, the inhibition of host innate immune defenses designed to limit bacterial replication, or both. To

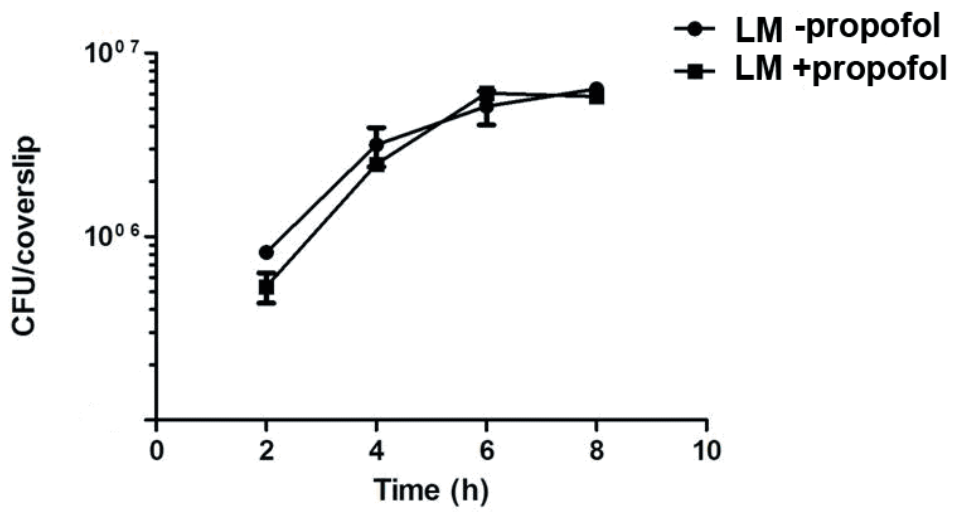


Figure 3.8: Propofol treatment does not increase LM invasion of or replication within Caco-2 cells. Caco-2 cells were plated onto glass coverslips and allowed to form a monolayer overnight. They were then infected with wild-type LM in the presence of propofol or Intralipid vehicle control, and coverslips were removed at the indicated timepoints. Cells were lysed and lysates were plated on LB agar plates for viable bacteria. Data representative of 3 independent experiments.

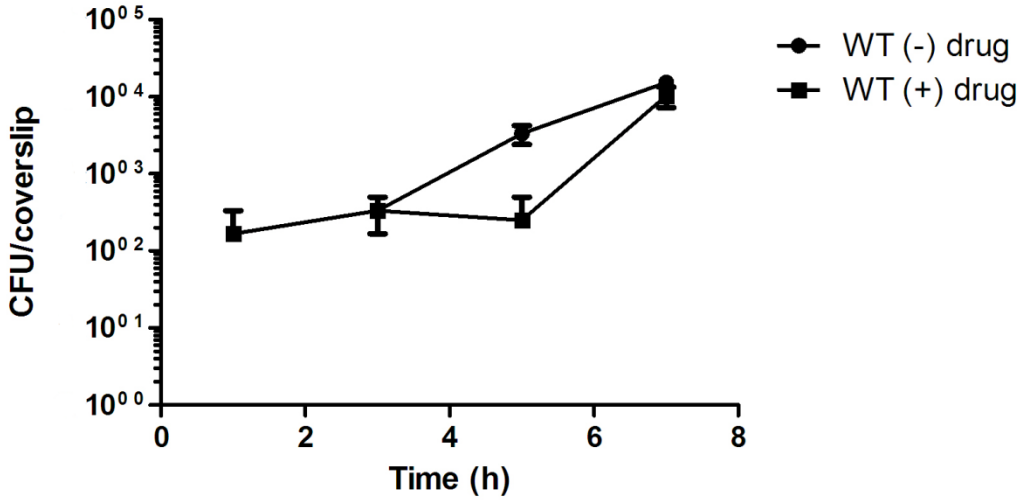


Figure 3.9: Propofol treatment does not significantly affect invasion or intracellular growth within J774 macrophage-like cells. J774 macrophage-like cells were plated on glass coverslips and allowed to form a monolayer overnight. Cells were infected in the presence or absence of propofol, and coverslips were removed at the indicated timepoints. Bacterial burdens were enumerated as described in *Materials and Methods*. Data representative of 3 independent experiments.

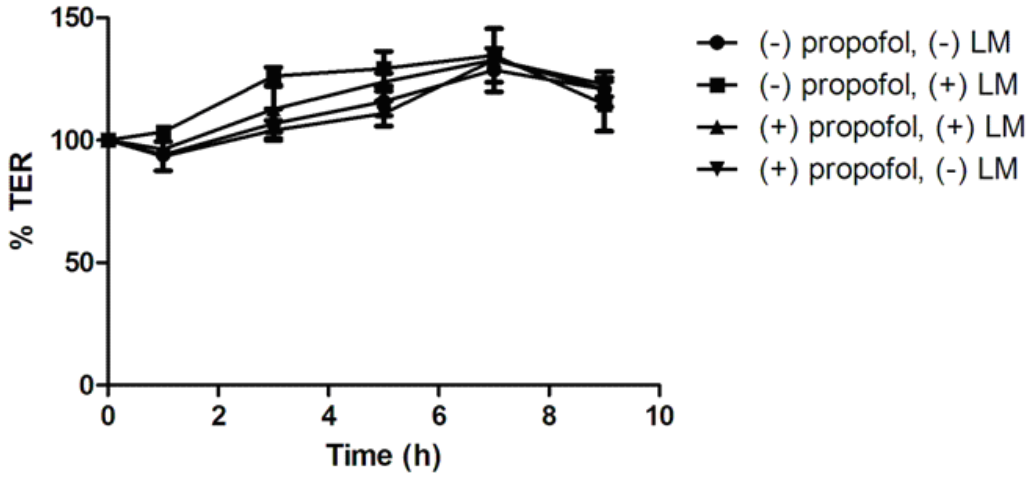


Figure 3.10: Neither propofol treatment nor LM infection result in changes in tight junction integrity. Caco-2 cells were plated in transwells for 1 week to allow for tight junction formation. Cells were infected +/- propofol and/or LM and measurements were taken at the indicated timepoints. Data representative of 4 independent experiments.

determine if propofol reduces host clearance of bacteria, 2 x 10^4 CFU of LM were inoculated directly into the blood stream of drug and vehicle treated animals, thus bypassing the intestinal barrier and proceeding immediately to systemic infection. At early time points post-infection, bacterial burdens in drug treated and non-drug treated animals were similar (Figure 3.11). However, as the infection progressed to 72 hours, propofol-treated animals showed significant increases in the numbers of bacteria associated with the liver and spleen, a phenotype that became even more pronounced at 96 hours post-infection when bacterial numbers began to decrease in vehicle treated control mice (Figure 3.11). Propofol sedated infected animals were visibly more ill than the vehicle treated controls (data not shown), with 3 out of 10 animals succumbing to infection by 96 hours. Propofol did not impair the ability of mice to control infection when exposed to attenuated LM strains, as no difference was observed in bacterial burdens in the organs of mice intravenously infected with an in-frame *actA* deletion mutant which is defective for cell-to-cell spread[140] and more than 1000-fold less virulent than wild type LM (Figure 3.12). Propofol treatment also did not enhance the ability of wild-type LM to replicate in isolated primary bone marrow-derived (Figure 3.13) or peritoneal (Figure 3.14) macrophages *in vitro*. Propofol thus appears to increase host susceptibility to microbial infection via bacterial persistence within the intestine and increased bacterial translocation across the intestinal barrier, but additionally is able to directly inhibit host immune responses that limit bacterial replication within tissues, irrespective of barrier-mediated effects.

Propofol enhances bacterial translocation to the brain

One manifestation of systemic LM infections is bacterial crossing of the blood-brain barrier[141]. Given that propofol exposure appeared to enhance bacterial translocation across the intestinal barrier, infected mice were examined for LM translocation across the blood-brain

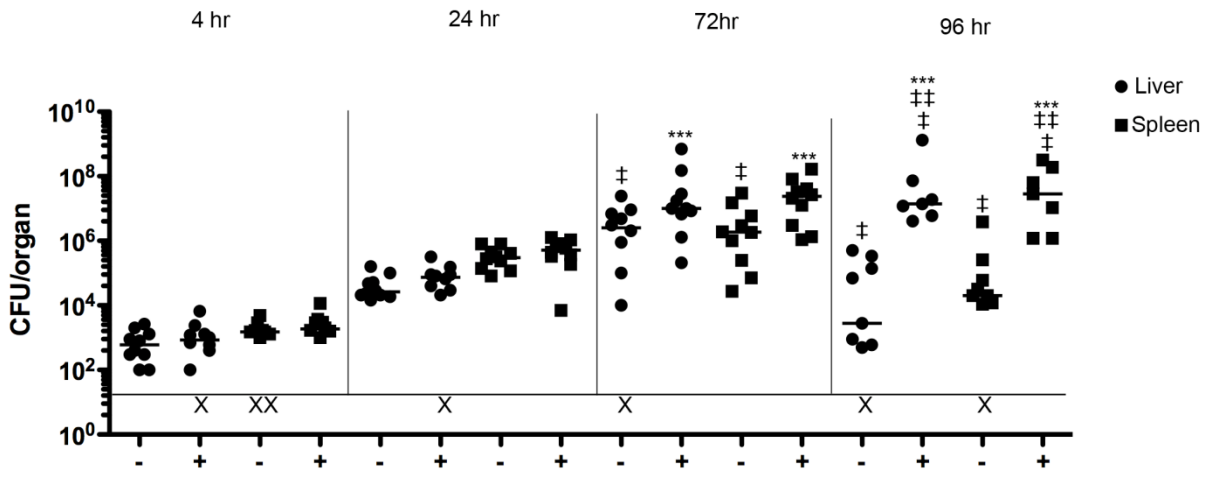


Figure 3.11: Propofol inhibits host clearance of LM 72-96h post-intravenous challenge. Mice were infected intravenously with $2x10^4$ CFU of wild-type LM with or without propofol anesthesia. Animals were sacrificed and organs harvested at the indicated timepoints. Though control animals were largely able to clear the infection after 96h, propofol-treated animals were not, with a significant proportion of drug-treated animals succumbing to infection. *** $p<0.0005$; ‡ indicates dead animal.

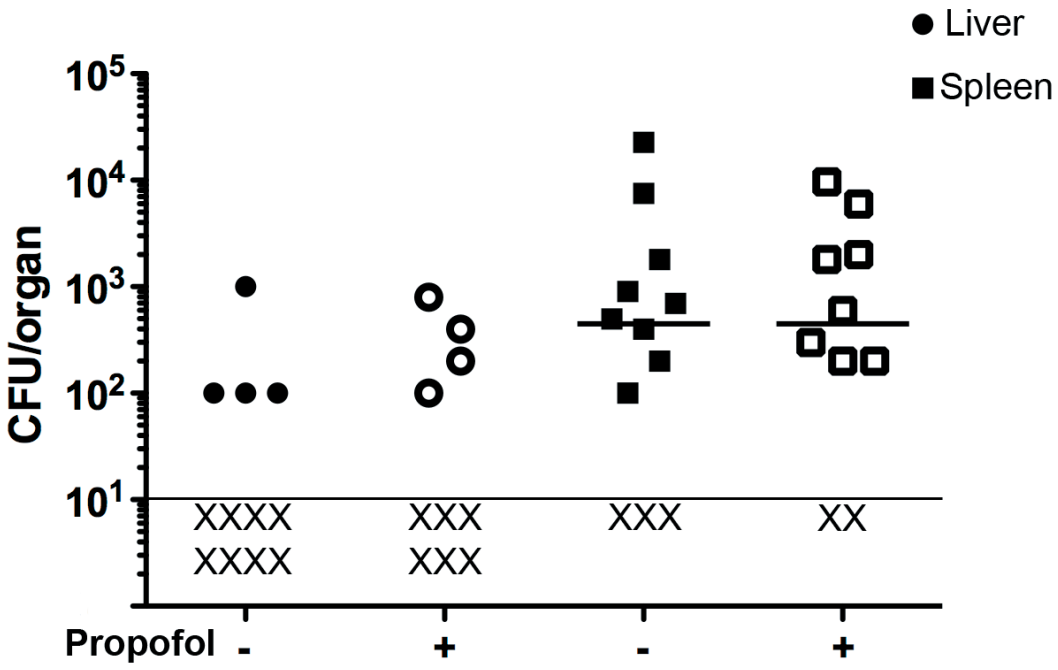


Figure 3.12: Cell-to-cell spread is required for LM to cause productive infection, regardless of propofol treatment. Animals were intravenously infected with $2x10^4$ CFU of the *ΔactA* strain of LM which is defective for the ability to polymerize actin to spread from cell to cell. Animals were sacrificed after 72h and organs harvested and processed for bacterial burden enumeration.

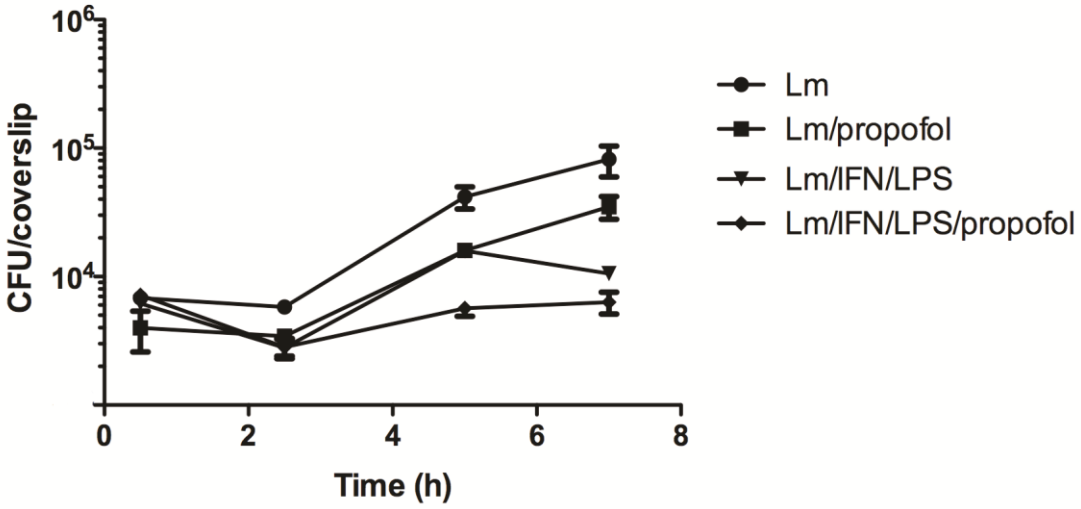


Figure 3.13: Propofol treatment does not increase killing of LM in bone marrow-derived macrophages. Primary bone marrow-derived were isolated from female Swiss-Webster mice. Macrophages were cultured in tissue culture dishes containing glass coverslips and infected with LM in the presence or absence of 50μM propofol. Coverslips were removed at the indicated time point, macrophages were lysed, and dilutions of the cell lysates were plated for viable CFU. Data shown is representative of 3-4 independent experiments

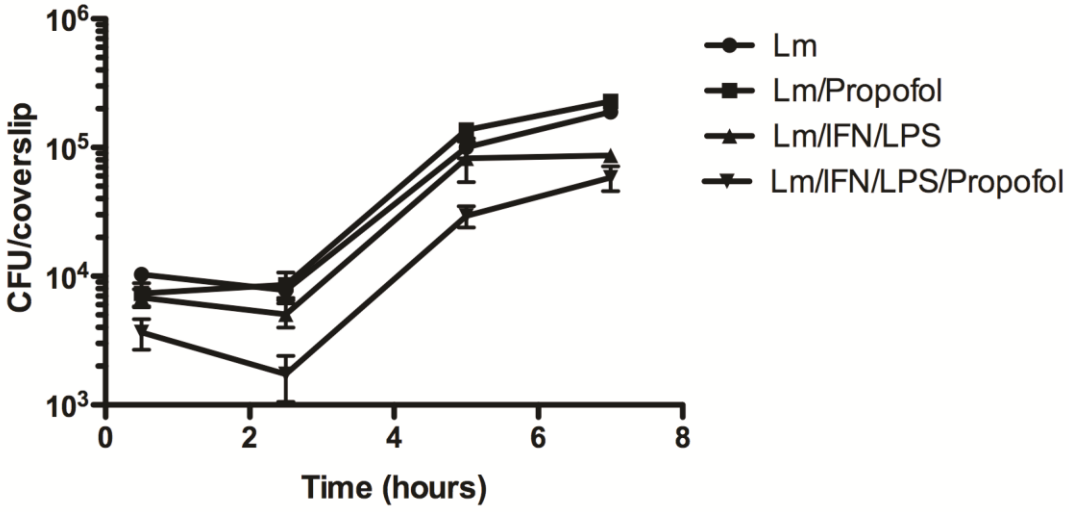


Figure 3.14: Propofol does not affect efficiency of LM killing by peritoneal macrophages. Data representative of 2 independent experiments.

barrier. Propofol exposure was found to significantly increase both the percentage of animals exhibiting brain infections as well as the bacterial burdens within the brains (Figure 3.15). The infection of drug-treated animals with a 10-fold lower dose of LM resulted in the recovery of bacteria from the brains of 50% of the sedated animals, in comparison with no evidence of brain infection for animals infected in the absence of drug (Figure 3.16).

Propofol exposure increases host susceptibility to infection up to 96 hours after recovery from sedation

Propofol is the preferred anesthetic induction agent for many invasive procedures because it is fast-acting with a very brief duration of anesthesia and presents few severe side-effects upon recovery[58,93,142,143]. Our previous studies indicated that brief induction of anesthesia with propofol in immunocompetent mice impacted their ability to clear infection, but it was unclear if this effect was only present when animals were exposed to infectious agents while sedated. To determine if the influence of propofol on host immunity extends beyond periods of sedation, mice were briefly anesthetized for 5 minutes with propofol and allowed to recover for 24 or 96 hours before intravenous infection with a sublethal dose ($2x10^3$ CFU) of LM. Animals intravenously infected 24 hours after recovery from sedation still exhibited significantly increased bacterial burdens in the livers and spleens 96 hours after LM infection in comparison to vehicle treated controls (Figure 3.17, left panel). Differences in bacterial burdens were still detectable in animals infected 96 hours after propofol exposure (Figure 3.17, right panel), and continued up to 7 days post-drug exposure (Figure 3.18), however the magnitude of the difference observed after 7 days of recovery was much reduced. These results show that

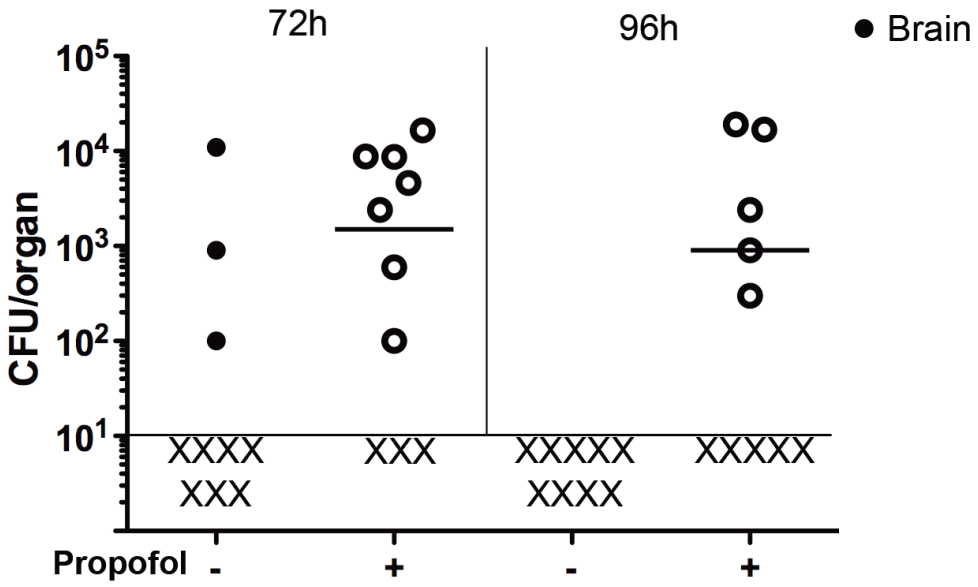


Figure 3.15: Propofol treatment increases LM translocation to the brain. Mice were intravenously infected with 2 x 10^4 CFU LM in the presence and absence of propofol exposure. Graph depicts bacterial burdens present in the brain at the indicated time points post-infection. Data shown is from two independent experiments.

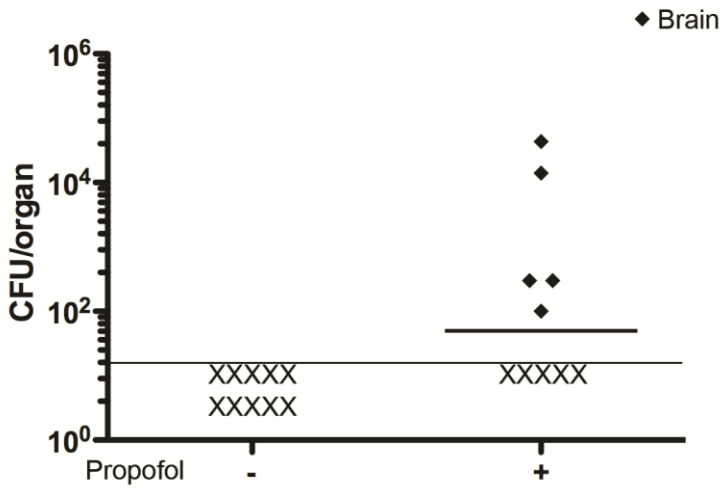


Figure 3.16: : Propofol increases the efficiency of LM translocation across the blood-brain barrier even at low infectious doses. Mice were intravenously infected via tail vein with a low dose ($2x10^3$ CFU) of LM in the presence or absence of propofol. At three days post-infection the animals were euthanized, and bacterial burdens determined in the brain. Data shown is representative of two independent experiments.

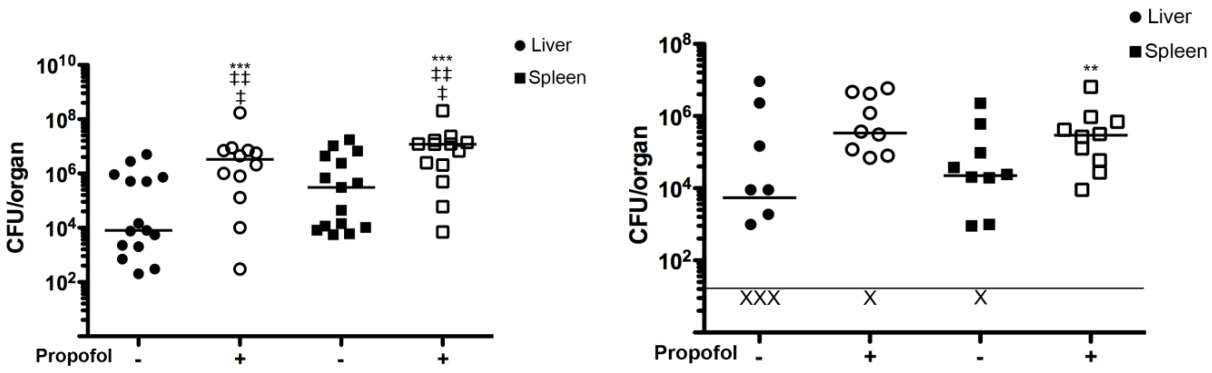


Figure 3.17: Propofol decreases host clearance of LM from tissues for up to four days post-sedation. Animals were anesthetized with propofol and allowed to recover from sedation for 24h (left panel) or 96h (right panel). They were then infected intravenously with $2x10^3$ CFU of LM and sacrificed 96h post-infection. X: burdens undetectable; ‡: dead animal. ** $p<0.005$, *** $p<0.0005$.

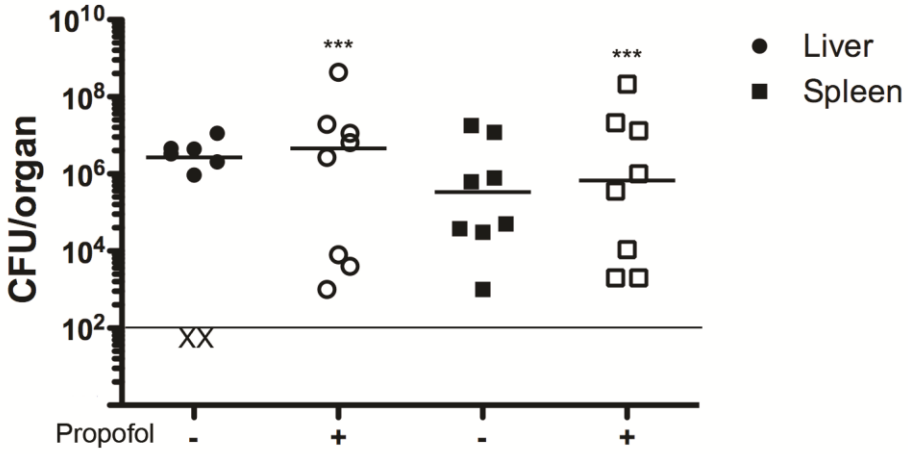


Figure 3.18: Propofol does not significantly alter host clearance of LM from target tissues 7 days post-sedation. Mice were briefly anesthetized with 18.75 mg/kg propofol (approximately five minutes of sedation) and allowed to recover for 7 days before intravenous infection with $2x10^3$ CFU of LM. Animals were euthanized at three days post-infection and bacterial burdens were determined in the liver and spleen. Data shown is representative of two experiments.

although the sedative effects of propofol are short-acting, the effects of the drug on host infection susceptibility continue for several days after drug exposure.

Alternate anesthetics and drugs targeting the GABA-A receptor do not recapitulate the effects of propofol on host innate immunity

Pentobarbital, similar to propofol, is an anesthetic that targets the GABA-A receptor[133], whereas the anesthetic ketamine binds to the N-methyl-D-aspartate (NMDA) receptor[144] and induces anesthesia via an alternate mechanism. All three anesthetics have been evaluated for their effects on immune modulation *in vitro* and *in vivo*[36,38,48]. However, none of them have been evaluated directly for immunomodulation *in vivo* in the context of an infection. To determine whether sedation in general influenced host susceptibility to microbial infection, mice were orally inoculated with LM following exposure to pentobarbital or ketamine. Both pentobarbital and ketamine sedation were found to increase bacterial burdens in livers and spleens by an average of 100- to 10,000-fold at 72 hours post-infection compared to vehicle treated controls (Figure 3.19). While a number of control animals had no detectable bacteria recovered from either liver or spleen, both pentobarbital and ketamine treatment groups had mice that succumbed to infection (Figure 3.19). These results indicated that anesthesia with both pentobarbital and ketamine may alter barrier permeability, a known side effect of these two drugs when referring to physiological parameters such as gut motility in anesthetized patients[29,125].

To determine if pentobarbital and ketamine exposure influenced immune clearance of LM from target organs, animals were intravenously infected with a sub-lethal dose of LM in the presence or absence of drug exposure. After 72 hours of infection, there was no significant

difference in bacterial burdens recovered from livers and spleens of pentobarbital or ketamine treated animals in comparison with vehicle-treated controls (Figure 3.20). This was in contrast to animals exposed to propofol, who exhibited significant increases in bacterial burdens in both liver and spleen (Figure 3.20). Mice exposed to the GABA-A receptor-selective agonists muscimol and topiramate or the receptor antagonists securinine and picrotoxin exhibited no consistent differences in bacterial burdens in target organs following either oral or intravenous inoculation, although statistically significant differences were observed for some drugs in some organs (Figures 3.21 & 3.22.). Thus, although propofol targets GABA-A receptors, the drug's inhibition of host immune clearance of bacteria from infected tissues is not replicated by other drugs that target GABA-A receptors or by alternative anesthetics such as pentobarbital or ketamine.

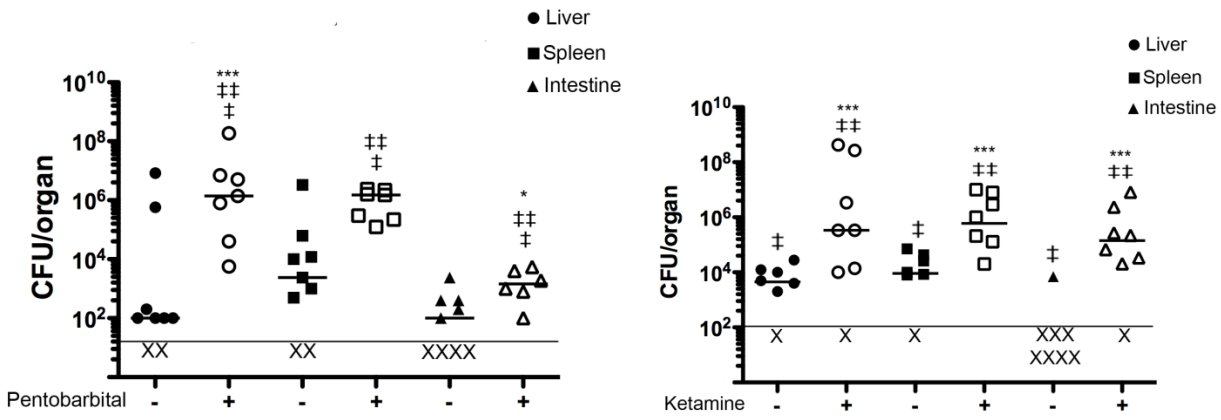


Figure 3.19: The anesthetics sodium pentobarbital and ketamine increase host susceptibility to oral infection. Mice were orally infected with 10^8 CFU of LM following intravenous injection of vehicle solution, pentobarbital (left), or ketamine (right). Graphs depict bacterial burdens in liver, spleen, and intestine at three days post-infection. Data shown is from two independent experiments. X: undetectable bacterial burden; ‡: dead animal; *** $p<0.0005$.

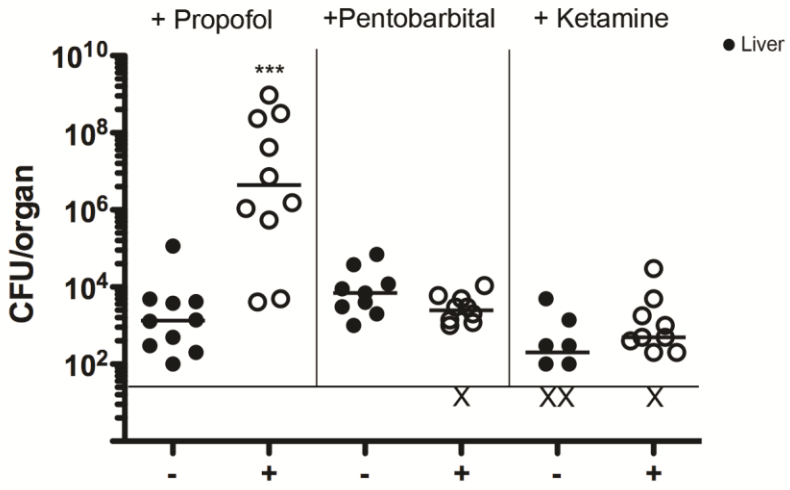


Figure 3.20: Sedation with the sodium pentobarbital and ketamine does not increase host susceptibility to intravenous infection, whereas propofol does. Mice were intravenously infected with $2x10^3$ CFU of LM following intravenous injection of vehicle solution, propofol, pentobarbital, or ketamine. Graph depicts bacterial burdens in the liver at three days post-infection. Data shown is from two independent experiments. *** $p<0.0005$.

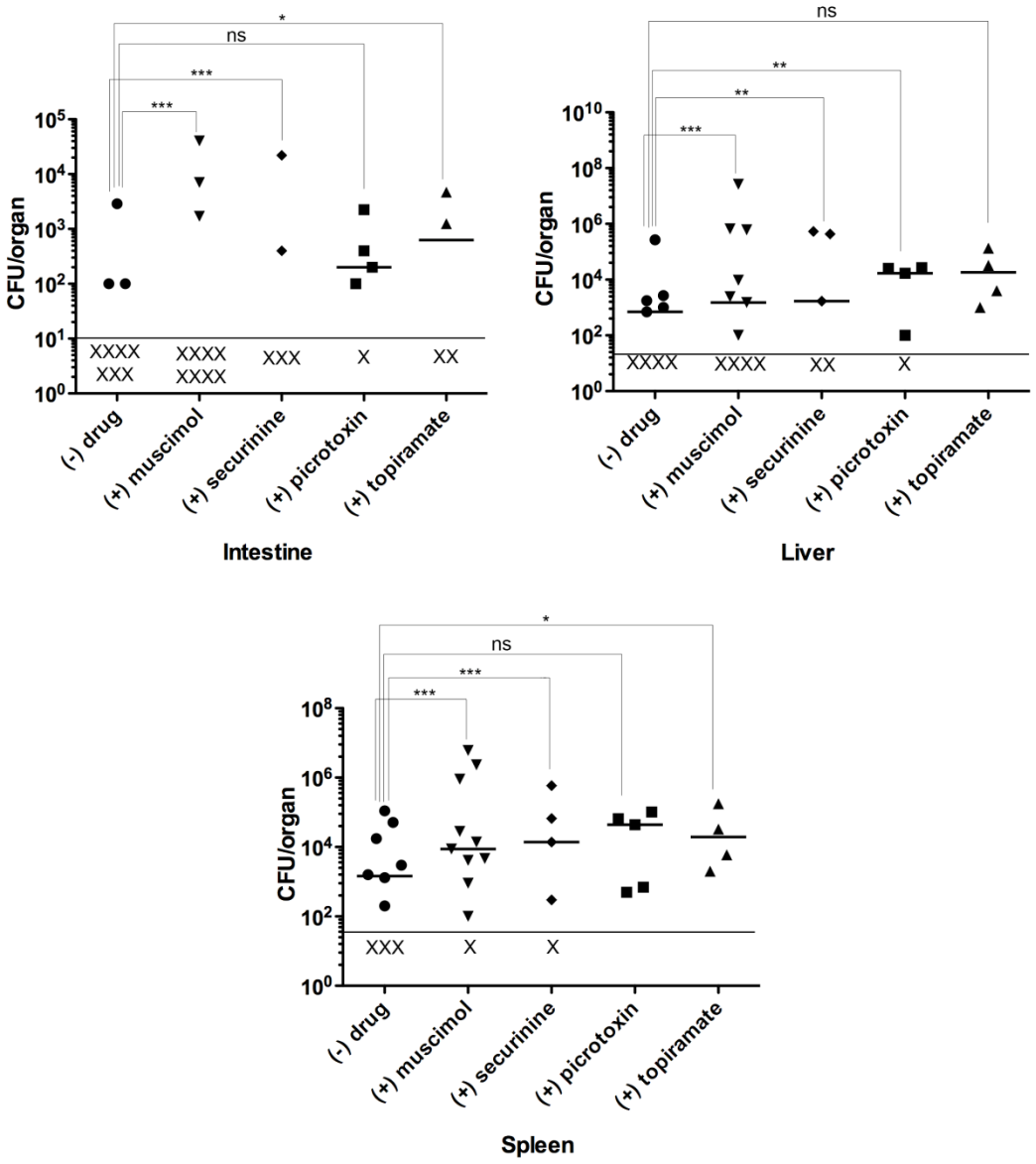


Figure 3.21: GABA-A receptor-specific agonists and antagonists do not increase host susceptibility to oral infection with LM. Animals were intravenously administered the GABA-A receptor agonists muscimol or topiramate, PBS vehicle, or the GABA-A receptor antagonists securinine and picrotoxin via tail vein injection. Mice were subsequently infected with 10^8 CFU intragastrically. Animals were euthanized at three days post-infection and bacterial burdens were determined for the liver, spleen, and intestine. Data is representative of two independent experiments. * $p<0.05$, ** $p<0.005$, *** $p<0.0005$. X: burden undetectable.

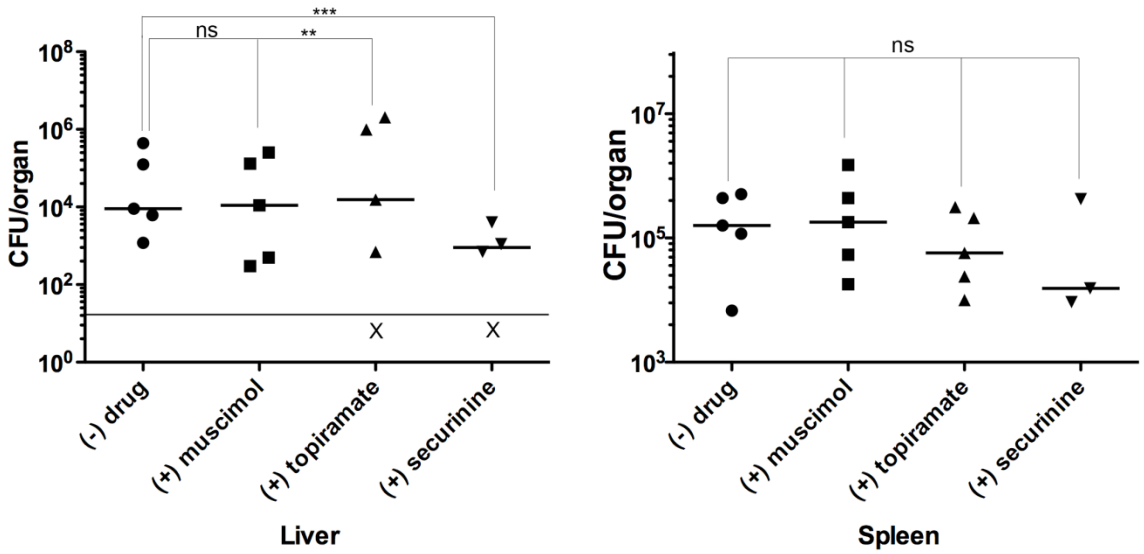


Figure 3.22: GABA-A receptor-specific drugs do not exacerbate LM infection in an IV model of listeriosis. Animals were infected with $2x10^4$ CFU of LM in the presence or absence of the IV injected GABA-A agonists and antagonists shown above. Animals were sacrificed at 72h days post-infection. ** $p<0.005$, *** $p<0.0005$.

3.4 Discussion

Previous studies showed that propofol as well as other anesthetics can be immunomodulatory *in vivo* and i*n vitro*[33,34,37,53,55]. However, the studies described in this chapter are the first to characterize the effects of propofol anesthesia in a physiologically relevant infection model. We have provided evidence that the commonly used anesthetic propofol significantly increased the risk and severity of microbial infection, through both oral and intravenous routes. Propofol treatment increased LM translocation across the intestinal barrier in an InlA-independent manner, and allowed for bacterial persistence within the intestine (Figures 3.1, 3.2, and 3.4). Anesthetized animals were additionally unable to efficiently clear infection when LM was administered intravenously (Figure 3.11). These results indicate that propofol affects host susceptibility through multiple mechanisms, including an increase in barrier permeability as well as by directly preventing host clearance of infection.

Studies published by Czuprynski et al indicated that the efficiency of LM oral infection in mice was increased significantly by sodium pentobarbital anesthesia[37]. Sodium pentobarbital is a commonly used veterinary anesthetic agent[145], and is occasionally used to sedate animals prior to oral inoculation with infectious agents[146]. Czuprynski observed that, though oral infection of mice with wild type LM was not very efficient, intraperitoneal anesthesia with sodium pentobarbital greatly increased the efficiency and reproducibility of infection[147,148]. Studies assessing the reduction of gut acidity or motility, two known side effects of pentobarbital *in vivo*[125], could not recapitulate the decrease in host resistance seen in anesthetized mice orally infected with LM[37]. Additionally, mice infected IV with LM did not display any alterations in resistance to infection, regardless of pentobarbital administration. This led Czuprynski's group to conclude that pentobarbital anesthesia possibly altered the environment of the gut to be more

favorable to bacterial translocation, but did not directly affect immunity[37], contrary to many published reports[36,53,55]. These studies warranted further characterization of the links between anesthetics and host infection susceptibility.

Sodium pentobarbital and other barbiturate anesthetics are no longer commonly used in hospitals due to the large number of side effects, especially regarding their addictive potential[125]. Propofol, in contrast, is the drug of choice to induce anesthesia before surgery and in intensive care units (ICUs) throughout the US[58,62,149,150] for many reasons including its lack of addictive properties[151]. Though propofol and sodium pentobarbital both bind to the GABA-A receptor to induce anesthesia, propofol represented a more clinically relevant alternative to study the interactions between anesthesia and infection. Preliminary studies showed that propofol increased host susceptibility to oral infection with LM similar to the reported effect of sodium pentobarbital (Figures 3.1, 3.2), and the disparity in numbers of bacteria in the intestine at early times post-infection (Figure 3.1) indicated that propofol increased bacterial translocation across the intestinal barrier. Additionally, mice infected with the *ΔinlA ΔinlB* strain of LM showed large amounts of bacteria in the intestine at 72h post infection, despite the fact that LM was unable to invade intestinal epithelial cells through interaction with E-cadherin, which represents a classical portal of entry for LM into the intestine[137] (Figure 3.5). This was surprising, as propofol is not known to increase intestinal permeability or gut motility as does sodium pentobarbital[151], and the InlA-E-cadherin interaction is thought to be important for LM to successfully penetrate the intestinal barrier[139]. These results imply that the increased presence of LM in the intestines of propofol-treated mice cannot be explained by alteration of gut physiology or by enhanced translocation across intestinal epithelial cells dependent on E-cadherin-InlA

M cells are antigen-sampling cells of the mucosal associated lymphoid tissue (MALT) that represent a first line of defense against pathogens invading the intestinal barrier[152]. M cells differentiate from intestinal epithelial cells located in intestinal crypts when exposed to lymphoid stimulation, such as association with macrophages, T cells, and B cells in the intestinal lumen[152-155]. Microbes and microbial antigens are phagocytosed by M cells and are transcytosed through associated macrophages deeper into the MALT[154]. LM is able to breach the intestinal barrier through various interactions, including transcytosis through M cells[153,156] (Figure 3.23). Although the interaction of LM with intestinal epithelial cells requires InlA-E-cadherin binding, at least one group has shown that InlA is not required for LM transcytosis across M cells[153]. While most non drug-treated animals did not display bacteria in the livers and intestines when orally infected with the *ΔinlA ΔinlB* strain of LM, the same strain was able to cause much more productive infection in anesthetized animals (Figure 3.5). This phenomenon may be caused by propofol interacting with M cells to increase LM transcytosis and/or survival within macrophages after crossing the intestinal barrier. Although propofol is not thought to affect barrier permeability[151], the increased presence of LM in the intestines and brains (Figure 3.15) of anesthetized animals suggests that propofol somehow promotes bacterial translocation across distinct physiological barriers. Propofol has also been shown to sequester reactive oxygen and nitrogen species[39,52,78], as well as prevent NF-κB translocation to the nucleus[90]. Both of these effects could increase LM survival in the host.

Propofol treatment resulted in the decreased ability of the host to clear infection when bacteria were delivered intravenously (Figure 3.11), which will be discussed in further detail in Chapter 4. Interestingly, this effect was not duplicated in primary macrophage culture (Figures 3.12, 3.13), suggesting that macrophages are not the only cell type affected by propofol

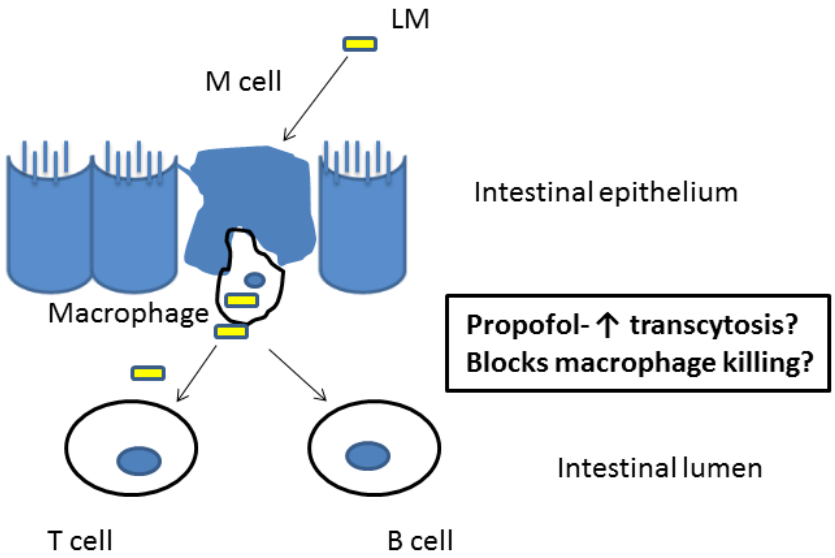


Figure 3.23: Diagram of how LM translocates across M cells. Propofol may increase LM translocation across the intestinal epithelium through M cells by potentially increasing invasion or by blocking macrophage killing.

treatment. This implies that communication between multiple cell types may be important for propofol-dependent immunomodulation.

Although the sedative effects of propofol are short acting, even brief exposures to the drug were sufficient to increase host susceptibility to both oral and intravenous LM infection for up to 96 hours post sedation (Figure 3.17). In spite of its short half-life, the metabolites of propofol can persist in tissues for long periods of time[143]. Propofol is a highly lipophilic drug, and once introduced into the bloodstream is rapidly distributed into tissues[143]. The drug is then rapidly perfused out and metabolically modified in the liver by the Cytochrome P450 2A6 enzyme[157]. The 4 main metabolic species excreted in the urine are shown in Figure 3.28; most undergo glucuronidation to become water soluble, and 72% of the drug is excreted as metabolites with only 0.3% excreted as unmodified propofol within 24 hours[158]. However, some quantity of the drug may remain in slowly perfused tissues with high fat content[151,159], though it is unknown if the species that remain in these tissues are active propofol or its metabolites. Within 5 days of administration, 88% of propofol is excreted in the urine[160]. Our results indicated that animals remain more susceptible to systemic LM infection from 24h to 96h after recovery from sedation (Figure 3.16), with this effect essentially disappearing when infected 7 days post-recovery (Figure 3.17). This corresponds with the tissue elimination rate of propofol. It may be that the amount of propofol remaining in tissues after 24h (28%) is enough to affect the immune response and skew the host towards being immunocompromised. However, there would appear to be a threshold to this effect: the quantity of propofol or its metabolites remaining in tissues may need to be somewhere between 12% and 28% in order to affect immunity, which is why there was no effect seen in anesthetized animals 7 days post-recovery (Figure 3.18). Further

studies must be undertaken to gauge whether propofol or its metabolites persist in the liver or other tissues, and if they are active in modulating immune responses.

The alternative anesthetics pentobarbital and ketamine were examined with regards to their potential to exacerbate LM infection in hosts. While both anesthetics increased host susceptibility to oral infection with LM (Figure 3.19), only propofol additionally reduced host immune clearance of bacteria from sites of infection (Figure 3.20). This implies that pentobarbital and ketamine both decrease resistance to infection by increasing barrier permeability, a known side effect of both drugs[29,145], as opposed to acting directly on host immunity. Interestingly, while both propofol and pentobarbital bind to GABA-A receptors to mediate anesthesia, only propofol decreased clearance of bacteria from target organs. Additional experiments using the GABA-A receptor agonists mucimol and topiramate or the antagonists securinine and picrotoxin showed no effect on host susceptibility to oral or intravenous LM infection (Figures 3.21, 3.22). GABA-A receptors are expressed on a variety of immune cells, including macrophages and T cells[161,162], and changes in receptor expression or activation patterns have been associated with immunomodulation[163]. However, the effect of propofol on immune clearance may potentially be mediated through an alternate receptor recognized by propofol but not by the other GABA-A receptor agonists and antagonists examined in this study. These findings indicate that drugs that target the central nervous system may have additional, distinct influences on host immune function, with implications for successful host clearance of infection

CHAPTER FOUR-

Propofol treatment and immunomodulation: effect of drug exposure on host serum cytokine profiles immune cell recruitment and differentiation in target organs

4.1 Summary

In the previous chapter, it was shown that propofol treatment inhibited host clearance of LM from target organs, thus raising the possibility that the drug influences host immunity. In this chapter, studies were carried out to probe this mechanistically by examining organ pathology, serum cytokine secretion patterns, and by immunophenotyping the spleens of anesthetized vs. control animals. Propofol anesthesia induced gross pathological alterations in the livers and spleens of infected animals. Anesthetized animals displayed more areas of necrosis, fibrosis, and inflammation compared with infected controls. Propofol treatment was also found to alter serum cytokine and chemokine levels throughout infection. While propofol did not impair $CCR2^{+}$ inflammatory monocyte recruitment to target sites of infection, significant reductions in the numbers of differentiated macrophages and TNF and iNOS-producing dendritic cells (TipDCs) were observed in drug exposed animals. Interestingly, both differentiated $F4/80^{+}$ macrophages and TipDCs derive from the same $CCR2^{+}$ inflammatory monocyte progenitor population circulating in the bloodstream, indicating that propofol may affect immune cell differentiation at target sites of infection.

4.2 Introduction

LM has been used as a model pathogen by immunologists for decades, and the host immune response against this pathogen is very well characterized[103,109,112,116,131,140,164-172]. Adaptive immunity is required to fully clear LM infection, and is primarily mediated by CD8+ cytotoxic T-cells[103,116,166]. However, without an effective innate immune response, animals rapidly succumb to infection[102,131,173,174]. Proper macrophage function is critically important for mediating uptake of LM in tissues or in the blood[109,175], killing intracellular bacteria as they attempt to escape the phagosome to replicate in the cytosol[103,109,112], and for antigen presentation to effectively prime T-cells[109,176,177]. Another cell type, the TNF- and iNOS-producing dendritic cell (TipDC) also has been shown to be crucial in protective host immunity against listeriosis[104,105,112,178-180]. Both cell types are derived from monocyte precursor populations circulating in the bloodstream, termed inflammatory monocytes and characterized by the presence of chemokine receptor 2 (CCR2) on their cell surface[104,105,112,131,172,178].

The intracellular infectious cycle of LM can be divided into various stages, including invasion into host cells, escape from the phagolysosome, and cytosolic replication phases[101,103,109,117,127,171]. LM occupies different intracellular niches throughout its virulence lifecycle, and at each phase activates various arms of innate immune defenses. Particularly important for our studies are innate immune effector functions performed by macrophages during intracellular invasion and replication of LM within the host cell cytosol. LM that enters the bloodstream directly are taken up within minutes by cells of myeloid lineage in the spleen, including dendritic cells, F4/80^{+} macrophages, and to a lesser extent, neutrophils[102]. While neutrophils have been shown to be dispensable for clearing LM infection in the spleen[131],

macrophages and dendritic cells are essential[103,104,131]. Initial infection of splenic myeloid cells by LM causes cytokine and chemokine secretion into the bloodstream[103,105,112,164,165,175], and the recruitment of $CCR2^+$ inflammatory monocytes into target organs[104,112,115,131,178,179] through the chemokine monocyte chemoattractant protein-1 (MCP-1). Once inflammatory monocytes reach sites of infection in the spleen, a variety of inflammatory signals induce their differentiation into effector cells such as activated $F4/80^+$ macrophages or TipDCs[104,178,181] (Figure 1.5).

Our studies have shown that propofol treatment decreased the ability of the host to clear infection from target organs in an IV model of listeriosis (chapter 3). The current studies seek to further define how propofol modifies initial immune responses to LM infection through serum cytokine assays as well as flow cytometric analysis of splenic tissue. It was found that propofol caused massive dysregulation of T_h1 cytokines important for clearance of LM infection. We also found that propofol significantly reduced the numbers of differentiated macrophages and TNF and iNOS-producing dendritic cells (TipDCs) in drug-exposed animals.

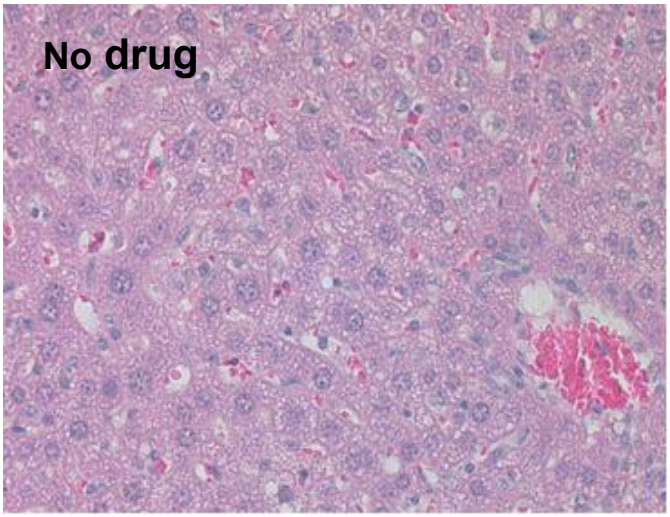
4.3 Results

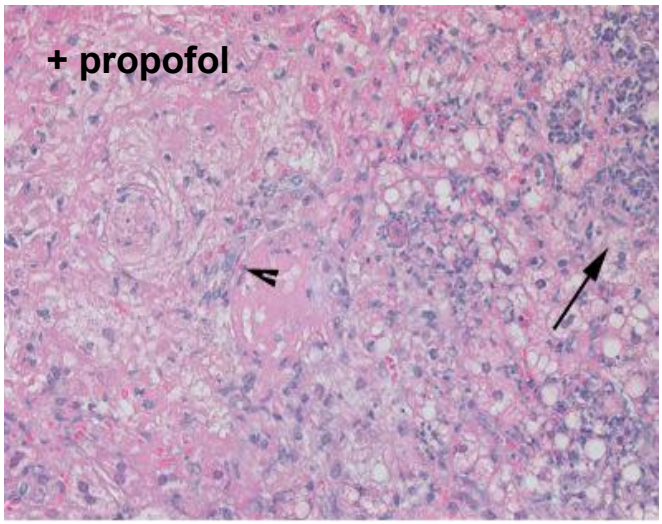
Exposure to propofol during LM infection results in pathological alterations in organ structure

LM primarily colonizes and replicates in the liver and spleen during systemic infection[103,165]. Systemic infection induces large amounts of proinflammatory immune cell recruitment[103,105,112,166], which, if compensatory anti-inflammatory mechanisms are not activated, could lead to large unresolving foci of inflammation as well as areas of necrosis in target organs. In order to assess if propofol anesthesia led to alterations in the histopathology of the intestine, liver and spleen after oral or IV inoculation of LM, hemotoxylin and eosin (H&E) stains were performed on a number of organ samples taken from animals infected in the presence and

absence of propofol, animals given propofol alone, and uninfected, non-sedated animals. Liver sections from animals infected IV with LM showed gross pathological differences depending on if the animal was anesthetized. Infected animals displayed some inflammatory foci in the absence of propofol, but hepatocytes were regularly spaced and no necrosis was observed (Figure 4.1, left). However, anesthetized animals had larger and more numerous inflammatory foci, as well as anucleated areas characteristic of liver necrosis (Figure 4.1, right and bottom). Additionally, the presence of fatty vacuoles in animals infected with LM in the presence of propofol was suggestive of metabolic disruption in the liver (Figure 4.1, bottom panel).

The spleen is a compartmentalized organ that is organized into red pulp, consisting primarily of erythrocytes, and white pulp, consisting primarily of lymphocytes[182]. While this organization was maintained in animals infected with LM in the absence of propofol (Figure 4.2, left panel) and in animals exposed to propofol in the absence of infection (Figure 4.3), the spleens of animals infected and exposed to propofol exhibited significant levels of fibrosis as well as a loss of compartmentalization and dissolution of spleen structure (Figure 4.2, right panel). The spleens of these animals were also significantly smaller in size (~40% smaller on average) with evident areas of cell necrosis (Figure 4.4).





	(-) propofol	(+) propofol
Inflammatory foci	3.8 ± 7.95	19.5 ± 13.38
Necrotic lesions	0	2.25 ± 1.26

Figure 4.1: Propofol treatment in conjunction with systemic LM infection results in increased foci of inflammation and areas of necrosis in the liver. Mice were infected with $2x10^4$ CFU of WT LM via tail vein. Livers were harvested at 72h, fixed, and processed for histology. Arrowhead: necrotic section; arrow: focus of inflammation. Though hepatocytes are regularly spaced and nuclei readily visible in the top panel, propofol treatment resulted in a number of anucleated, necrotic areas (arrowhead, bottom panel). Additionally, anesthetized animals exhibited numerous fatty vacuoles (bottom panel, area around arrow) indicative of metabolic disruption. Chart represents average numbers of inflammatory foci and necrotic lesions in the presence or absence of drug treatment. Representative images from 5 animals/group.

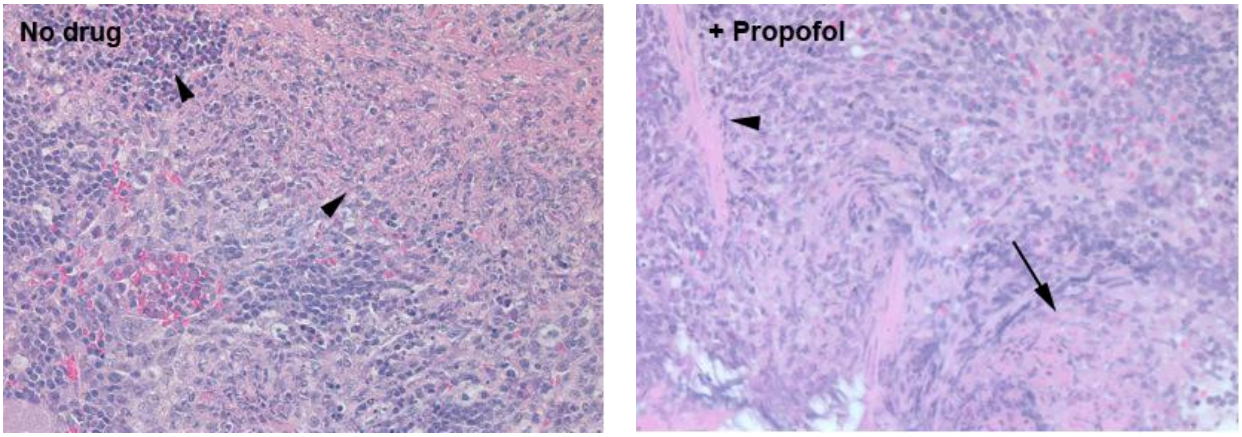


Figure 4.2: Propofol treatment results in splenic necrosis, fibrosis, and loss of architecture in an IV model of LM infection. Mice were intravenously infected with $2x10^4$ CFU of LM in the presence or absence of propofol. Spleens were isolated at 72 h post-infection, formalin fixed, and stained with hematoxylin and eosin (3 spleens per treatment group). Arrowheads, left panel: compartmentalization of red pulp and white pulp. Arrowhead, right panel: fibroid deposit; arrow: splenic necrosis.

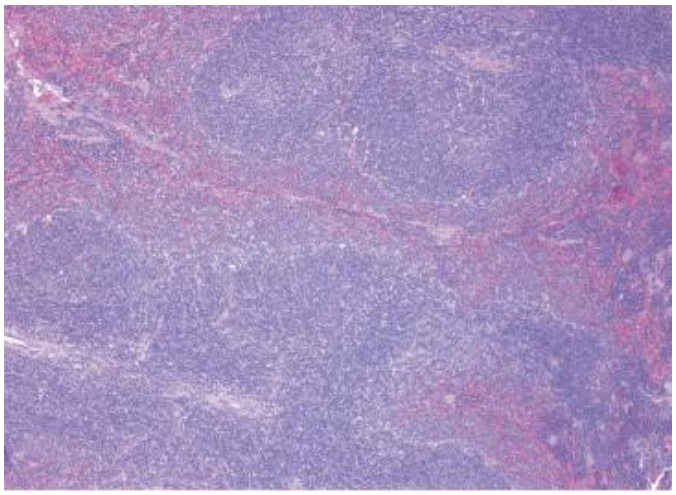


Figure 4.3: Propofol treatment alone does not affect splenic architecture. Mice were anesthetized with propofol and allowed to recover from sedation. They were euthanized after 72h, spleens were isolated, fixed, and processed for H&E staining. Representative image from 3 animals.

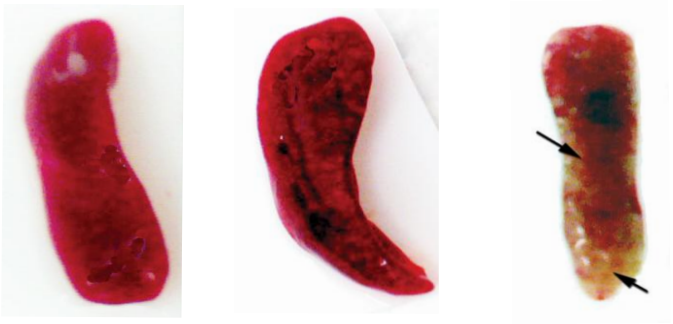


Figure 4.4: Propofol treated animals have smaller, discolored spleens 3 days after IV challenge with LM. Spleens isolated from infected animals exposed to propofol weighed approximately 40% less than the spleens obtained from animals infected in the absence of drug, and exhibited gross signs of necrosis (arrows). Left: naïve mouse, middle: LM infected control mouse, right: propofol-treated, LM infected mouse.

Propofol alters the expression of host cytokines and chemokines in response to LM infection.

Given that propofol sedation increased host susceptibility to LM infection, we examined the expression patterns of key serum cytokines and chemokines associated with immunity to LM[103] (Figure 4.5). Lipoteichoic acid, a LM cell-wall component shed by extracellular bacteria, is recognized by TLR-2 at the cell surface[183,184]. TLR-2 activation induces transcription and ultimately secretion of the proinflammatory cytokines TNF-α, IL-1β, IL-6, and IL-12, as well as the chemokines KC (neutrophil chemoattractant in mice) and MCP-1 (monocyte chemoattractant)[114,184]. The production of the IL-10 anti-inflammatory cytokine is also increased in response to inflammation[185]. IFN-γ, a proinflammatory cytokine critical for priming the Th1 inflammatory response, is produced by NK cells, T cells, and splenic macrophages in response to systemic LM infection as well[186]. Following intravenous infection, serum cytokine levels of IL-1b, IL-6, IL-12, and TNF-α and of the anti-inflammatory cytokine IL-10 appeared similar in anesthetized and control animals during the first 48 hours of infection (Figures 4.6-4.8). Levels of IL-1b, IL-6, and TNF-α increased by 72 hours post-infection in drug treated animals (Figures 4.6 & 4.7) even though overall bacterial burdens were similar at this time point to those observed for vehicle treated controls (Figure 3.11). Interestingly, the chemokine MCP-1, which contributes to monocyte recruitment to sites of infection, was significantly higher at all time points in the propofol-treated groups, as were the eosinophil chemoattractant eotaxin and the neutrophil chemoattractant KC (Figure 4.9). IFN-γ was elevated in propofol-treated groups in comparison to control animals at 24 hours post-infection, but then steadily decreased to the levels observed for control animals at 48 and 72 hours post-infection (Figure 4.10). As infection progressed in the host, propofol treatment seemed to be detrimental to keeping IFN-γ levels high enough to aid the host in effective bacterial clearance (Figure 4.10). In contrast to IFN-γ, levels

of IL-10 increased significantly at 72 hours post-infection in drug treated animals (Figure 4.8). As IL-10 is anti-inflammatory and its production directly antagonizes the action of IFN-γ [187], this may reflect a mechanism by which propofol increases susceptibility to infection. Additionally, TGF-β levels in the presence of propofol were moderately lower at later time points compared with control animals, indicating a potential inability in anesthetized animals to control inflammation (Figure 4.11). Propofol thus modifies host immune signaling in response to LM infection as indicated by the altered patterns of expression of multiple cytokines and chemokines.

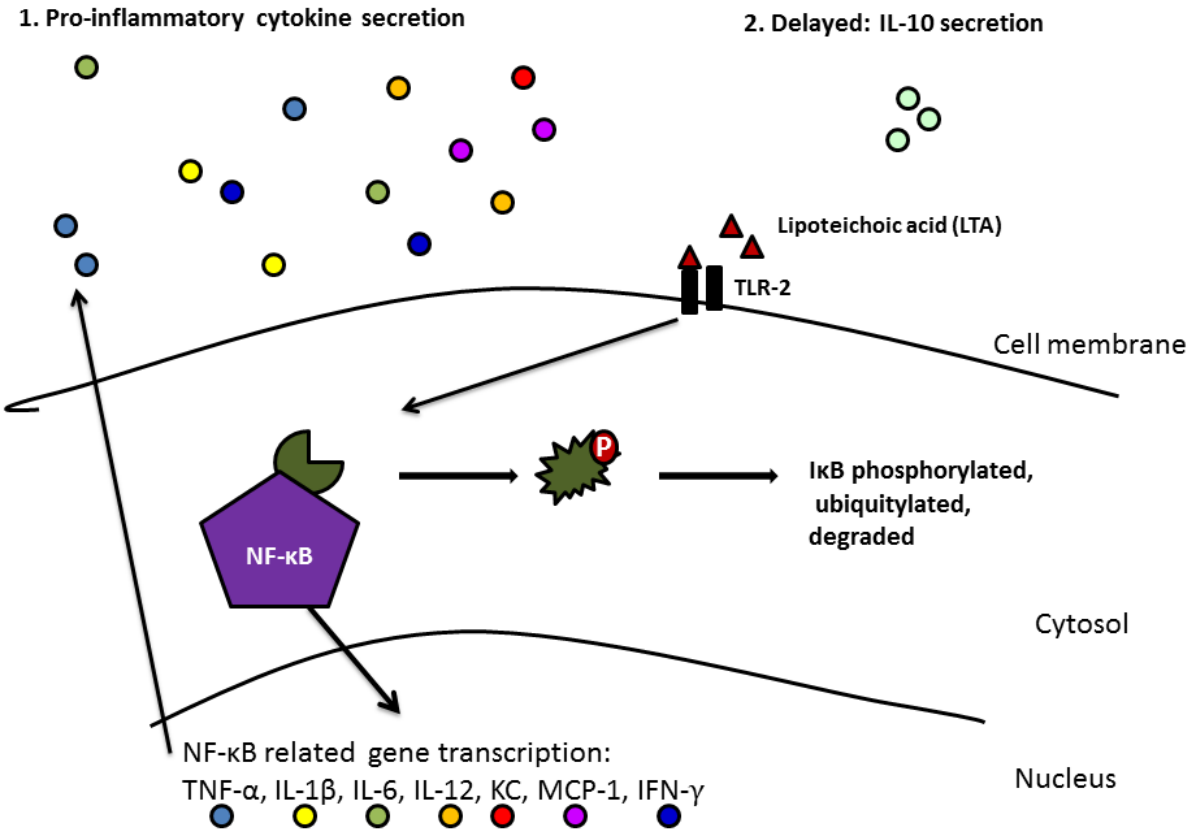


Figure 4.5: Model of LM-induced cytokine secretion by macrophages (NF-κB-dependent pathway). Lipoteichoic acid binding to TLR-2 induces NF-κB-dependent pro-inflammatory cytokine transcription. Initial responses include secretion of the cytokines listed above, with IL-10 secretion occurring as a delayed second step to dampen down inflammation.

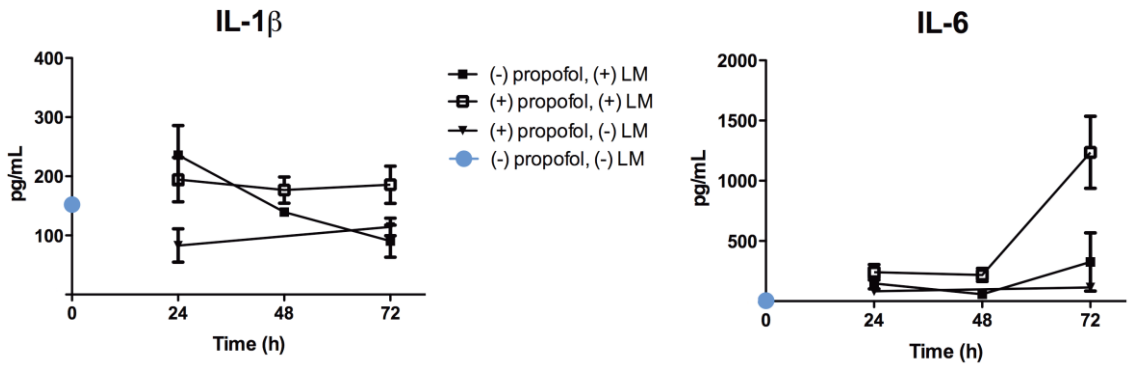


Figure 4.6: Propofol treatment causes increases in serum cytokine levels of IL-1β, IL-6 at late times in infection. Mice were intravenously infected with $2x10^4$ CFU of LM in the presence and absence of propofol sedation. Serum samples were collected via cardiac puncture and run on Bioplex (Bio-Rad Laboratories, St. Louis, MO) magnetic bead assays. Data points represent 3-5 animals per treatment group per time point. Propofol treatment caused no changes in serum levels of IL-1β throughout infection, in contrast to control infected animals that started with higher levels

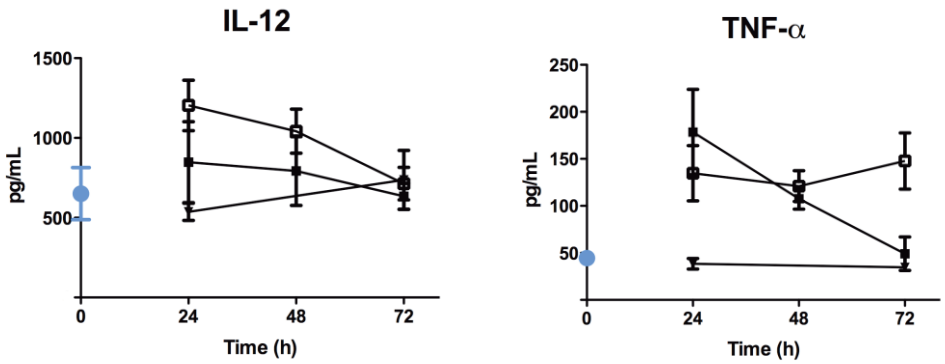


Figure 4.7: Propofol causes alterations in serum IL-12 levels, changes in pattern of TNF-α expression late in infection. While IL-12 levels were moderately higher in propofol-treated animals compared to infected controls, they returned to serum concentrations equal to controls at 72h post-infection. In contrast, TNF-α started out at relatively equivalent serum concentrations between control infected and propofol-treated animals, but remained at the same levels throughout infection, whereas control infected animals exhibited lower levels at later times in infection. Circle: naïve animals, triangle: propofol-only treatment, closed square: vehicle treated, LM-infected treatment, open square: propofol-treated, LM-infected treatment.

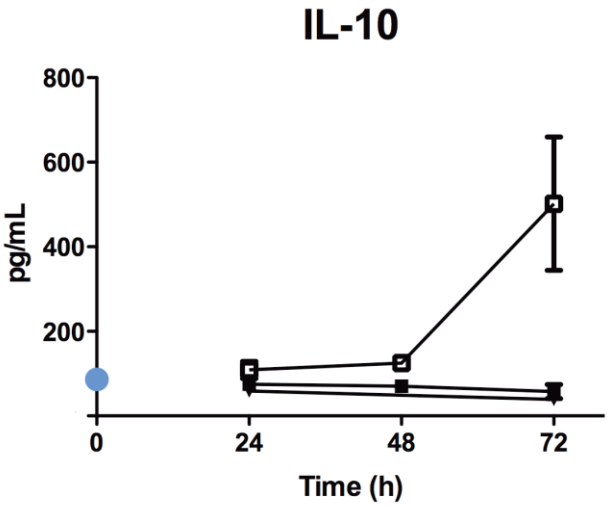


Figure 4.8: Propofol treatment induces spike in serum levels of the anti-inflammatory cytokine IL-10 at late times in infection. IL-10 levels remained low in both control and experimental groups through the first 48h of infection. However, while in control infected groups IL-10 stayed low at all timepoints assayed, propofol treatment caused IL-10 levels to spike late in infection, potentially indicating a role for IL-10 in attempting to resolve excessive inflammation. Circle: naïve animals, triangle: propofol-only treatment, closed square: vehicle treated, LM-infected treatment, open square: propofol-treated, LM-infected treatment.

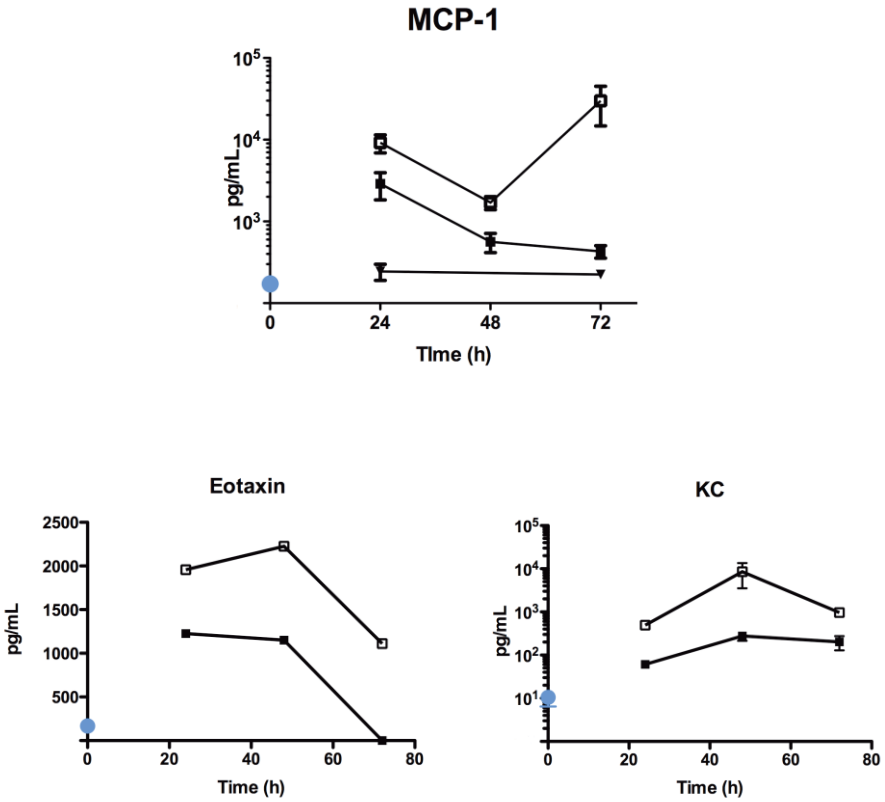


Figure 4.9: Propofol increases serum chemokine levels through both early and late timepoints in infection. Circle: naïve animals, triangle: propofol-only treatment, closed square: vehicle treated, LM-infected treatment, open square: propofol-treated, LM-infected treatment.

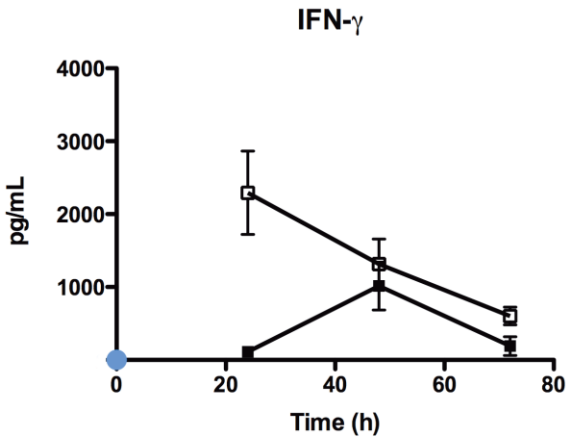


Figure 4.10: Propofol treatment changes the pattern of IFN-γ expression. In the absence of propofol, IFN-γ expression peaks at 48h post-infection. In contrast, propofol treatment induces high IFN-γ production at early times post-infection, but serum levels steadily decrease as the infection progresses. Circle: naïve animals, triangle: propofol-only treatment, closed square: vehicle treated, LM-infected treatment, open square: propofol-treated, LM-infected treatment.

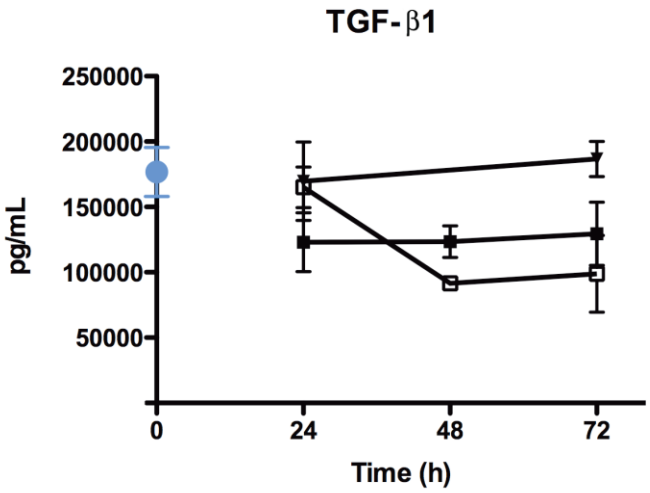


Figure 4.11: Propofol moderately decreases TGF-β levels at later times in LM infection. Though TGF-β levels started out moderately higher at early times post-infection compared to control animals, propofol treatment resulted in lower levels of TGF-β as the infection progressed. Circle: naïve animals, triangle: propofol-only treatment, closed square: vehicle treated, LM-infected treatment, open square: propofol-treated, LM-infected treatment.

Propofol exposure alters splenocyte populations following LM infection

Macrophages, dendritic cells, and neutrophils are three immune cell types known to be important in controlling LM infection [104,109,112,114,131,174,179,180,184-186,188,189]. Macrophages in particular contribute to clearance of LM infection through phagocytosis, antigen presentation, and direct killing of bacteria in infected cells[109]. Macrophages present at sites of LM infection within the spleen are of two types: resident macrophages present in the marginal zone of the spleen[186] and macrophages that differentiate after recruitment of $CCR2^{+}$ inflammatory monocytes[105,112,131,175,190]. Recruitment of inflammatory monocytes can make the spleen larger[182], which can be seen visually without a microscope. Spleens from infected animals not exposed to propofol were significantly larger than both naïve animals as well as propofol-treated infected animals, suggesting differences in inflammatory cell composition of the spleen.

Flow cytometry was used to examine the numbers of immune effector cells including neutrophils, dendritic cells, macrophages, inflammatory monocytes, and TGF-β-positive cells in the spleens of infected mice in the presence and absence of drug treatment. One published report indicated high levels of the monocyte chemoattractant MCP-1 could be detrimental to host immunity to LM by abolishing chemokine gradients and decreasing inflammatory monocyte recruitment to sites of infection[170] Based on this, initial experiments focused on whether propofol affected inflammatory monocyte recruitment to the spleen. As we observed exponentially elevated levels of MCP-1 in sera of propofol-treated infected animals compared with infected controls (Figure 4.9), it was logical to investigate whether too much MCP-1 abrogated inflammatory monocyte recruitment to the spleen. At early times in LM infection, there appeared to be no difference between control and anesthetized animals in inflammatory monocyte ($CCR2^{+}$/ Ly-$6C^{hi}$) recruitment to the spleen (Figure 4.12). There was also no

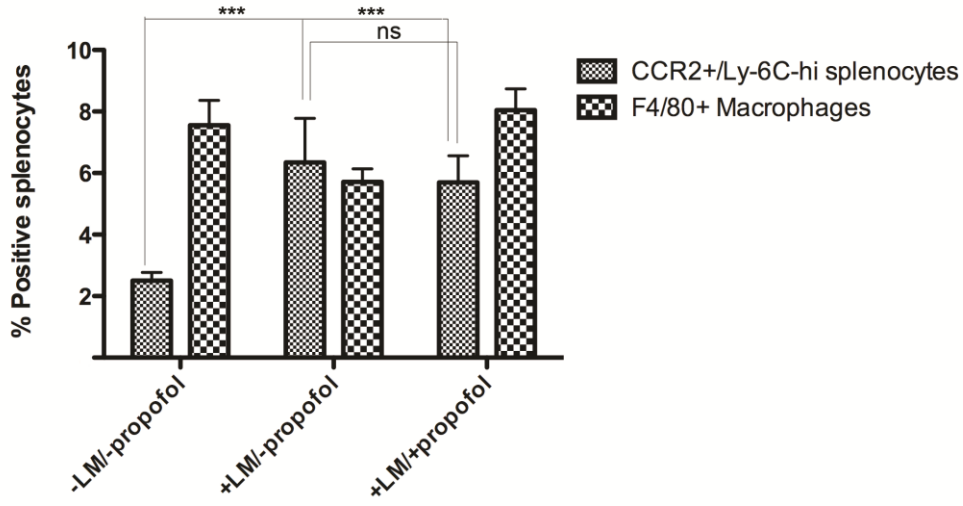


Figure 4.12: Propofol treatment does not alter inflammatory monocyte recruitment to the spleen at early times after LM infection. Mice were left uninfected or infected intravenously with $2x10^4$ CFU of *LM* in the presence or absence of propofol and sacrificed after 24h. Spleens were isolated and processed for FACS analysis. No significant difference was observed between control infected and propofol-treated infected mice in levels of $CCR2^+/Ly\text{-}6C^{hi}$ inflammatory monocytes recruited to the spleen, nor in numbers of differentiated $F4/80^+$ macrophages. Representative data from 2 independent experiments with 4 animals per treatment group per experiment. *** $p<0.0005$, ns: not significant.

difference observed in levels of differentiated F4/80^{+} macrophages between treatment groups. However, as the infection progressed to 72h, while inflammatory monocyte recruitment remained similar between controls and anesthetized animals, propofol-treated animals displayed 2 to 3-fold fewer differentiated macrophages compared with infected control animals (Figure 4.13). Neutrophil and dendritic cell numbers were comparable in the spleens of uninfected and LM infected mice in the presence or absence of propofol treatment (Figure 4.14). In contrast, significant reductions were again seen in the numbers of differentiated macrophages at 72 hours post-infection as well as in the number of TGF-β^{+} cells in the spleens of drug treated animals (Figure 4.14). Overall, mice sedated with propofol exhibited approximately 3-fold fewer differentiated macrophages and 10-fold fewer TGF-β^{+} cells within the spleen (Figure 4.14). Interestingly, the majority of TGF-β^{+} cells were not macrophages (Figure 4.15), neutrophils, or DCs (data not shown), indicating that propofol influences TGF-β production in other lymphocytes.

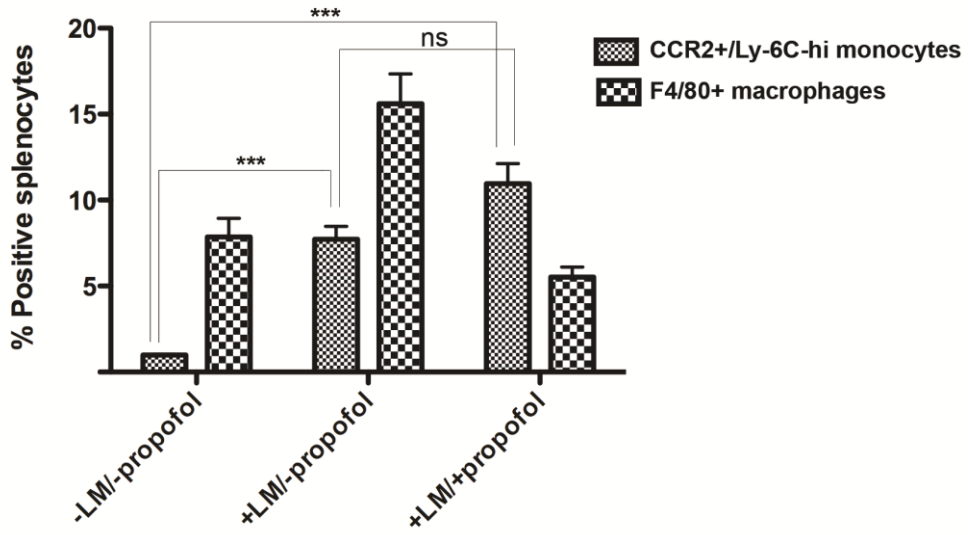


Figure 4.13: Propofol treatment does not alter inflammatory monocyte recruitment to the spleen at late times in LM infection, but significantly decreases numbers of F4/80$^+$ mature macrophages. Mice were infected as described in Figure 7 and animals were sacrificed at 72h. Spleens were isolated and processed for FACS analysis. While no significant difference in inflammatory monocyte recruitment was apparant between control and propofol-treated groups at 72h, anesthetized animals exhibited less than half the number of differentiated F4/80$^+$ macrophages compared to controls. Representative data from 2 independent experiments with 3-4 animals per treatment group per experiment. *** $p<0.0005$, ns: not significant.

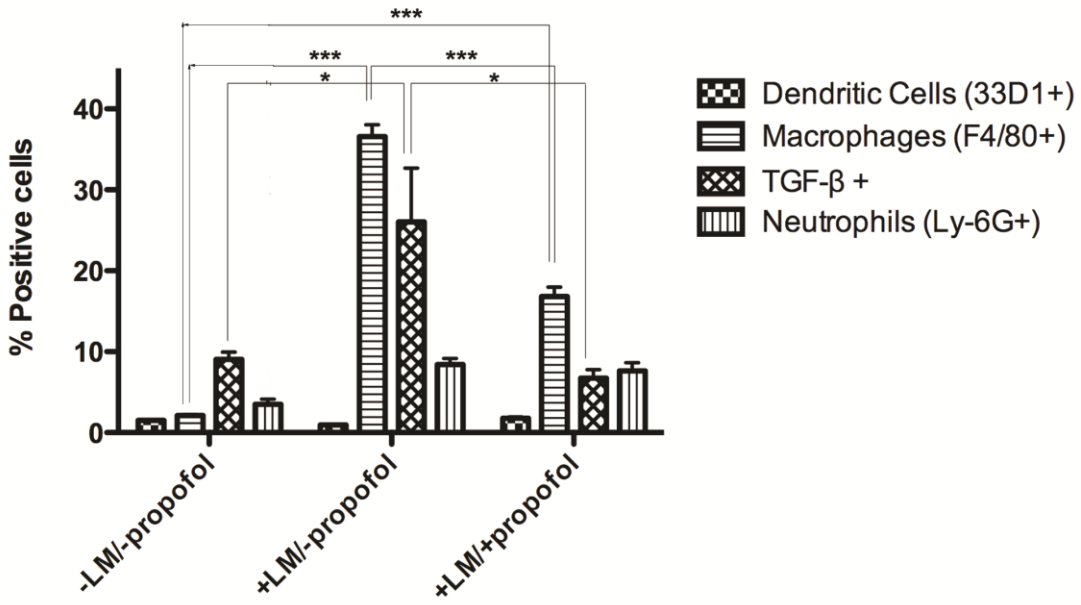


Figure 4.14: While exposure to propofol does not significantly alter levels of dendritic cells or neutrophils, anesthetized animals display fewer mature macrophages and TGF-β^+ cells in the spleen. Animals were infected as described in Figure 7 and sacrificed 72h post-infection. Spleens were isolated and processed for flow cytometry. While no significant differences were observed in neutrophil or classical dendritic cell levels, propofol treatment resulted in fewer than half the number of differentiated macrophages and 10-fold fewer TGF-β^+ cells. * $p<0.05$, *** $p<0.0005$.

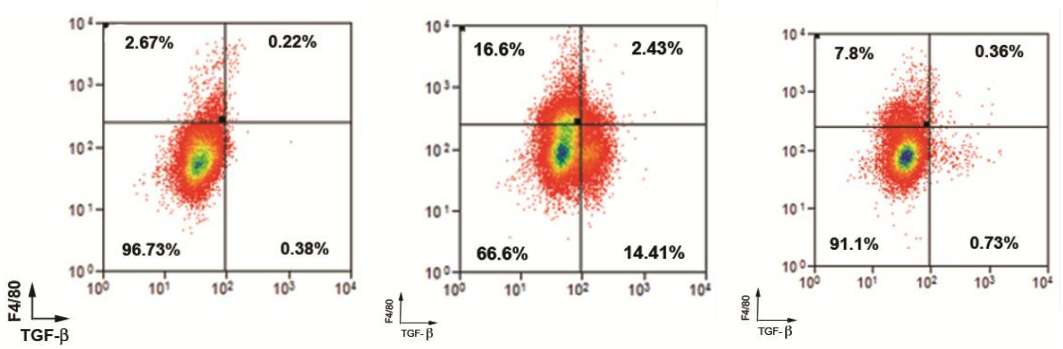


Figure 4.15: Propofol treatment reduces the number of TGF-β$^+$ splenocytes by 10-fold, and these cells are not macrophages. Animals were infected as described in Figure 7 and animals were sacrificed 72h post-infection. Spleens were isolated and processed for flow cytometry. Intracellular TGF-β staining indicated that most TGF-β$^+$ cells were not macrophages (above), neutrophils or DCs (data not shown). Left: naïve mice, middle: vehicle-treated infected mice, right: propofol-treated infected mice. Data shown representative of 2 independent experiments with 3-4 animals per treatment group per experiment.

In order to further understand how propofol affects immune cell populations at sites of LM infection, flow cytometry was used to assess for presence of TipDCs. Immunophenotyping of TipDCs requires analysis of the following markers indicating a functional TipDC: presence of CD11b, CD11c, Mac3 (extracellular), TNF-α, and iNOS (intracellular)[104]. Pamer et al identified 3 splenocyte populations present in the spleens of LM-infected mice with different levels of the cell surface markers Cd11b and CD11c. $CD11b^{hi}CD11c^{low}$ cells are monocytes/macrophages, granulocytes, and NK cells[191]. $CD11b^{low}CD11c^{hi}$ cells represent classical plasmacytoid dendritic cells[108,192]. The population shown by various functional assays to be TipDCs was initially characterized as $CD11b^{int}CD11c^{int}$. These cells were further confirmed as TipDCs through staining with the marker Mac3, indicating cells of $CCR2^{+}$ monocytic lineage[104]. Anesthetized mice displayed half the number of $CD11b^{int}$ $CD11c^{int}$ splenocytes compared with controls 48h post-infection with LM (Figure 4.16). This trend was also shown in Mac-3^{+} $CD11b^{int}$ cells (Figure 4.17). At late times post-infection, propofol treatment results in a massive loss of the differentiated TipDC population, represented by $CD11b^{+}CD11c^{+}$ cells which also stain positive for TNF-α and NOS2 (marker of iNOS production) (Figures 4.18 & 4.19). Propofol thus increases host susceptibility to infection by altering both immune signaling and the populations of immune effector cells present at foci of infection. The flow cytometry data presented indicate that propofol may block differentiation of $CCR2^{+}$ inflammatory monocytes into discrete effector populations, including $F4/80^{+}$ mature macrophages and functional TNF-α and iNOS producing dendritic cells important for clearing LM infection.

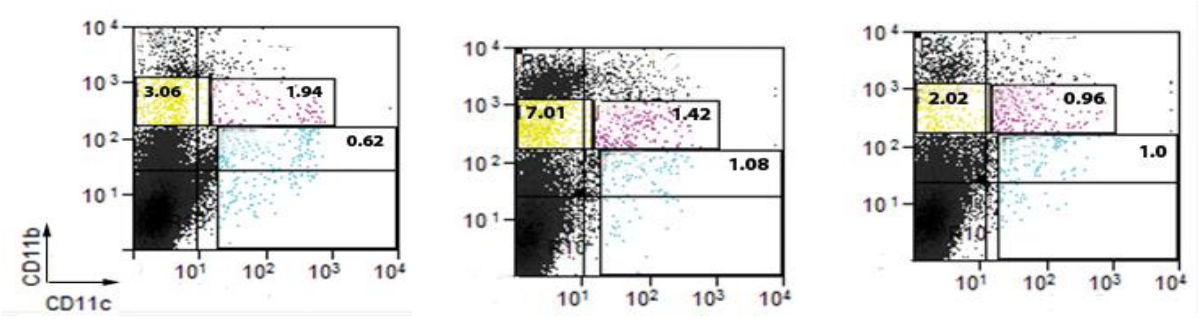


Figure 4.16: Propofol decreases CD11b^{int}CD11c^{int} TipDC splenocyte population in the context of LM infection. Mice were left uninfected or infected IV with 2x10^4 CFU of LM in the presence or absence of propofol anesthesia. Mice were sacrificed at 48h post infection and spleens were isolated and processed for flow cytometry. CD11b^{int}CD11c^{int} splenocytes have been shown to be TipDCs (Pamer et al, 2003), and propofol treatment significantly decreased presence of this group. Yellow: CD11b^{hi}CD11c^{low}, purple: CD11b^{int}CD11c^{low}, blue: CD11b^{low}, CD11c^{hi}. Left: naïve mice, middle: vehicle-treated LM infected mice, right: propofol-treated, LM infected mice.

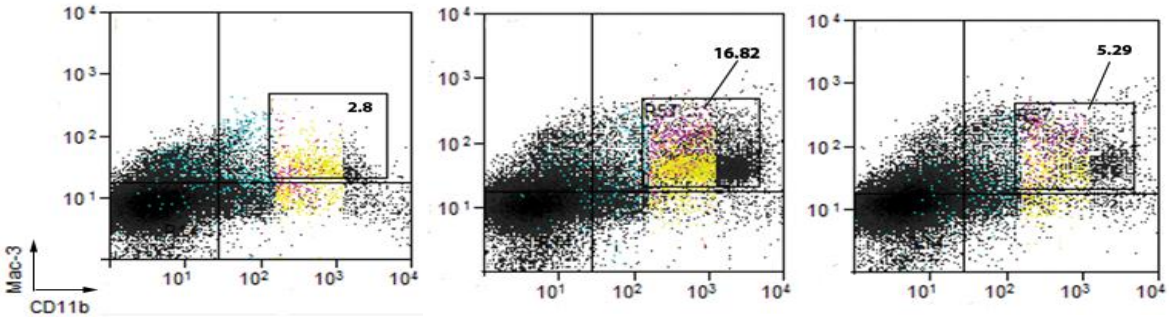


Figure 4.17: Propofol anesthesia decreases Mac-3^{+}/CD11b^{int} cells by 3-fold. Animals were infected and spleens harvested as described in Figure 4.16. Representative data from 2 independent experiments with 4 animals per treatment group per experiment.

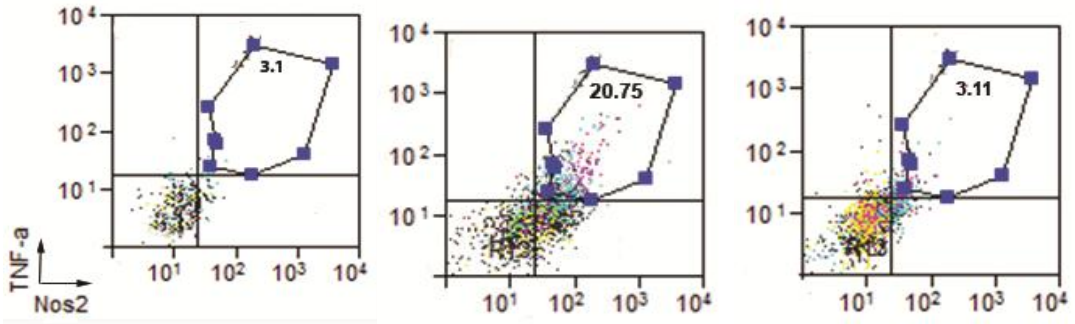


Figure 4.18: Propofol treatment significantly decreases presence of differentiated TipDCs at late times post-infection. Mice were left uninfected or intravenously infected with $2x10^4$ CFU of LM in the presence or absence of propofol and sacrificed 64h post-infection. Spleens were harvested and processed for flow cytometry. Cells were gated on the $CD11b^+CD11c^+$ population, and subsequently gated on TNF-α^+Nos2^+ cells. Left: naïve mice, middle: vehicle treated, LM infected mice, right: propofol-treated, LM infected mice.

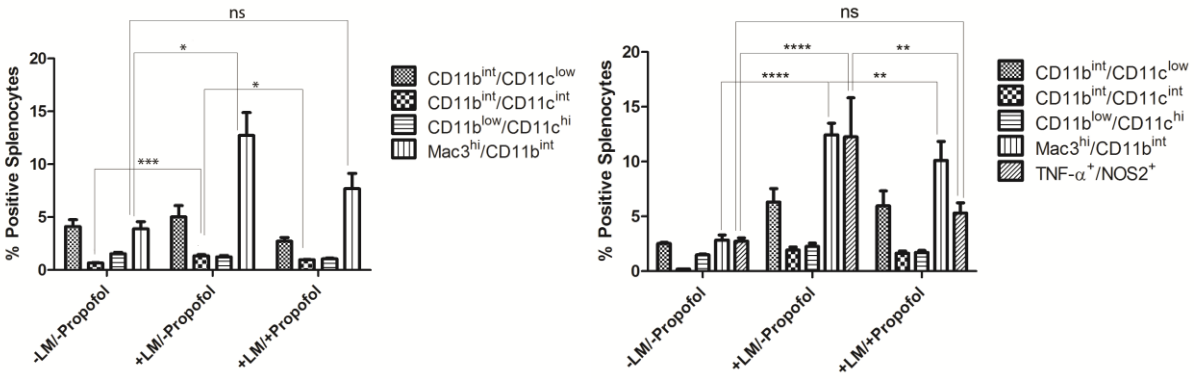


Figure 4.19: Propofol decreases the presence of TipDCs early and late in LM infection. Mice were infected and sacrificed as described in Figure 4.18. Propofol decreased TipDC precursor populations 48h post-infection (left), and significantly lowered functional TipDCs 64h post-infection (right). * $p<0.05$, *** $p<0.0005$, ns: not significant.

4.4 Discussion

There have been a number of *in vitro* studies focused on how anesthesia in general and propofol in particular may affect various aspects of immunity, including macrophage phagocytosis and generation of an oxidative burst [32,35-38,44,47]. Reports have often been conflicting as to whether propofol treatment was protective or detrimental to immune function[38,42,52,193]. Our *in vivo* results (chapter 3) showed that propofol dramatically increased host susceptibility to systemic LM infection, but the mechanism was unclear. Here, it was shown that propofol treatment in conjuction with LM infection causes a number of alterations in innate immune signaling and effector functions. Propofol treatment induced necrosis and fibrosis in livers and spleens of LM infected animals, which was significantly lessened in control animals. Anesthetized animals also displayed altered patterns of serum cytokine and chemokine expression throughout the course of infection. Particularly striking were the differences in pro- and anti-inflammatory cytokine levels late in infection in control animals and propofol-treated hosts (Figures 4.6-4.11). Though exposure to propofol did not prevent inflammatory $CCR2^+$ monocyte recruitment to the spleen (4.12 & 4.13), anesthetized animals displayed fewer differentiated macrophages, TGF-β^+ cells, and TipDCs in the spleen compared to infected controls (4.14, 4.16-4.19). These studies show that brief anesthesia with propofol in conjunction with LM infection increases host susceptibility to infection through altering cytokine and chemokine expression patterns, as well as potentially affecting immune cell differentiation.

Organ Pathology

Hematoxylin and eosin (H&E) staining of liver and spleen specimens from infected animals showed that propofol treatment greatly increased the severity of organ pathology (Figures 4.1 & 4.2). Liver sections from anesthetized animals contained significantly larger

numbers of inflammatory foci and areas of necrosis, where no cell nuclei were visible, compared with LM infected controls (Figure 4.1). As LM replication increases in the liver, inflammatory cells such as monocytes and granulocytes are recruited in large numbers and form inflammatory foci[194]. Propofol treatment resulted in ~4-fold greater numbers of inflammatory foci in the liver compared with infected control animals (Figure 4.1), which corresponded with higher average bacterial burdens (Figure 3.11). The increased bacterial replication also resulted in large areas of necrosis (Figure 4.1). Interestingly, anesthetized animals also accumulated fatty vacuoles interspersed in the inflammatory foci (Figure 4.1, right panel), which is indicative of hepatic metabolic disruption and is a symptom of very severe listeriosis[194]. This pathological manifestation was absent in control animals (Figure 4.1, right), as well as in animals exposed to propofol alone (Figure 4.3).

The spleen is another target organ for LM, and bacteria are localized in the spleen shortly after IV infection[101]. Following infection, inflammatory monocytes and neutrophils are rapidly recruited to the spleen[195]. Though inflammatory cells were readily apparent in spleens of control infected animals, the separation between red pulp and white pulp was maintained (Figure 4.2, left panel). In contrast, propofol anesthesia in conjunction with LM infection caused dissolution of spleen structure (Figure 4.2, right panel): boundaries between red pulp and white pulp were not maintained, and additionally there were large areas of necrosis and fibrosis. The increase in necrosis could have been a function of increased bacterial replication in propofol-treated animals. However, the observed increases in fibrosis are less easily explained. Elevated TGF-β levels are associated with generation of fibroid deposits in organs following chronic inflammatory stimulation[196], but propofol-treated animals actually had lower levels of TGF-β in

the serum as well as the spleen (Figure 4.15). It may be that other as yet unidentified alterations in growth factor signaling are responsible for the increase in fibroids.

Serum cytokine analysis

Quantitation of serum levels of cytokines and chemokines in infected animals allows for characterization of the immune status of the host. Proinflammatory cytokines IL-1β, IL-6, tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) were examined in this study and are crucial for host clearance of LM[103]. IL-1β is responsible for proinflammatory cellular responses such as increasing cell-surface expression of adhesion molecules and elevating NO synthesis, along with physiological manifestations of infection such as fever, vasodilation, and lowered pain threshold[197]. Together with the induction of chemokine expression, the upregulation of cell-surface adhesins allows for the extravasation of inflammatory cells from circulation into infected tissues[197]. Propofol treatment caused serum levels of IL-1β to become elevated compared to infected controls at late times post-infection, but IL-1β levels in anesthetized mice were slightly lower than controls at 24h after LM infection (Figure 4.6). It is possible that the early disparity in IL-1β levels in drug-treated animals could affect the generation of a productive inflammatory response later in infection.

IL-6 is produced by macrophages upon LM infection and is important for generating the oxidative burst in neutrophils, among other functions[198]. Studies using mice deficient for IL-6 have shown that in order to effectively control bacterial replication at early times in LM infection, IL-6 dependent stimulation of neutrophils is essential[199]. Though some studies have proposed that neutrophils are nonessential for controlling LM infection[131], other studies have shown that neutrophils are crucial for immune defense against LM in the liver, but not the spleen[189,200]. Propofol treatment causes IL-6 levels to spike at 72h post-infection, while IL-6

levels in control animals are relatively stable throughout infection (Figure 4.6). Additionally, propofol caused elevation of the neutrophil chemoattractant KC (or CXCL1) at all time points examined (Figure 4.9). It could be that propofol together with LM infection causes IL-6 levels to increase late in infection in an attempt to stimulate additional proinflammatory responses, such as increased neutrophil recruitment to sites of infection, in a terminal attempt to control infection.

TNF-α is a proinflammatory cytokine with pleiotropic effects on immune cell activation and effector functions. This cytokine is crucial to host defense against listeriosis, as some reports suggest the incidence of invasive LM infection increase significantly in patients receiving anti-TNF-α antibody therapy[201] and in animal models of LM infection[202]. TNF-α is produced by macrophages and TipDCs in response to LM infection, and serves as a proinflammatory signaling molecule that primes NK cells to release IFN-γ, a key cytokine that induces macrophage activation[198]. TNF-α together with NO produced by TipDCs also serves to activate alloreactive T cells and prime T_h1-type immune responses[113]. Interestingly, propofol treatment actually increased levels of TNF-α in sera of mice between 48 and 72h post-infection in comparison to controls (Figure 4.7), but this was not enough to increase host resistance to bacterial replication. This may be because serum titers of TNF-α were actually slightly lower in anesthetized animals than in infected controls at early times post-infection (Figure 4.7), perhaps suggesting that high TNF-α levels are necessary early during infection to effectively control LM replication.

IFN-γ secreted by NK cells and T cells is crucial for activating antilisterial macrophage effector functions[109,114,169,186,198]. After LM invades resting macrophages, TNF-α is secreted and activates resting T cells and NK cells to produce and secrete IFN-γ[186]. IFN-γ in turn activates

resting macrophages to produce iNOS, increase antigen presentation on MHC molecules, and escalate cytokine and chemokine secretion that attract inflammatory cells to sites of

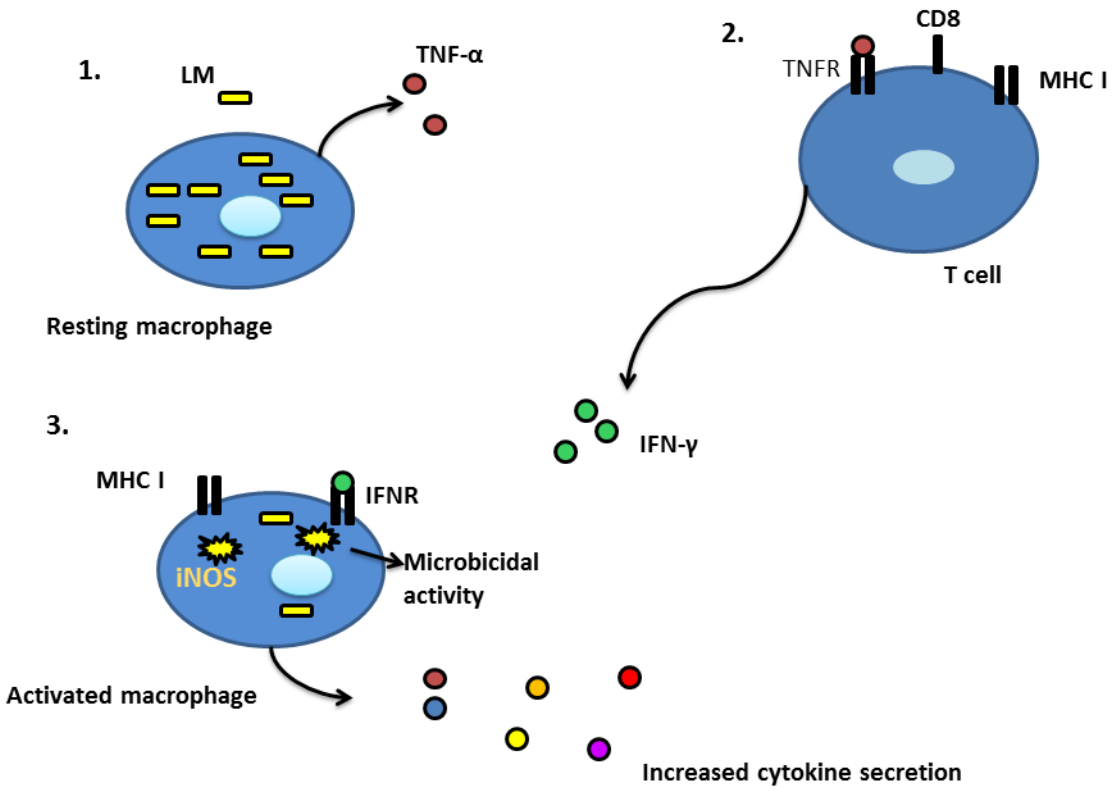


Figure 4.20: IFN-γ- induced macrophage activation during LM infection.

infection[186,203,204] (Figure 4.20). Some in vitro studies have indicated that propofol exposure induces IFN-γ production in NK cell culture in the absence of infection[41]. This may be responsible for the elevated levels of IFN-γ in the sera of propofol-treated animals at 24h post-infection with LM (Figure 4.10). However, drug treatment reduced IFN-γ levels rapidly between 24h and 72h post-infection, in spite of the fact that LM replication in target organs steadily increased in that time (Figure 3.11). Propofol also altered the pattern of expression of IFN-γ in control mice, IFN-γ levels peaked at 48h before returning to baseline at 72h, in contrast to drug-treated animals where IFN-γ peaked at 24h. It is possible that propofol inhibits IFN-γ secretion from T cells and NK cells at later times in infection, thus increasing host susceptibility to LM.

Monocyte recruitment/differentiation

Macrophages play a pivotal role in limiting LM infections[112,105,131], and high serum expression of the monocyte chemoattractant protein MCP-1 has been associated with defects in the recruitment of monocytes to sites of infection, and increased host susceptibility to LM [105,112,131,170]. This is because chemokines operate by establishing a gradient for inflammatory cells to follow in order to migrate to sites of infection[205], and when chemokine levels become too elevated in the blood, this gradient is abolished. While propofol exposure was found to increase serum titers of MCP-1 to levels similar to those associated with monocyte recruitment defects (Figure 4.9), there was no appreciable difference in the numbers of monocytes recruited to the spleen at either 24 or 72 hours following LM infection in the presence or absence of drug treatment (Figures 4.12 & 4.13). Instead, propofol exposure significantly reduced the number of differentiated F4/80^{+} macrophages at sites of infection (Figures 4.13 & 4.14). Additionally, some studies have shown that mice lacking the receptor for MCP-1 (CCR2) on monocytes are highly susceptible to LM because inflammatory monocytes are unable to reach sites of

infection[104]. Interestingly, propofol treatment recapitulated this phenotype in spite of intact trafficking of $CCR2^+$ monocytes, perhaps indicating that exposure to propofol blocks macrophage cell differentiation and/or induces cell death.

Another effector cell population critical in host resistance to *L.monocytogenes* infection is the TNF and iNOS producing dendritic cell (TipDC). Only described fairly recently[104,105,108,179,206], TipDCs are important in signaling: they prime T cells for proliferation and IFN-γ production in a TNF-α and iNOS-dependent manner[207], and are thus crucial for macrophage activation. Classical dendritic cells (DCs) display their effector functions (including antigen presentation) and morphology at all times, in both the steady state as well as during inflammation[207]. In contrast, TipDCs only differentiate in response to inflammation, and are derived from $CCR2^+$ inflammatory monocytes recruited to sites of infection[107,208]. While conventional dendritic cells can be derived from both lymphoid and myeloid precursors, TipDCs can only be derived from inflammatory monocytes whose immediate precursors are only stem cells of myeloid origin[208]. Exposure to propfol caused a precipitous drop in TipDC maturation (Figures 4.16-4.19). Interestingly, differentiation of monocytes into TipDCs is dependent on the presence of IFN-γ[207], which propofol caused to drop significantly from 24h to 72h post-infection with *LM* (Figure 4.10). Propofol thus may block differentiation of $CCR2^+$ inflammatory monocytes into two types of mature effector cells crucial to controlling LM infection: macrophages and TipDCs. In the case of TipDC differentiation, propofol may disrupt this process in an IFN-γ-dependent manner.

IL-10 and TGF-β

Another notable effect of propofol exposure was the reduction in TGF- β^+ cells associated with infected spleens (Figure 4.15), as well as a reduction in serum cytokine levels of

active TGF-β late in infection (Figure 4.11). High levels of TGF-β have been suggested to be beneficial for LM immune clearance[209], and recent findings have indicated that, far from being purely anti-inflammatory, TGF-β can skew the inflammatory response based on local signals[210-212]. The 10-fold reduction in TGF-β^+cells observed in spleens of propofol-treated animals may further exacerbate immune defects associated with the reduction in mature macrophages and TipDCs[209,212].

Intriguingly, though levels of TGF-β were lower in the presence of propofol at 72h (Figures 4.11 & 4.15), IL-10 serum levels spiked between 48h and 72h post-infection in anesthetized animals (Figure 4.8). IL-10 acts on many different immune cell types to suppress inflammation, and deficiencies in IL-10 have been associated with unregulated inflammatory responses[213]. Many studies have shown that activation of the TGF-β pathway leads to transcriptional upregulation of many genes required for anti-inflammatory responses, including IL-10[214,215]. However, IL-10 is also known to be produced by endogenous T regulatory cells (T_{reg})[216], and this is not always dependent on induction of TGF-β[217,218]. It is possible that the elevated IL-10 in the sera of propofol-treated mice is produced by endogenous T_{regs}. Alternatively, toll-like receptor ligands such as LTA from gram-positive bacteria have been shown to induce non-classical activation of macrophages into regulatory macrophages that secrete IL-10 in response to inflammation[219]. Perhaps propofol induces increased differentiation of regulatory macrophages, preventing a productive proinflammatory response.

Our studies suggest that propofol treatment may skew the immune response to induce an immunocompromised state in the host during infection. It is evident that the proinflammatory responses necessary for successful host clearance of LM infection are altered through propofol administration. Serum cytokine levels indicate altered patterns of secretion in the presence of

propofol, and far fewer inflammatory cells crucial for immune defense against LM are present at sites of infection. Together, these results suggest that propofol alters immune signaling through a variety of mechanisms, and further studies must be conducted to fully understand the complex molecular crosstalk behind these phenomena.

CHAPTER FIVE

Effects of propofol treatment on infections caused by additional pathogens: *Streptococcus pyogenes, Salmonella enterica* serovar Typhimurium, and *Staphylococcus aureus*

5.1 Summary

Thus far, propofol has been shown to suppress host immunity in the context of infection with *LM*. This chapter aims to characterize the effect of propofol on other pathogens, occupying both intracellular and extracellular host replication niches, that activate different arms of the immune response. Brief anesthesia with propofol was found to increase the susceptibility of mice to systemic infection with methicillin-resistant *Staphylococcus aureus* (MRSA). Propofol-treated animals infected with *S. aureus* via the tail vein were more likely to exhibit gross inflammation of skin at the base of the tail, had greater numbers of abscesses and larger areas of inflammation in the kidney, and exhibited higher bacterial burdens in the liver, spleen, and kidney than non-drug treated animals. Preliminary studies also indicated that propofol-treated animals were more susceptible to infection with *Streptococcus pyogenes* and *Salmonella enterica* serovar Typhimurium, as indicated by increased frequency of tail vein lesions with *S. pyogenes* and by significantly increased bacterial burdens in the livers and spleens of *S.* Typhimurium infected animals. These studies indicate that propofol increases host susceptibility to a variety of infectious agents with replication niches extending from outside host cells to intracellular vacuoles as well as the cytosol. These findings suggest that propofol affects host immunity in ways that are broadly relevant to a variety of infectious agents.

5.2 Introduction

Propofol has been shown to increase susceptibility of hosts to systemic infection with LM (Chapter 3) and to decrease the number of macrophages and TipDCs present at sites of infection (Chapter 4). However, in order to determine if propofol predisposes hosts to infection in general or only to infection with LM, infection with alternate human pathogens was examined. The three pathogens examined in the studies below occupy different replication niches in infected hosts and allow for further study on how propofol affects host susceptibility to infection.

Salmonella enterica serovar Typhimurium is an intracellular gram-negative pathogen that can cause severe systemic infection in mice similar to typhoid fevers caused by *Salmonella enterica* serovoar Typhi in humans[220,221]. Animal models for *S.* Typhimurium infection include intragastric inoculation as well as intraperitoneal injection to establish systemic infection[222-224]. Infectious doses vary greatly in each model, with oral infection usually requiring between 10^7 and 10^9 CFU of *S.* Typhimurium to establish infection[222,223]. However, *S.* Typhimurium is highly virulent in mice when inoculated systemically and doses required to establish infection intraperitoneally are commonly around 1-500 CFU[224,225]. Interestingly, in published studies mice are rarely infected intravenously with *S.* Typhimurium when examining systemic infection. This may be because intraperitoneal injection is inherently easier to perform on a mouse due to the larger surface area of the peritoneum compared with the tail vein, but it does not represent a physiologically relevant mode of entry for the pathogen.

S. Typhimurium uses secreted effectors to form a *Salmonella*-containing vacuole as a replication niche within infected host cells, including macrophages. As *S.* Typhimurium is an intracellular pathogen, it is subject to a number of the same immune defense mechanisms as *LM*, including iNOS-mediated intraphagosomal killing[226,227] and activation of T_H1-type immune

responses induced by IFN-γ and TNF-α signaling[228]. However, upstream TLR-dependent immune activation differs between the two pathogens; LM as a gram-positive pathogen mostly activates TLR-2[229], while the gram-negative *S.* Typhimurium primarily activates TLR-4[230]. In spite of the fact that *LM* and *S.* Typhimurium occupy different intracellular niches, they activate similar NF-κB-dependent transcriptional profiles during infection[71,113,230], and require different components of the immune response to effectively clear infection.

Streptococcus pyogenes is an extracellular gram-positive pathogen that is commonly found colonizing the skin and oropharynx[231], but can cause infection in immunocompromised hosts. Infections can range from mild (impetigo) to more serious invasive manifestations (toxic shock syndrome, necrotizing fasciitis)[232]. Necrotizing fasciitis in particular is rare, and is characterized by skin necrosis caused by invasive *S. pyogenes*[233]. Animal models for *S. pyogenes* infection vary between establishing skin infections and more systemic infection through different inoculation routes. Skin infections are induced in hairless mice or by removing fur at the area of inoculation, and 10^7-10^8 CFU of *S. pyogenes* are injected subdermally. Infection is allowed to progress for a number of weeks, and lesion size is measured at various time intervals[234,235]. Systemic infection is established through subcutaneous, intraperitoneal and intravenous routes of inoculation. Infectious doses commonly range from 10^7-10^8 CFU per mouse, and animals are sacrificed 2-4 days after infection, due to the induction of a strong cytokine response leading to early lethality[120,236,237].

Invasive disease is accomplished by the coordinated action of many virulence factors. Transcriptional upregulation of multiple toxins allow *S. pyogenes* to transition from local to systemic infection, and a number of these toxins interfere with neutrophil-mediated killing[238]. As *S. pyogenes* is an extracellular pathogen, neutrophils and the corresponding chemokine

associated with neutrophil recruitment (IL-8) are critical for clearance of infection[232,239]. Additionally, increased production of TGF-β leading to induction of T regulatory cells and the induction of T_H17 immune responses are important for preventing cytokine storm associated with toxic shock syndrome caused by serious *S. pyogenes* infections[240,241].

Staphylococcus aureus is a gram positive coccus that is the causative agent of a broad spectrum of diseases[242]. Methicillin-resistant *Staphylococcus aureus* (MRSA), a common multidrug resistant variant of *S. aureus*, is a major nosocomial pathogen in hospitals around the world[119,243,244]. MRSA commonly presents as skin and soft tissue infections as well as invasive systemic infections which result in more deaths annually than any other single infectious agent in the US[245,246]. *S. aureus* is resistant to many environmental stressors, including drying and high salt concentrations, enabling it to successfully colonize the skin and nasal mucosa[244]. In susceptible hosts, however, *S. aureus* is very prone to causing infections, of which MRSA infections are especially hard to treat due to their resistance to many antibiotics. Owing to their place as common infectious agents in hospitals and in the community, significant effort has been put into the development of animal models for *S. aureus*-related diseases. Similar to *S. pyogenes*, animal models for *S. aureus* infection include those investigating skin infection, systemic disease, and sepsis through the induction of toxic shock syndrome (TSS)[247-249]. Skin infections are established through subcutaneous or subdermal injection of ~10^7 CFU per mouse, while systemic infection commonly requires intraperitoneal or retro-orbital injection of 10^7-10^8 CFU[247,250]. The lethal dose for Balb/C mice, which are slightly more susceptible to infection than the Swiss Webster mice used in the studies in this thesis, is $2x10^8$ CFU per animal[248]. *S. aureus* frequently targets the kidney as a preferred site of replication, and in mouse models of systemic infection, the measurement of abscess formation is a readout for the severity of

infection[251]. Innate immune defense against *S. aureus* is primarily mediated by neutrophils that directly kill bacteria at sites of infection[242,252] and dendritic cells that act to coordinate innate and adaptive immune responses[253].

We have used both intracellular and extracellular, gram-positive and gram-negative pathogens in oral and intravenous infection models, and demonstrate that exposure to propofol decreases host resistance to infection with multiple pathogens that occupy different host niches.

5.3 Results

Propofol treatment increases host susceptibility to MRSA infection

One of the most common strains causing infection outside hospitals is the community acquired methicillin resistant *S. aureus* (CA-MRSA) strain USA300[119]. This strain was used in IV infections in mice and the effects of propofol anesthesia were examined. Mice were infected with 1-3x10^6 CFU of USA300 in the presence or absence of propofol anesthesia, and sacrificed 7 days post-infection. Drug-treated mice were 100- to 1000-fold more susceptible to systemic infection with USA300 (Figure 5.4). Interestingly, though the USA300 strain of *S. aureus* does not usually target the liver or spleen as preferred replication niches (F. Alonzo, private communication), propofol treatment significantly increased the number of viable bacteria isolated from these organs (Figure 5.1). Additionally, although the preferred infectious dose for reproducible systemic *S. aureus* infection in many studies is ~10^7 CFU inoculated intravenously per mouse[247,250], a 10-fold lower dose was sufficient to establish infection in anesthetized animals. Propofol-treated animals also displayed larger areas of inflammation and abscess formation in the kidney and no distinct boundary between the kidney cortex and medulla (Figures 5.2 & 5.3),

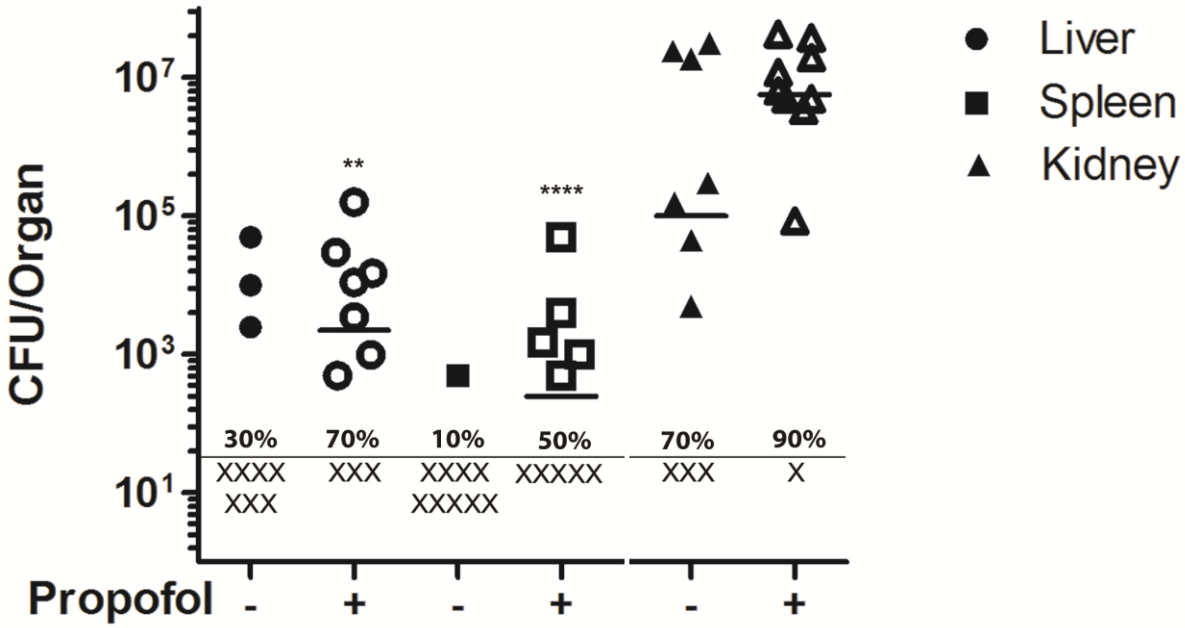


Figure 5.1: Propofol treatment increases severity of MRSA infection. Mice were intravenously infected with 1-3x10^6 CFU of the USA300 strain of MRSA. Animals were euthanized at 7 days post-infection and organs were processed for enumeration of viable bacteria. Percentages indicate proportion of infected animals within each group. ** $p<0.005$, **** $p<0.0001$, X: undetectable bacterial burden.

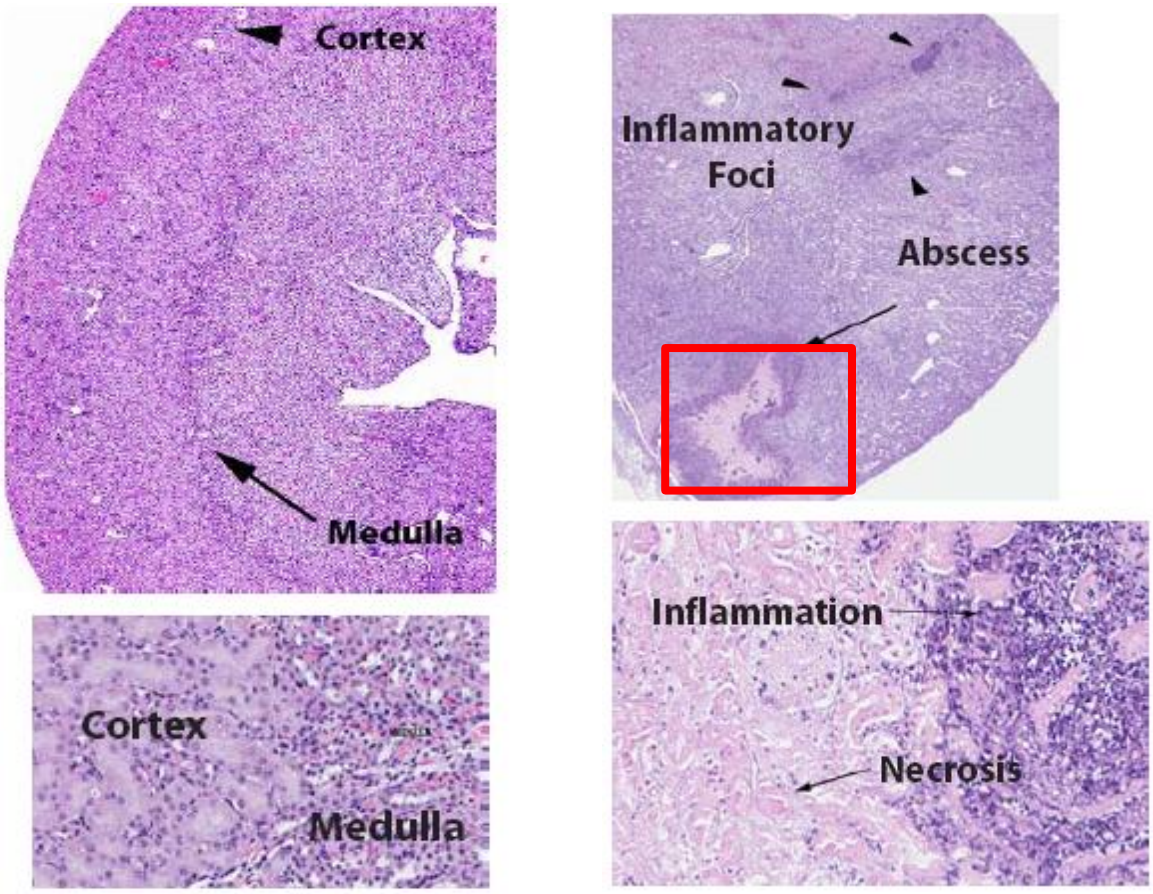


Figure 5.2: Propofol increases area of inflammation and abscess formation in the kidneys of MRSA-infected mice. Animals were intravenously infected 1-3x10^6 CFU of the USA300 strain of *S. aureus*. Animals were sacrificed 7 days post-infection, and kidneys were isolated, fixed, and processed for hematoxylin and eosin (H&E) staining. Propofol treatment induced more severe inflammation, loss of kidney compartmentalization between the cortex and medulla, and abscess formation. Left: images mouse infected with MRSA without propofol. Right: images from 2 mice infected with MRSA and anesthetized with propofol. Top: 2x magnification; bottom: 20x magnification.

Treatment	Avg. area of inflammation (μm^2) / section	Avg. % inflammation + necrosis/ section
(-) Propofol	$6.4x10^6$	8.4±6.9
(+) Propofol	$2.0x10^7$	35.6±12.33

Figure 5.3: Propofol anesthesia increases areas of kidney inflammation and abscess formation in MRSA-infected mice. Mice were infected and kidneys harvested and processed as indicated in Figure 5.2. Areas of inflammation and abscess formation in the kidney were quantitated. Propofol treatment significantly increased the percentage of the kidney section with inflammatory lesions or abscess formation. Levels of inflammation expressed as percentage of section ± std. error. Average taken from 5-6 animals per group.

indicating that propofol treatment makes the environment of various organs more conducive to bacterial replication.

Propofol anesthesia increases severity of systemic salmonellosis

S. Typhimurium is an enteric pathogen, and patients often contract infection through ingestion of contaminated food products[254]. For this reason, as well as to compare the effects of oral infection with *S.* Typhimurium with LM, mice were orally infected with $5x10^6$ CFU of wild-type *S.* Typhimurium in the presence or absence of propofol anesthesia and sacrificed 72h post-infection. Anesthetized mice were found to have significantly higher numbers of *S.* Typhimurium in their livers, spleens, and intestines, and all of the animals were infected compared with only ~20% of infected controls (Figure 5.4). This experiment has only been performed once, however, and must be repeated to verify these results.

Oral infection with *S.* Typhimurium indicated that propofol increased the severity of infection, but, similar to the experiments with LM, it was unclear if this was due to effects of propofol on intestinal barrier permeability or on host immune responses. To determine if propofol anesthesia increases host susceptibility to systemic infection with *S.*Typhimurium, 6-8 week old female Swiss Webster mice were intravenously infected with 1000 CFU of *S.* Typhimurium and sacrificed 6 days post-infection. This timepoint was chosen because prior to this, the animals did not appear very ill. Salmonellosis progresses more slowly than listeriosis in mice[222], therefore sacrificing animals at 72h or 96h post-infection was not informative. Preliminary results indicated that propofol increased host susceptibility to infection by approximately 100-fold in the livers and spleens of drug-treated animals in comparison to Intralipid-treated controls (Figure 5.5). Interestingly, one animal in the drug-treated group succumbed to infection by the 6^{th} day after infection, while there were no fatalities in the vehicle- treated group.

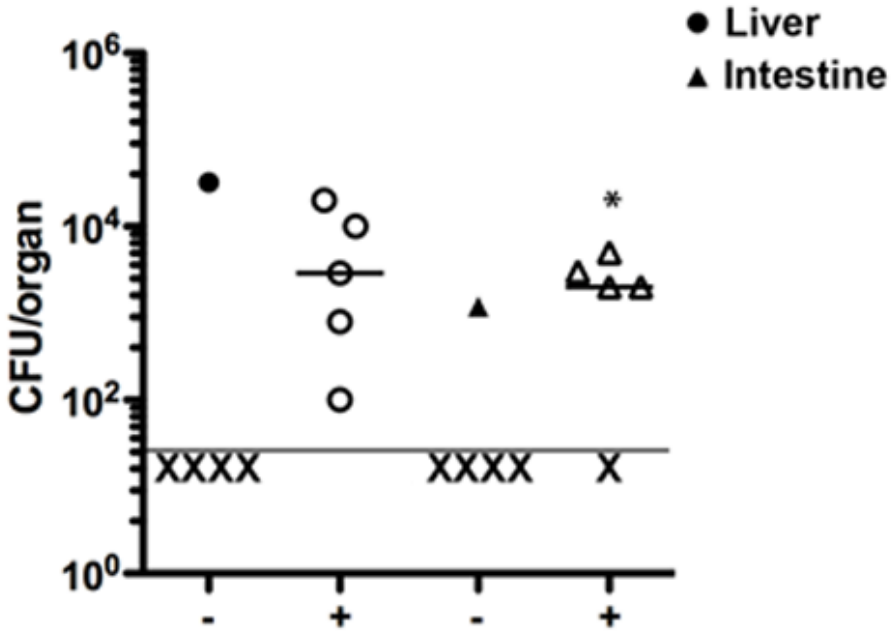


Figure 5.4: Propofol anesthesia increases host susceptibility to oral infection with *S.* Typhimurium. Animals were infected with $5x10^6$ CFU of *S.* Typhimurium and sacrificed 6 days post-infection. Livers and intestines were harvested, homogenized, and plated for viable CFU. *** $p<0.0005$, * $p<0.05$.

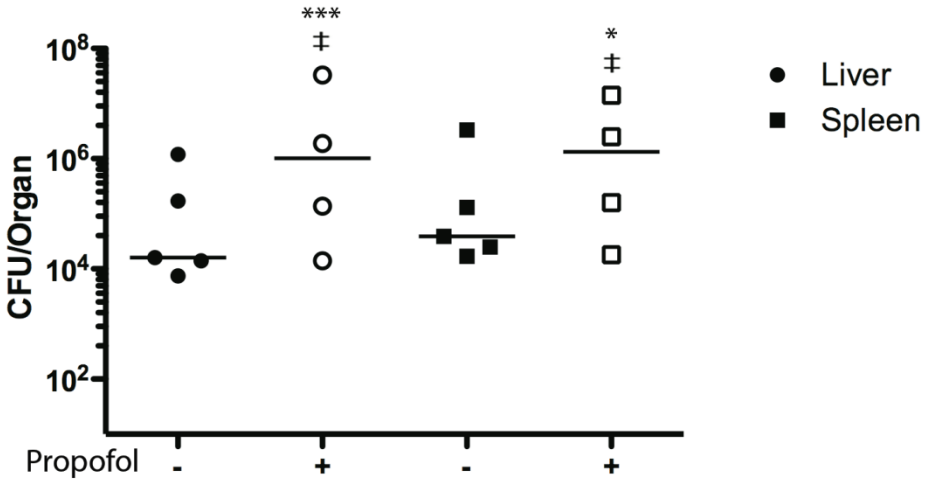


Figure 5.5: Propofol anesthesia decreases host resistance to IV *S.* Typhimurium infection. Mice were intravenously infected with 1000 CFU of a kanamycin-resistant strain of *S.* Typhimurium. Animals were euthanized at 6 days post-infection, and livers and spleens isolated and processed for isolation of viable bacteria. * $p<0.05$, ***$p<0.0005$, ‡ dead animal.

Additionally, there were sizeable variations in bacterial burdens among the individual animals given propofol (Figure 5.5), suggesting that optimization of infectious dosing and/or time course of infection is required.

Anesthetized animals display increased frequency of necrotic lesions in *S. pyogenes* infection

Our initial experiments with *S. pyogenes* attempted to isolate viable CFU from target organs after intravenous infection to compare the effects of propofol anesthesia on disease progression. However, *S. pyogenes* did not survive for very long in PBS solution (approximately 10 minutes), and although the intravenous infectious dose was intended to be 10^7 CFU per mouse, the actual dose was closer to 10^4 CFU. When organs were harvested, homogenized, and plated, most did not contain any viable CFU of *S. pyogenes* regardless of propofol treatment. This is probably due to the extremely low infectious dose that was actually used for inoculation. The only differences that were visible between anesthetized and control infected animals were in the presence of necrotic lesions at the tail vein injection site. One manifestation of severe *S. pyogenes* infection is necrotizing fasciitis, which is characterized by skin inflammation and tissue necrosis involving both skin and often underlying muscle tissue[233]. Systemic infection with a clinical isolate of *S. pyogenes* led to increased presence of injection-site necrosis in propofol-treated animals. Mice were intravenously infected with 10^4 CFU of *S. pyogenes* and animals were sacrificed at 12 days post-infection. Pictures of infected animals were taken at both 6 days and 9 days post-infection. 6 of 10 animals anesthetized with propofol displayed necrosis-type symptoms at the injection site and/or the base of the tail, becoming progressively worse as the infection proceeded (Figure 5.6). Inflammation and necrosis was present in 2 of 10 infected animals given vehicle, and most did not show overt signs of pathology in the tail (Figure 5.7).

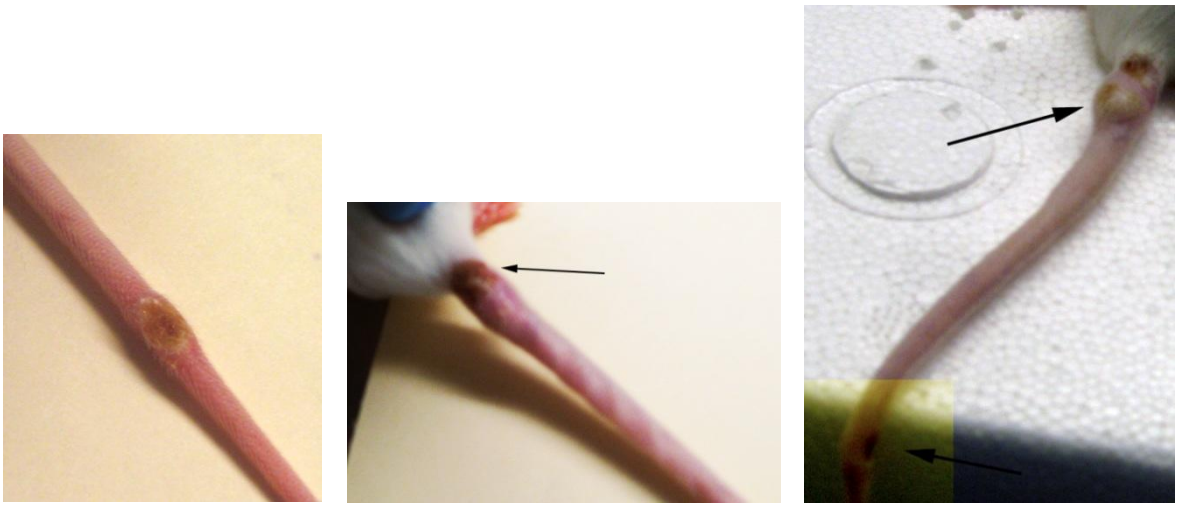


Figure 5.6: Propofol increases presence of necrotic lesions in *S. pyogenes* infected mice. Adolescent female Swiss Webster mice were infected IV with 10^6 CFU of the clinical isolate MGAS5005. Images were taken 6 (left), 9 (middle) or 12 (right) days post-infection.

Figure 5.7: In the absence of propofol, injection site necrosis is less frequent. Mice were given Intralipid vehicle and infected with 10^6 CFU of MGAS5005. Animals were sacrificed at 12 days-post infection. Picture of representative animal at 9 days post-infection.

5.4 Discussion

Previous experiments have shown that propofol treatment increases host susceptibility to bloodstream infection with LM (Chapter 3), potentially through altering cytokine secretion patterns and preventing differentiation of cells crucial to immune defense against microbes (Chapter 4). However, questions remained as to whether propofol treatment ubiquitously increased the severity of microbial infections in hosts, or if the effect was specific to LM. LM represents one class of pathogen: gram-positive, intracytosolic pathogens that require macrophages primarily for innate immune clearance[103]. The experiments conducted in this chapter aimed to investigate whether propofol anesthesia decreases host resistance to other classes of pathogens, including common nosocomial pathogens.

S. aureus, *S. pyogenes*, and *S.* Typhimurium initiate infection in susceptible hosts through the coordinated action of different virulence factors, and due to the fact they occupy different host niches, they activate different innate immune pathways. The following is a discussion on how propofol anesthesia may disrupt specific aspects of host immune defense against these particular pathogens.

Staphylococcus aureus

Methicillin resistant *Staphylococcus aureus* is a common nosocomial pathogen responsible for a spectrum of infections with different levels of invasivity[119]. *S. aureus* is a gram-positive, extracellular pathogen that commonly establishes skin infections as well as more invasive bloodstream infections and necrotizing pneumonia[245,253]. In order to effectively combat infection, mobilization of neutrophils and dendritic cells are crucial. Neutrophils are professional phagocytes that are released into circulation from the bone marrow upon maturation, and migrate to sites of infection in response to chemotactic factors such as IL-8 and KC, or CXCL-1[255].

Once there, they phagocytose bacteria and expose them to a variety of microbicidal compounds, including proteolytic enzymes, defensins, and ROI/RNIs[242]. The increase in bacterial burdens associated with MRSA-infected animals exposed to propofol indicates that drug treatment enhances bacterial replication in target organs. Enhanced replication and increased efficiency of organ colonization was observed even in organs not generally targeted by the USA300 strain of *S. aureus* (F. Alonzo, private communication), such as the liver and spleen (Figure 5.1). A number of explanations could account for the observed increases in organ-associated bacterial burdens, based on the critical role of neutrophils in clearance of *S. aureus* infection. It was previously shown that propofol treatment enhanced serum cytokine levels of CXCL-1 (Figure 4.4). As recruitment of immune cells to sites of infection and inflammation depend on signaling via a chemokine gradient, it is possible that when levels of CXCL-1 reach a certain threshold, the gradient is abolished and thus neutrophils may traffic less efficiently. This is especially possible in the kidney, the primary replication niche for *S. aureus* once it enters the bloodstream. Additionally, the antioxidant properties of propofol may come into play once again with regards to neutrophil-mediated killing of *S. aureus*. Similar to *S. pyogenes*, it is possible that sequestration of ROI/RNIs within the lysosomal compartment of neutrophils or a blockage in NO-dependent immune signaling is partially responsible for the decreased host resistance to systemic *S. aureus* infection.

Another possible mechanism through which propofol may increase host susceptibility to *S. aureus* infection is through interference with neutrophil extracellular traps (NETs). NETs are extracellular structures derived from dying neutrophils made of chromatin with attached

neutrophilic granules that may contain microbicidal activity[256], though this is refuted by some studies[257]. NETs form a web-like structure surrounding their parent cells that can entrap

extracellular bacteria[256], and their formation is dependent on IL-8 (or CXCL-8) signaling[258]. *S. aureus*, however, expresses a nuclease that enables it to degrade NETs that may entrap the bacteria[259]. Propofol may impair the activity of NETs in two ways: first, propofol may block neutrophil recruitment to sites of infection by altering CXCL-1 expression patterns, thus impairing NET formation triggered by microbial products. Additionally, as propofol was shown to increase bacterial replication in target organs (Figure 5.1), the elevated numbers of *S. aureus* could potentially be producing larger amounts of the nuclease that degrades NETs than can be handled by the host immune system. All of these hypotheses warrant further study to fully appreciate the different means by which propofol may increase host susceptibility to *S. aureus* bloodstream infections.

***Salmonella* Typhimurium**

Salmonella enterica serovar Typhimurium causes infection in mice that resembles the typhoid fever caused by severe *S.* Typhi infection in humans[220], and its ability to be virulent in mice in extremely small doses[226] attests to the efficacy of its many immune evasion mechanisms. Upon infection of macrophages, *S.* Typhimurium increases transcription of genes that enable delivery of a number of effector proteins that rearrange the host cell's actin cytoskeleton to allow formation of the Salmonella-containing vacuole (SCV)[260]. By creating this special intracellular niche, *S.* Typhimurium avoids the toxic environment of the mature endo-lysosome, which contains high concentrations of reactive oxygen and nitrogen species, and evades microbial killing. Additionally, once *S.* Typhimurium invades host cells, it secretes gene products that prevent antigen presentation via MHC molecules on dendritic cells, thus disrupting coordination between innate and adaptive immune responses[261].

In spite of the many methods employed by *S.* Typhimurium to evade immune defenses, immunocompetent hosts readily clear infection[221]. One recently described mechanism by which macrophages resist *S.* Typhimurium infection is through activation of a pro-inflammatory, non-necrotic cell death pathway dependent on caspase-1, termed pyroptosis[262]. Apoptosis, or programmed cell death, is characterized by cytoplasmic and nuclear condensation and a preservation of an intact cell membrane[263]. Cellular contents are packaged in apoptotic bodies that contain exposed receptors targeting the cell for phagocytosis, and thus apoptotic cells are not inflammatory in vivo[263]. In contrast, due to the release of proinflammatory contents upon pyroptotic cell death, immune cells are recruited to sites of infection and the innate immune response is activated, allowing effective clearance of bacteria[262]. In the presence of propofol, however, *S.* Typhimurium seems to be more capable of replicating in the target organs of the liver and spleen. Though previous chapters have shown that propofol treatment does not affect monocyte recruitment to the spleen, exposure to propofol might affect either differentiation of macrophages or possibly their mode or frequency of cell death (Chapter 4), and these represent an important cell type for clearance of *S.* Typhimurium[228]. This indicates that though inflammatory precursor cells may be effectively recruited to sites of infection, they may not be able to differentiate into effector cells. Thus the same mechanisms co-opted by *LM* to increase the severity of infection in the presence of propofol could hold true in the case of systemic *S.* Typhimurium infection as well.

Streptococcus pyogenes

Streptococcus pyogenes causes both mild diseases such as strep throat and impetigo, as well as more serious clinical manifestations such as necrotizing fasciitis by transitioning from a dormant state where it colonizes mucosal surfaces to invading tissues and causing disease[264]. A

number of transcriptional regulators that respond to environmental signals coordinate the expression of virulence genes, enabling *S. pyogenes* to first colonize host tissues, persist within those tissues, and finally spread to other organs[265]. At each of these stages, *S. pyogenes* is confronted by localized and then systemic host immune responses. *S. pyogenes* is an extracellular pathogen, and in the case of skin infection, autophagy by non-immune cells exposes *S. pyogenes* to intracellular immune defenses and aids in host clearance of infection[256,266].

Autophagy is the degradation of cytoplasmic components by eukaryotic cells, and is accomplished by sequestering a portion of cytoplasm into an intracellular autophagosome, after which autophagosomal contents are degraded through fusion with the lysosome[267]. Once *S. pyogenes* invades the cytosol of nonphagocytic cells, bacteria are rapidly trapped in autophagosomes and killed through autophagosome-lysosome fusion by exposure to toxic reactive oxygen and nitrogen species[266]. As autophagosomal machinery is commonly induced in epithelial cells upon establishment of *S. pyogenes* skin infections[268], it is possible that intereference with autophagy or lysosomal degradation could exacerbate infection. Propofol has been shown in a variety of studies to decrease transcription of iNOS and act as an antioxidant by sequestering reactive oxygen and nitrogen intermediates (ROIs/RNSs)[39,46,64]. Anesthesia with propofol may act to dampen down the effects of toxic ROI and RNI species through its antioxidant functions, decrease autophagosomal killing of *S. pyogenes*, and thus exacerbate the necrotizing faciitis seen in mice (Figure 5.3).

Additionally, NO is a potent immune signaling molecule, and propofol treatment decreased the presence of TipDCs that produce iNOS in LM-infected animals (Figure 4.18). TipDCs have mainly been examined in the context of LM and influenza infections[107,179,269], both

of which are intracellular pathogens. Further studies are needed to determine whether or not TipDCs are important to clearance of extracellular microbes as well.

Finally, NETs formed by dying neutrophils are important for clearance of *S. pyogenes* as well as *S. aureus*. However, similar to *S. aureus*, *S. pyogenes* produces a number of DNases that help it evade killing by NETs. For example, SpnA, a cell wall-anchored DNase, promotes *S. pyogenes* survival after subcutaneous inoculation of mice[270] and in the whole blood samples from humans[271] through dissolution of NETs. Thus these virulence factors are expressed during both cutaneous and systemic infection with *S.* pyogenes. Propofol may act to increase bacterial replication at the injection site as evidenced by larger areas of skin necrosis (Figures 5.6 & 5.7), which in turn would upregulate the expression of DNases and other proteins that quickly act to dissolve NETs. Propofol may also again disrupt neutrophil recruitment to sites of infection, effectively preventing sufficient NET formation.

The preliminary studies conducted within this chapter indicate that propfol's effect on increasing susceptibility of mice to infection is not limited to LM and thus has global implications for modulating host immunity. *S.* Typhimurium, *S. pyogenes*, and *S. aureus* represent pathogens that occupy very different replication niches and induce very different immune responses from each other, yet infection with all three pathogens is aggravated in the presence of propofol. The examination of cytokine and chemokine secretion patterns in the context of infection with these pathogens may elucidate common immunomodulatory mechanisms employed by propofol to decrease host resistance to infection. Further studies are needed in order to precisely determine the mechanisms by which propofol aggravates the phenotypes of these infections.

CHAPTER SIX

Conclusions and plans for future studies

Microbial infections are serious complications in hospital settings, and patients undergoing surgery or who are in the ICU for extended periods of time are more likely to be exposed to infectious agents. One overlooked variable in preventing infection risk in patients may be the anesthetic drug used to sedate them. Propofol is a commonly used anesthetic in surgery and the ICU[22,58,150,159,272,273], and is the primary drug investigated in the preceding studies. The work presented in this thesis serves to provide an in-depth understanding of how propofol anesthesia increases susceptibility to bacterial infection in immunocompetent hosts. Through *in vivo* studies assessing bacterial burdens, serum cytokine levels, immunophenotyping via flow cytometry, and histopathology of affected organs, it is shown that brief periods of propofol anesthesia cause a transient immunocompromised state in the host.

Effects of propofol on oral LM infection

Studies by Czuprynski[37] showing that sodium pentobarbital anesthesia increased the susceptibility of mice to oral infection with LM formed the basis for many of our original experiments. Initial studies examined how propofol anesthesia, which is commonly used in hospitals[58,150], influenced the progression of listeriosis in the host. Using an oral model of LM infection, it was shown that brief anesthesia with propfol increased host susceptibility to infection by an average of 10,000-fold, similar to levels reported by Czuprynski for infections in association with sodium pentobarbital[37] (Figures 3.1-3.4). Further experiments suggested that propofol increased the severity of oral infection with LM in a manner independent of the interaction of the LM internalin A protein interacting with E-cadherin on intestinal epithelial

cells (3.5-3.8, 3.10). However, our studies utilizing an oral infection model of LM did not allow us to differentiate between the effects of propofol on increasing intestinal barrier permeability and directly influencing immune clearance of bacteria. In order to address this, further studies were performed using an IV model of infection where bacteria were injected directly into the bloodstream, eliminating translocation across the intestinal barrier.

Effects of propofol on systemic LM infection

Upon establishment of systemic IV infection, propofol was found to prevent host clearance of LM from target organs (Figure 3.11), although fully virulent strains were required for propofol to increase the severity of infection (Figure 3.12). Propofol exacerbated LM infection as determined 3-4 days after IV inoculation, even though sedation itself only lasted for ~5 minutes. Therefore, questions remained as to how long after administration propofol could continue to affect host susceptibility to infection. Additional IV infection studies confirmed that propofol prevented host clearance of LM from target organs for infections initiated up to 4 days post-sedation (Figure 3.17), indicating that propofol impairs the ability of the host to clear infection long after recovery from sedation. This is particularly important in patients anesthetized with propofol and recovering from surgery, as recovery can often last for days. Throughout this time, patients are exposed to a variety of pathogens present in hospital settings, and though no longer sedated, they may be more susceptible to contracting infection due to exposure to propofol.

Alternate anesthetics and infection susceptibility

One interesting observation in Czuprynski's studies on sodium pentobarbital anesthesia was that he could not reproduce the increase in severity of infection observed in the presence of pentobarbital after oral inoculation when he instead injected LM directly into the bloodstream[37].

A caveat to this observation was that pentobarbital anesthesia was induced in mice through intraperitoneal injection, which may not allow as many tissues to be exposed to the drug as intravenous delivery[274]. For this reason, as well as to study the effects of pentobarbital using a more physiologically relevant drug delivery system, IV infection studies with LM in this thesis were carried out in the presence of IV pentobarbital sedation. Surprisingly, pentobarbital did not increase the susceptibility of mice to LM when inoculated IV but did increase susceptibility following intragastric infection (Figures 3.19 & 3.20), in agreement with Czuprynski's results and suggesting that pentobarbital acts to increase intestinal permeability to bacteria while not affecting host clearance of bacteria from tissues. Furthermore, though they bind to the same GABA-A receptor in the CNS, only propofol and not pentobarbital prevented host clearance of bacteria from target organs in an IV model of LM infection (Figure 3.20). These studies demonstrated two key results: the first being that pentobarbital mediates an increase in severity of oral LM infection primarily through affecting intestinal barrier permeability, and second, that propofol's inhibition of LM clearance is not apparently mediated through the GABA-A receptor (Figures 3.20-3.22) . Further studies are warranted to identify different host target(s) propofol may be acting on to affect the host immune response.

Modification of immune responses by propofol

Histological analysis of LM target organs indicated greater inflammatory cell recruitment as well as increased pathological alterations in organ structure when infected animals were exposed to propofol (Figures 4.1-4.4). This led to studies examining whether propofol modifies the expression patterns of key cytokines and chemokines in the sera of LM infected animals, and it was shown that drug exposure did indeed alter serum levels of these immune signaling molecules (Figures 4.6-4.11). An emerging pattern in these results was that many

proinflammatory cytokines and chemokines were elevated in propofol-treated animals relative to infected controls at later times post-infection. This suggested that propofol anesthesia allowed the immunological environment of the host to be more amenable to bacterial replication, and the elevated levels of proinflammatory mediators endeavored to compensate for this in a terminal attempt to control infection. However, the elevated levels of proinflammatory signaling molecules were accompanied by a simultaneous increase in serum levels of the anti-inflammatory IL-10 (Figure 4.8), which may actually further prevent host clearance of LM from target organs.

MCP-1 and inflammatory monocyte recruitment and differentiation

The monocyte chemoattractant MCP-1 was found to be elevated in the serum at all times during infection in anesthetized animals when compared to infected controls (Figure 4.9). Though some studies have suggested that high MCP-1 levels serve to decrease inflammatory monocyte recruitment to sites of inflammation, thus worsening the progression of infection[170], this was not the case in the presence of propofol (Figures 4.12 & 4.13). Instead, propofol treatment decreased the presence of mature macrophages and TNF- and iNOS-producing dendritic cells (TipDCs) at sites of infection (Figures 4.14, 4.16-4.18). Since some mature macrophages and all TipDCs differentiate from inflammatory monocytes, and the spleens of anesthetized animals infected with LM were ~40% smaller on average than infected controls (Figure 4.4), it is possible that propofol treatment blocks differentiation of inflammatory monocytes into effector cells important for clearance of LM from target organs. Additionally, propofol may induce cell death of mature macrophages and TipDCs due to blockage of their differentiation programs or due to an as yet unidentified mechanism. Further studies must be

conducted to fully understand how propofol decreases the presence of inflammatory effector cells present at sites of LM infection.

Propofol and infection with alternate pathogens

Studies conducted with LM suggested that propofol increased susceptibility to listeriosis by interfering with productive immune responses (Chapters 3&4). However, LM is an intracellular pathogen that activates a specific program of innate immune defense that relies heavily on macrophages and TipDCs for effective clearance of infection[103,104,275]. To explore whether propofol increased host susceptibility to bacterial infections other than with LM, it was necessary to conduct experiments with alternate pathogens.

Staphylococcus aureus* and *Streptococcus pyogenes

When animals were anesthetized with propofol and infected with the methicillin-resistant USA300 strain of *S. aureus*, it was found that drug exposure increased the severity of infection (Figures 5.1-5.3). Anesthetized animals displayed higher bacterial burdens in livers, spleens, and kidneys (Figure 5.1), and propofol treatment also resulted in more serious organ pathology in infected kidneys (Figure 5.2 & 5.3). While control animals displayed discrete foci of inflammation, the underlying structure of the kidney was preserved and there was a distinct separation between the cortex and medulla of the kidney (Figure 5.2). In contrast, the kidneys of propofol-treated animals had larger areas of inflammation, as well as necrotic abscess formation in some cases (Figures 5.3 & 5.4). Additionally, the structural integrity of the kidney was disrupted and there was no longer any separation between the cortex and medulla due to spreading inflammation (Figure 5.3). These results, combined with findings using an *S. pyogenes* infection model indicating larger areas of necrosis in propofol-treated animals (Figures 5.7 & 5.8), suggest that propofol can increase host susceptibility to gram-positive pathogens

other than LM. Interestingly, *S. aureus* and *S. pyogenes* are both extracellular pathogens, and clearance of these pathogens relies largely on neutrophil-mediated microbicidal activity[239,242,259,276] and not macrophages. This implies that propofol may modify neutrophil-dependent immune responses in the context of infections that particularly depend on this cell type for immune defense. Data showing that propofol interferes with expression patterns of the neutrophil chemoattractant KC during LM infection (Figure 4.9) points toward a role for drug exposure in impeding neutrophil trafficking to sites of inflammation.

***Salmonella enterica* serovar Typhimurium**

S. Typhimurium shares many similarities with LM in that it is an intracellular pathogen, and macrophages are an essential cell type for effective bacterial clearance[74,262]. However, its occupation of a slightly different intracellular replication niche (vacuole vs. cytosol for LM), combined with the fact that it is a gram-negative pathogen offers the opportunity to study how propofol may impede host clearance of *S.* Typhimurium in a slightly different manner than during LM infection. Propofol anesthesia prior to *S.* Typhimurium infection resulted in significantly increased bacterial burdens *in vivo* after both oral and IV inoculation (Figures 5.5 & 5.6). This showed that, similar to LM infection, propofol affected both permeability of the intestinal barrier to bacteria as well as directly interfered with host clearance of bacteria from target organs during *S.* Typhimurium infection. These studies were preliminary, and must be optimized and repeated. Further studies must be conducted to determine if, as is the case during LM infection, propofol's potential interference with the differentiation of macrophages and TipDCs are involved in increasing host susceptibility to salmonellosis.

The studies in this thesis have shown that propofol influences the host immune response in a manner that allows for uncontrolled bacterial replication in the case of LM infection (Figure

6.1). Further, the ability of propofol to increase host susceptibility to infection is not confined to infection with one pathogen, but are present during infection with various classes of pathogens

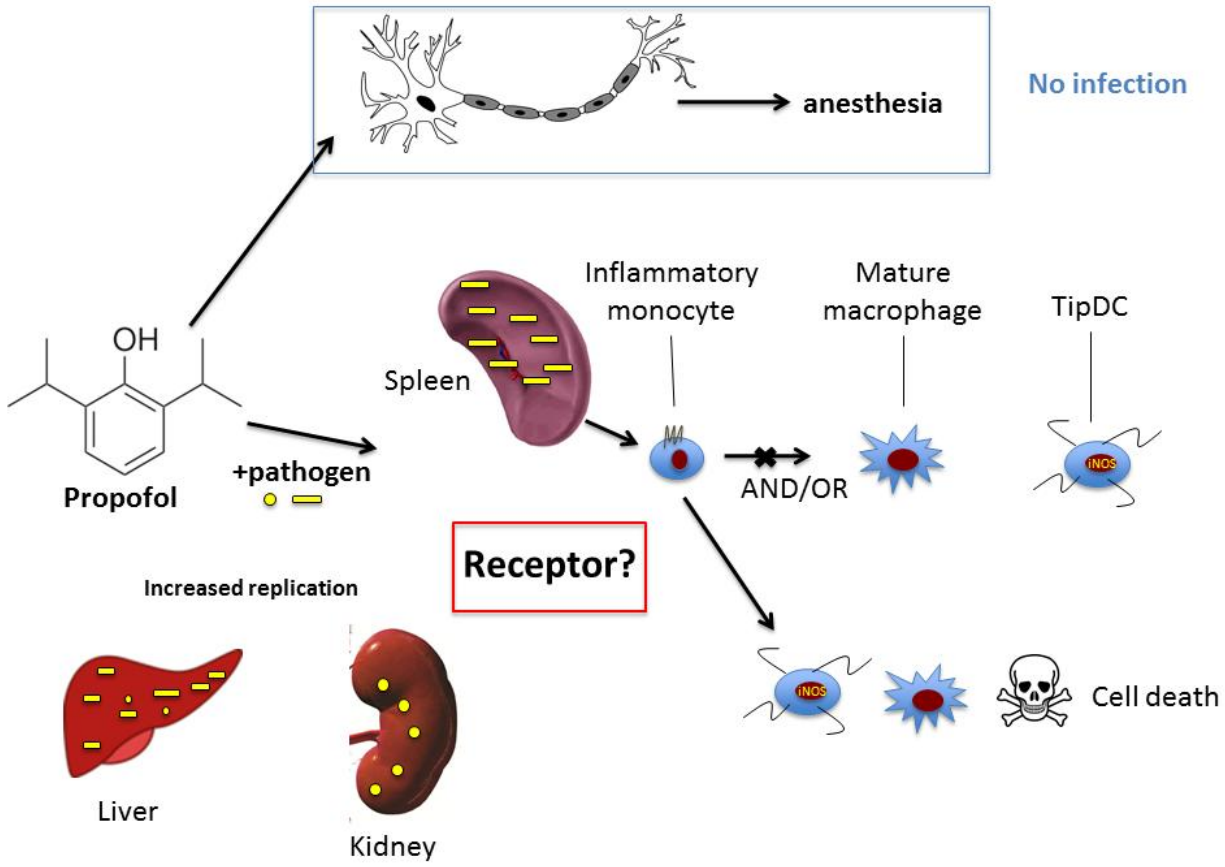


Figure 6.1: Model. Model of the effects of propofol on host susceptibility to microbial infection. Propofol binds to the GABA-A receptor on neurons to mediate anesthesia. Based on the ability of propofol to modulate host immunity up to 7 days post-sedation, it is possible that immunomodulatory metabolites persist within the liver. Upon infection, propofol increases bacterial translocation across physiological barriers and inhibits bacterial clearance. Propofol treatment does not inhibit the recruitment of inflammatory monocytes to sites of infection, although drug exposure reduces differentiated macrophages and TipDCs present in spleens. Thus, propofol may inhibit monocyte differentiation into macrophages and TipDCs and/or induce cell death. While the receptor that induces anesthesia is known (GABA-A), the receptor(s) that modulate immunity as a result of propofol exposure is not known but may act independently of GABA-A receptors.

that activate different arms of the innate immune response and occupy different replication niches in the host. Future studies will focus on understanding how propofol increases susceptibility to *S. aureus, S. pyogenes,* and *S.* Typhiumurium infections through affecting the immune response. Additionally, further investigations must be conducted to probe how propofol influences immunity at a molecular level. Does propofol act to disrupt differentiation programs in inflammatory monocytes? If so, how is this accomplished? Descriptions of future experiments to answer these questions will be discussed in the next section.

Future studies

This section will briefly describe experimental approaches to understanding how propofol modifies immune responses in the context of infection, with both LM as well as other pathogens.

Studies with alternate pathogens

The preliminary studies outlined in Chapter 5 lead to many questions regarding the specific mechanisms by which propofol increases host susceptibility to infection with *S. aureus, S. pyogenes,* and *S.* Typhimurium. The next steps will be to characterize the effect of propofol on the innate immune response to each of these three pathogens. In order to do this, experimental approaches similar to those employed with LM will be used.

S. aureus

Future studies with *S. aureus* could assess how propofol modifies cytokine and chemokine expression during infection through the use of Bioplex assays (described in *Materials and Methods*, Chapter 2). Additional experiments may be undertaken to determine if there is a defect in neutrophil recruitment to *S. aureus*-infected tissues, and if TipDCs are important in host clearance of infection. To do this, flow cytometry will be performed on *S. aureus*-infected

kidneys from animals given propofol or Intralipid vehicle solution. Innate immune cells of lymphoid origin, such as NK cells and conventional dendritic cells, are also known to be important in killing *S. aureus* directly and in priming adaptive immune responses[277-279]. Thus, future experiments could also involve probing whether or not propofol affects the presence of these cell types at sites of infection, as well as using flow cytometry to investigate the activation states of NK cells present in the kidneys of infected animals given propofol.

S. pyogenes

Prior to moving forward with characterizing how propofol increases host susceptibility to *S. pyogenes* infection, our animal model must be optimized to ensure reproducible systemic infection of mice in the presence or absence of propofol anesthesia. Once animals are reliably infected, *S. pyogenes* target organs of the liver, spleen, and lung can be harvested and processed for bacterial burden enumeration. If, as anticipated, there is evidence of increased host susceptibility to *S. pyogenes* infection in the presence of propofol, serum cytokine and chemokine levels could be measured and flow cytometry performed to look at the same targets as in *S. aureus* infection (described above).

S. pyogenes is often the etiologic agent of skin infections[237], and based on previous results indicating larger areas of injection site necrosis on the surface of the tails of anesthetized mice, it would be informative to find out if propofol affects the severity of *S. pyogenes* skin infections. These experiments could be carried out in hairless mice infected with a virulent clinical isolate of *S. pyogenes* (courtesy of Dr. Michael Federle). If propofol does aggravate skin infections with *S. pyogenes*, it may be an indicator that propofol increases host susceptibility to local as well as systemic infections.

S. Typhimurium

As mentioned in the discussion, *S.* Typhimurium has a virulence lifecycle with intriguing similarities to and differences from LM in that though both are intracellular pathogens, they occupy different intracellular host niches. Thus, many of the same experimental approaches used in our studies with LM are pertinent here. Experiments with *S.* Typhimurium could be conducted using both oral and IV infection models. First, the preliminary experiments in Chapter 5 (Figures 5.5 & 5.6) must be repeated to verify that propofol increases susceptibility to both oral and IV *S.* Typhimurium infection. Pending those results, future investigations could use Bioplex assays to assess whether propofol disrupts serum cytokine and chemokine expression patterns during both oral and IV *S.* Typhimurium infection. Comparisons could be made with the cytokine data obtained during LM infection to identify patterns in propofol-dependent disruption of cytokine expression. Additional studies could investigate if propofol interferes with immune cell recruitment to the intestinal epithelial barrier during oral *S.* Typhimurium infection through fluorescence microscopy of intestinal tissue, allowing further exploration into how propofol may increase barrier permeability. These experiments will be carried out in collaboration with Dr. Leo Lefrancois at the University of Connecticut. IV infection studies will center on immunophenotyping cells present in the liver and spleen after infection with *S.* Typhimurium in the presence or absence of propofol.

Mechanistic studies: propofol and immunomodulation

The studies presented in this thesis have broadly characterized how propofol modulates immune responses to make host organisms more susceptible to microbial infection. Future studies should be aimed at more deeply understanding the mechanisms by which propofol affects immunity.

Does propofol block cell differentiation or induce cell death?

Previous flow cytometry studies showed that propfol exposure decreased the presence of mature macrophages in the spleens of LM-infected animals, in spite of not affecting inflammatory monocyte recruitment (Figure 4.13). This led to the hypothesis that propofol may block the differentiation of inflammatory monocytes into mature macrophages and/or lead to cell death. In order to investigate the first part of the hypothesis, one potential experiment could attempt to differentiate primary murine bone marrow cells into macrophages *ex vivo* in the presence of propofol. If cells are incapable of differentiating into macrophages due to propofol exposure, this experiment could inform more detailed studies looking at specific differentiation markers through flow cytometry and western blotting techniques. To examine whether or not propofol anesthesia leads to cell death in the presence of LM, spleens of LM-infected animals will be isolated and stained with markers for apoptotic and necrotic cell death.

Does propofol decrease other immune cell populations during infection?

The flow cytometry studies conducted to this point have only looked at innate immune cells of myeloid origin (monocytes, macrophages, TipDCs). In order to more fully characterize the different populations of immune cells present at sites of infection upon propofol administration, flow cytometry could again be used to look at natural killer (NK) cells, NKT cells, and conventional dendritic cells that are derived from lymphoid progenitors. All of these cell types are known to play important roles in immune clearance of LM[106,180,198], and it would be interesting to see if propofol treatment alters the proportion of these cells at sites of inflammation.

It was also observed that propofol caused a late spike in serum levels of IL-10 (Figure 4.8), while simultaneously decreasing TGF-β in the serum (Figure 4.11) and the spleen (Figure

4.15). One possible reason for this is that IL-10 could be produced by endogenous T_{regs} in a TGF-β-independent manner[217,218]. To test this, a timecourse study could be performed to assess the kinetics of T_{reg} expansion and IL-10 secretion during infection, and if this persists even when TGF-β levels decrease due to propofol exposure. Other studies could use mice with conditional knockouts of the TGF-β receptor on T_{regs} to see if activation by this cytokine is in fact essential for expansion.

If not the GABA-A receptor, what is the immunological target of propofol?

One of the more interesting findings from the work presented in this thesis is that propofol induces anesthesia through binding to the GABA-A receptor, but it does not appear to be the receptor through which propofol influences host immunity (Figures 3.20-3.22). Ultimately, pharamacological manipulation of the GABA-A receptor in the context of LM infection is not sufficient to discount the role of GABA in mediating the phenotype seen during propofol administration. To address this, studies must be conducted in transgenic mice that are macrophage-specific GABA-A receptor knockouts. Through infection of these mice in the presence or absence of propofol, it can be definitively determined whether or not modulation of the GABA-A receptor is critical for propofol to increase host susceptibility to infection.

In the event that the GABA-A receptor is not involved in the propofol/infection phenotype, other studies must be conducted to identify how propofol increases susceptibility to infection on a molecular level. In order to identify host targets of propofol pertinent to immune modulation, multiple questions need to be addressed. Once injected into the bloodstream, propofol is quickly broken down into different metabolic species, many of which persist in fatty tissues for days[143,157]. Initial studies could determine what metabolic species of propofol are in the liver and other fatty tissues at 12h, 24h, and 96h post-sedation, and whether the proportion of

metabolites changes with infection. These studies would require collaboration with experts in pharmacokinetic studies. Depending on the localization of these primary metabolites in tissues, the next step will be to narrow down targets in a tissue specific manner.

Concluding remarks

This dissertation contains many novel findings describing the unanticipated effects of propofol anesthesia on increasing host susceptibility to infection. Patients in hospitals worldwide are commonly sedated with propofol, and the work presented above could significantly inform clinical practice. A deeper understanding of the consequences of anesthetizing patients with propofol could substantially decrease the incidence of nosocomial infections and patient mortality.

Propofol was found to perturb immune responses long after animals recover from sedation, but not necessarily through activation of the GABA-A receptor. Most pharmacological agents are thought to act through discrete receptor-ligand interactions, but the work in this dissertation shows that at least for propofol, this is not the case. This leads to many questions about the nature of how drugs actually work in the body- Are drug metabolites active at alternate sites from where the original drug binds? What properties of various drug molecules lend themselves to pleiotropic effects on different body systems? Is it possible to predict when the action of a drug will be limited to its identified target and when other targets are also involved? And finally, can studies be conducted that expose regulatory networks between drug metabolism and immunity?

This thesis is also a foundation from which to further study links between the nervous system and the immune system. The sedative state is limited to the nervous communication, but

it is not fully known how drugs that act on the nervous system can influence immune responses at disparate sites. Many neurotransmitters are known to influence immunity in artificial systems, but the neuro-immunological axis is less studied in the context of infection *in vivo*. This dissertation provides a model system and foundational studies upon which to build a larger narrative regarding metabolism, immunity, and CNS communication.

CITED LITERATURE

1. Dudeck, M.A., *et al.* National Healthcare Safety Network (NHSN) report, data summary for 2009, device-associated module. *American journal of infection control* **39**, 349-367 (2011).
2. Kirkland, K.B., Briggs, J.P., Trivette, S.L., Wilkinson, W.E. & Sexton, D.J. The impact of surgical-site infections in the 1990s: attributable mortality, excess length of hospitalization, and extra costs. *Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America* **20**, 725-730 (1999).
3. Danesh, J., Wong, Y., Ward, M. & Muir, J. Risk factors for coronary heart disease and persistent infection with Chlamydia pneumoniae or cytomegalovirus: a population-based study. *Journal of cardiovascular risk* **6**, 387-390 (1999).
4. Perencevich, E.N., *et al.* Health and economic impact of surgical site infections diagnosed after hospital discharge. *Emerging infectious diseases* **9**, 196-203 (2003).
5. Wong, E.S. The price of a surgical-site infection: more than just excess length of stay. *Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America* **20**, 722-724 (1999).
6. Holtz, T.H. & Wenzel, R.P. Postdischarge surveillance for nosocomial wound infection: a brief review and commentary. *American journal of infection control* **20**, 206-213 (1992).
7. Engels, E.A., *et al.* Early infection in bone marrow transplantation: quantitative study of clinical factors that affect risk. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **28**, 256-266 (1999).
8. Wong, B.C., *et al.* Differential Helicobacter pylori infection rates in two contrasting gastric cancer risk regions of South China. China Gastric Cancer Study Group. *Journal of gastroenterology and hepatology* **14**, 120-125 (1999).
9. Barnard, B. Fighting Surgical Site Infections. in *Infection Control Today* (Virgo Publishing, Phoenix, AZ, 2002).
10. Schwulst, S.J. & Mazuski, J.E. Surgical prophylaxis and other complication avoidance care bundles. *The Surgical clinics of North America* **92**, 285-305, ix (2012).
11. Clayton, J.L., Bazakas, A., Lee, C.N., Hultman, C.S. & Halvorson, E.G. Once is Not Enough: Withholding Postoperative Prophylactic Antibiotics in Prosthetic Breast Reconstruction is Associated with an Increased Risk of Infection. *Plastic and reconstructive surgery* (2012).
12. Karaman, E., Alimoglu, Y., Aygun, G., Kilic, E. & Yagiz, C. Effect of septoplasty and per-operative antibiotic prophylaxis on nasal flora. *B-Ent* **8**, 13-19 (2012).
13. Calcagnetti, D.J. & Holtzman, S.G. Intake of sweet water attenuates restraint-stress-induced potentiation of morphine analgesia in rats. *Brain research bulletin* **29**, 859-864 (1992).
14. Kornman, K.S., Newman, M.G., Holtzman, S. & Matheson, J.E. Field testing as clinical trial methodology in periodontics. *Journal of periodontology* **63**, 1064-1071 (1992).
15. Holtz, G. & Patton, G.W., Jr. Dual-image video endoscopy. *Journal of gynecologic surgery* **8**, 69-71 (1992).
16. Girard, T.D., *et al.* Efficacy and safety of a paired sedation and ventilator weaning protocol for mechanically ventilated patients in intensive care (Awakening and Breathing Controlled trial): a randomised controlled trial. *Lancet* **371**, 126-134 (2008).

17. Payen, J.F., *et al.* Current practices in sedation and analgesia for mechanically ventilated critically ill patients: a prospective multicenter patient-based study. *Anesthesiology* **106**, 687-695; quiz 891-682 (2007).
18. Dudeck MA, H.T., et. al. National Healthcare Safety Network (NHSN) Report, Data Summary for
2010, Device-associated Module. (CDC.gov, 2010).
19. Hackman, R.J., Holtzman, G.L. & Walter, P.E. Color vision testing for the U.S. Naval Academy. *Military medicine* **157**, 651-657 (1992).
20. Schifilliti, D., Grasso, G., Conti, A. & Fodale, V. Anaesthetic-related neuroprotection: intravenous or inhalational agents? *CNS drugs* **24**, 893-907 (2010).
21. Bein, B. Clinical application of the cardioprotective effects of volatile anaesthetics: PRO--get an extra benefit from a proven anaesthetic free of charge. *European journal of anaesthesiology* **28**, 620-622 (2011).
22. Ostermann, M.E., Keenan, S.P., Seiferling, R.A. & Sibbald, W.J. Sedation in the intensive care unit: a systematic review. *JAMA* **283**, 1451-1459 (2000).
23. Xu, Z., *et al.* Propofol-induced sleep: efficacy and safety in patients with refractory chronic primary insomnia. *Cell biochemistry and biophysics* **60**, 161-166 (2011).
24. Tanelian, D.L., Kosek, P., Mody, I. & MacIver, M.B. The role of the GABAA receptor/chloride channel complex in anesthesia. *Anesthesiology* **78**, 757-776 (1993).
25. Ebert, A., Haussleiter, I.S., Juckel, G., Brune, M. & Roser, P. Impaired facial emotion recognition in a ketamine model of psychosis. *Psychiatry research* (2012).
26. Reiner, G.N., Perillo, M.A. & Garcia, D.A. Effects of propofol and other GABAergic phenols on membrane molecular organization. *Colloids and surfaces. B, Biointerfaces* **101C**, 61-67 (2012).
27. Oakley, S., *et al.* Recognition of anesthetic barbiturates by a protein binding site: a high resolution structural analysis. *PloS one* **7**, e32070 (2012).
28. Lisman, J., Yasuda, R. & Raghavachari, S. Mechanisms of CaMKII action in long-term potentiation. *Nature reviews. Neuroscience* **13**, 169-182 (2012).
29. Bergman, S.A. Ketamine: review of its pharmacology and its use in pediatric anesthesia. *Anesthesia progress* **46**, 10-20 (1999).
30. Bloom, F.E. & Iversen, L.L. Localizing 3H-GABA in nerve terminals of rat cerebral cortex by electron microscopic autoradiography. *Nature* **229**, 628-630 (1971).
31. Vedula, L.S., *et al.* A unitary anesthetic binding site at high resolution. *The Journal of biological chemistry* **284**, 24176-24184 (2009).
32. Ben-Eliyahu, S., Shakhar, G., Rosenne, E., Levinson, Y. & Beilin, B. Hypothermia in barbiturate-anesthetized rats suppresses natural killer cell activity and compromises resistance to tumor metastasis: a role for adrenergic mechanisms. *Anesthesiology* **91**, 732-740 (1999).
33. Chang, H., Tsai, S.Y., Chang, Y., Chen, T.L. & Chen, R.M. Therapeutic concentrations of propofol protects mouse macrophages from nitric oxide-induced cell death and apoptosis. *Canadian journal of anaesthesia = Journal canadien d'anesthesie* **49**, 477-480 (2002).
34. Chen, R.M., *et al.* Propofol suppresses macrophage functions and modulates mitochondrial membrane potential and cellular adenosine triphosphate synthesis. *Anesthesiology* **98**, 1178-1185 (2003).

35. Chen, R.M., *et al.* Propofol reduces nitric oxide biosynthesis in lipopolysaccharide-activated macrophages by downregulating the expression of inducible nitric oxide synthase. *Archives of toxicology* **77**, 418-423 (2003).
36. Correa-Sales, C., Tosta, C.E. & Rizzo, L.V. The effects of anesthesia with thiopental on T lymphocyte responses to antigen and mitogens in vivo and in vitro. *International journal of immunopharmacology* **19**, 117-128 (1997).
37. Czuprynski, C.J., Faith, N.G., Steinberg, H. & Neudeck, B. Sodium pentobarbital anesthesia transiently enhances the severity of infection following intragastric, but not intravenous, inoculation of Listeria monocytogenes in mice. *Microbial pathogenesis* **35**, 81-86 (2003).
38. Heine, J., *et al.* Anaesthesia with propofol decreases FMLP-induced neutrophil respiratory burst but not phagocytosis compared with isoflurane. *British journal of anaesthesia* **85**, 424-430 (2000).
39. Hsing, C.H., *et al.* Anesthetic propofol reduces endotoxic inflammation by inhibiting reactive oxygen species-regulated Akt/IKKbeta/NF-kappaB signaling. *PloS one* **6**, e17598 (2011).
40. Humar, M., *et al.* Barbiturates directly inhibit the calmodulin/calcineurin complex: a novel mechanism of inhibition of nuclear factor of activated T cells. *Molecular pharmacology* **65**, 350-361 (2004).
41. Inada, T., Kubo, K. & Shingu, K. Promotion of interferon-gamma production by natural killer cells via suppression of murine peritoneal macrophage prostaglandin E production using intravenous anesthetic propofol. *International immunopharmacology* **10**, 1200-1208 (2010).
42. Inada, T., Kubo, K. & Shingu, K. Possible link between cyclooxygenase-inhibiting and antitumor properties of propofol. *Journal of anesthesia* **25**, 569-575 (2011).
43. Inada, T., Kubo, K., Ueshima, H. & Shingu, K. Intravenous anesthetic propofol suppresses prostaglandin E2 production in murine dendritic cells. *Journal of immunotoxicology* **8**, 359-366 (2011).
44. Inada, T., *et al.* Propofol depressed neutrophil hydrogen peroxide production more than midazolam, whereas adhesion molecule expression was minimally affected by both anesthetics in rats with abdominal sepsis. *Anesthesia and analgesia* **92**, 437-441 (2001).
45. Inada, T., *et al.* Effect of propofol and isoflurane anaesthesia on the immune response to surgery. *Anaesthesia* **59**, 954-959 (2004).
46. Kambara, T., Inada, T., Kubo, K. & Shingu, K. Propofol suppresses prostaglandin E(2) production in human peripheral monocytes. *Immunopharmacology and immunotoxicology* **31**, 117-126 (2009).
47. Li, Q., Zhang, L., Han, Y., Jiang, Z. & Wang, Q. Propofol reduces MMPs expression by inhibiting NF-kappaB activity in human MDA-MB-231 cells. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* (2011).
48. Ohta, N., Ohashi, Y. & Fujino, Y. Ketamine inhibits maturation of bone marrow-derived dendritic cells and priming of the Th1-type immune response. *Anesthesia and analgesia* **109**, 793-800 (2009).
49. Ploppa, A., *et al.* Dose-dependent influence of barbiturates but not of propofol on human leukocyte phagocytosis of viable Staphylococcus aureus. *Critical care medicine* **34**, 478-483 (2006).

50. Shiratsuchi, H., Kouatli, Y., Yu, G.X., Marsh, H.M. & Basson, M.D. Propofol inhibits pressure-stimulated macrophage phagocytosis via the GABAA receptor and dysregulation of p130cas phosphorylation. *American journal of physiology. Cell physiology* **296**, C1400-1410 (2009).
51. Tang, J., *et al.* Propofol inhibits the activation of p38 through up-regulating the expression of annexin A1 to exert its anti-inflammation effect. *PloS one* **6**, e27890 (2011).
52. Yu, G., *et al.* Propofol's effects on phagocytosis, proliferation, nitrate production, and cytokine secretion in pressure-stimulated microglial cells. *Surgery* **150**, 887-896 (2011).
53. Krumholz, W., *et al.* The effects of thiopentone, etomidate, ketamine and midazolam on several bactericidal functions of polymorphonuclear leucocytes in vitro. *European journal of anaesthesiology* **12**, 141-146 (1995).
54. Nishina, K., *et al.* The inhibitory effects of thiopental, midazolam, and ketamine on human neutrophil functions. *Anesthesia and analgesia* **86**, 159-165 (1998).
55. Humar, M., *et al.* Inhibition of activator protein 1 by barbiturates is mediated by differential effects on mitogen-activated protein kinases and the small G proteins ras and rac-1. *The Journal of pharmacology and experimental therapeutics* **311**, 1232-1240 (2004).
56. Karin, M., Liu, Z. & Zandi, E. AP-1 function and regulation. *Current opinion in cell biology* **9**, 240-246 (1997).
57. Saha, B., Jyothi Prasanna, S., Chandrasekar, B. & Nandi, D. Gene modulation and immunoregulatory roles of interferon gamma. *Cytokine* **50**, 1-14 (2010).
58. Miner, J.R. & Burton, J.H. Clinical practice advisory: Emergency department procedural sedation with propofol. *Annals of emergency medicine* **50**, 182-187, 187 e181 (2007).
59. Miner, J.R. & Krauss, B. Procedural sedation and analgesia research: state of the art. *Academic emergency medicine : official journal of the Society for Academic Emergency Medicine* **14**, 170-178 (2007).
60. Aouad, M.T., *et al.* A single dose of propofol at the end of surgery for the prevention of emergence agitation in children undergoing strabismus surgery during sevoflurane anesthesia. *Anesthesiology* **107**, 733-738 (2007).
61. Kim, Y.S., *et al.* A comparative study of emergence agitation between sevoflurane and propofol anesthesia in adults after closed reduction of nasal bone fracture. *Korean journal of anesthesiology* **63**, 48-53 (2012).
62. Kim, Y.H., Yoon, S.Z., Lim, H.J. & Yoon, S.M. Prophylactic use of midazolam or propofol at the end of surgery may reduce the incidence of emergence agitation after sevoflurane anaesthesia. *Anaesthesia and intensive care* **39**, 904-908 (2011).
63. Marik, P.E. Propofol: an immunomodulating agent. *Pharmacotherapy* **25**, 28S-33S (2005).
64. Chiu, W.T., Lin, Y.L., Chou, C.W. & Chen, R.M. Propofol inhibits lipoteichoic acid-induced iNOS gene expression in macrophages possibly through downregulation of toll-like receptor 2-mediated activation of Raf-MEK1/2-ERK1/2-IKK-NFkappaB. *Chemico-biological interactions* **181**, 430-439 (2009).
65. Gu, X., *et al.* Mechanisms mediating propofol protection of pulmonary epithelial cells against lipopolysaccharide-induced cell death. *Clinical and experimental pharmacology & physiology* **39**, 447-453 (2012).

66. Zhang, L., *et al.* Propofol induces proliferation and invasion of gallbladder cancer cells through activation of Nrf2. *Journal of experimental & clinical cancer research : CR* **31**, 66 (2012).
67. Hsing, C.H., Chou, W., Wang, J.J., Chen, H.W. & Yeh, C.H. Propofol increases bone morphogenetic protein-7 and decreases oxidative stress in sepsis-induced acute kidney injury. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **26**, 1162-1172 (2011).
68. Yeh, C.H., *et al.* Propofol inhibits lipopolysaccharide-induced lung epithelial cell injury by reducing hypoxia-inducible factor-1alpha expression. *British journal of anaesthesia* **106**, 590-599 (2011).
69. Ozkan, F., Senayli, Y., Ozyurt, H., Erkorkmaz, U. & Bostan, B. Antioxidant effects of propofol on tourniquet-induced ischemia-reperfusion injury: an experimental study. *The Journal of surgical research* **176**, 601-607 (2012).
70. Zwaferink, H., Stockinger, S., Reipert, S. & Decker, T. Stimulation of inducible nitric oxide synthase expression by beta interferon increases necrotic death of macrophages upon Listeria monocytogenes infection. *Infection and immunity* **76**, 1649-1656 (2008).
71. Henard, C.A. & Vazquez-Torres, A. Nitric oxide and salmonella pathogenesis. *Frontiers in microbiology* **2**, 84 (2011).
72. Shimada, K., *et al.* Caspase-1 dependent IL-1beta secretion is critical for host defense in a mouse model of Chlamydia pneumoniae lung infection. *PloS one* **6**, e21477 (2011).
73. Lewis, N.D., *et al.* Immune evasion by Helicobacter pylori is mediated by induction of macrophage arginase II. *J Immunol* **186**, 3632-3641 (2011).
74. Vazquez-Torres, A., *et al.* Analysis of nitric oxide-dependent antimicrobial actions in macrophages and mice. *Methods in enzymology* **437**, 521-538 (2008).
75. Carryn, S., Van de Velde, S., Van Bambeke, F., Mingeot-Leclercq, M.P. & Tulkens, P.M. Impairment of growth of Listeria monocytogenes in THP-1 macrophages by granulocyte macrophage colony-stimulating factor: release of tumor necrosis factor-alpha and nitric oxide. *The Journal of infectious diseases* **189**, 2101-2109 (2004).
76. Wang, W., *et al.* Interaction among nitric oxide, reactive oxygen species, and antioxidants during endotoxemia-related acute renal failure. *American journal of physiology. Renal physiology* **284**, F532-537 (2003).
77. Green, T.R., Bennett, S.R. & Nelson, V.M. Specificity and properties of propofol as an antioxidant free radical scavenger. *Toxicology and applied pharmacology* **129**, 163-169 (1994).
78. Kahraman, S. & Demiryurek, A.T. Propofol is a peroxynitrite scavenger. *Anesthesia and analgesia* **84**, 1127-1129 (1997).
79. Murphy, P.G., Myers, D.S., Davies, M.J., Webster, N.R. & Jones, J.G. The antioxidant potential of propofol (2,6-diisopropylphenol). *British journal of anaesthesia* **68**, 613-618 (1992).
80. Taniguchi, T., Kanakura, H., Takemoto, Y., Kidani, Y. & Yamamoto, K. Effects of ketamine and propofol on the ratio of interleukin-6 to interleukin-10 during endotoxemia in rats. *The Tohoku journal of experimental medicine* **200**, 85-92 (2003).
81. Brasier, A.R. The NF-kappaB regulatory network. *Cardiovascular toxicology* **6**, 111-130 (2006).

82. Pahl, H.L. Activators and target genes of Rel/NF-kappaB transcription factors. *Oncogene* **18**, 6853-6866 (1999).
83. Sun, Z. & Andersson, R. NF-kappaB activation and inhibition: a review. *Shock* **18**, 99-106 (2002).
84. Baldwin, A.S., Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annual review of immunology* **14**, 649-683 (1996).
85. Ghosh, S., May, M.J. & Kopp, E.B. NF-kappa B and Rel proteins: evolutionarily conserved mediators of immune responses. *Annual review of immunology* **16**, 225-260 (1998).
86. Vallabhapurapu, S. & Karin, M. Regulation and function of NF-kappaB transcription factors in the immune system. *Annual review of immunology* **27**, 693-733 (2009).
87. Song, X.M., *et al.* Effects of propofol on pro-inflammatory cytokines and nuclear factor kappaB during polymicrobial sepsis in rats. *Molecular biology reports* **36**, 2345-2351 (2009).
88. Li, Q., Zhang, L., Han, Y., Jiang, Z. & Wang, Q. Propofol reduces MMPs expression by inhibiting NF-kappaB activity in human MDA-MB-231 cells. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **66**, 52-56 (2012).
89. Wu, G.J., Chen, T.L., Chang, C.C. & Chen, R.M. Propofol suppresses tumor necrosis factor-alpha biosynthesis in lipopolysaccharide-stimulated macrophages possibly through downregulation of nuclear factor-kappa B-mediated toll-like receptor 4 gene expression. *Chemico-biological interactions* **180**, 465-471 (2009).
90. Jawan, B., *et al.* Propofol pretreatment attenuates LPS-induced granulocyte-macrophage colony-stimulating factor production in cultured hepatocytes by suppressing MAPK/ERK activity and NF-kappaB translocation. *Toxicology and applied pharmacology* **229**, 362-373 (2008).
91. Li, H., Tan, J., Zou, Z., Huang, C.G. & Shi, X.Y. Propofol post-conditioning protects against cardiomyocyte apoptosis in hypoxia/reoxygenation injury by suppressing nuclear factor-kappa B translocation via extracellular signal-regulated kinase mitogen-activated protein kinase pathway. *European journal of anaesthesiology* **28**, 525-534 (2011).
92. Xia, W.F., Liu, Y., Zhou, Q.S., Tang, Q.Z. & Zou, H.D. Protective effect of propofol and its relation to postoperation recovery in children undergoing cardiac surgery with cardiopulmonary bypass. *Pediatric cardiology* **32**, 940-946 (2011).
93. Xia, W.F., Liu, Y., Zhou, Q.S., Tang, Q.Z. & Zou, H.D. Comparison of the effects of propofol and midazolam on inflammation and oxidase stress in children with congenital heart disease undergoing cardiac surgery. *Yonsei medical journal* **52**, 326-332 (2011).
94. Olivier, V., Queen, J. & Satchell, K.J. Successful small intestine colonization of adult mice by Vibrio cholerae requires ketamine anesthesia and accessory toxins. *PloS one* **4**, e7352 (2009).
95. Czuprynski, C.J., Faith, N.G. & Steinberg, H. A/J mice are susceptible and C57BL/6 mice are resistant to Listeria monocytogenes infection by intragastric inoculation. *Infection and immunity* **71**, 682-689 (2003).
96. Freitag, N.E., Port, G.C. & Miner, M.D. Listeria monocytogenes - from saprophyte to intracellular pathogen. *Nature reviews. Microbiology* **7**, 623-628 (2009).
97. Tilney, L.G. & Portnoy, D.A. Actin filaments and the growth, movement, and spread of the intracellular bacterial parasite, Listeria monocytogenes. *The Journal of cell biology* **109**, 1597-1608 (1989).

98. Camejo, A., *et al.* The arsenal of virulence factors deployed by Listeria monocytogenes to promote its cell infection cycle. *Virulence* **2**, 379-394 (2011).
99. Freitag, N.E. From hot dogs to host cells: how the bacterial pathogen *Listeria monocytogenes* regulates virulence gene expression. *Future microbiology* **1**, 89-101 (2006).
100. Mostowy, S. & Cossart, P. Virulence factors that modulate the cell biology of Listeria infection and the host response. *Advances in immunology* **113**, 19-32 (2012).
101. Tilney, L.G., Connelly, P.S. & Portnoy, D.A. Actin filament nucleation by the bacterial pathogen, Listeria monocytogenes. *The Journal of cell biology* **111**, 2979-2988 (1990).
102. Williams, M.A., Schmidt, R.L. & Lenz, L.L. Early events regulating immunity and pathogenesis during Listeria monocytogenes infection. *Trends in immunology* (2012).
103. Pamer, E.G. Immune responses to Listeria monocytogenes. *Nature reviews. Immunology* **4**, 812-823 (2004).
104. Serbina, N.V., Salazar-Mather, T.P., Biron, C.A., Kuziel, W.A. & Pamer, E.G. TNF/iNOS-producing dendritic cells mediate innate immune defense against bacterial infection. *Immunity* **19**, 59-70 (2003).
105. Shi, C. & Pamer, E.G. Monocyte recruitment during infection and inflammation. *Nature reviews. Immunology* **11**, 762-774 (2011).
106. Naper, C., Shegarfi, H., Inngjerdingen, M. & Rolstad, B. The role of natural killer cells in the defense against Listeria monocytogenes lessons from a rat model. *Journal of innate immunity* **3**, 289-297 (2011).
107. Dominguez, P.M. & Ardavin, C. Differentiation and function of mouse monocyte-derived dendritic cells in steady state and inflammation. *Immunological reviews* **234**, 90-104 (2010).
108. Edelson, B.T. Dendritic cells in Listeria monocytogenes infection. *Advances in immunology* **113**, 33-49 (2012).
109. Shaughnessy, L.M. & Swanson, J.A. The role of the activated macrophage in clearing Listeria monocytogenes infection. *Frontiers in bioscience : a journal and virtual library* **12**, 2683-2692 (2007).
110. Park, J.B. Phagocytosis induces superoxide formation and apoptosis in macrophages. *Experimental & molecular medicine* **35**, 325-335 (2003).
111. Unanue, E.R. Inter-relationship among macrophages, natural killer cells and neutrophils in early stages of Listeria resistance. *Current opinion in immunology* **9**, 35-43 (1997).
112. Serbina, N.V., Jia, T., Hohl, T.M. & Pamer, E.G. Monocyte-mediated defense against microbial pathogens. *Annual review of immunology* **26**, 421-452 (2008).
113. Serbina, N.V., Shi, C. & Pamer, E.G. Monocyte-mediated immune defense against murine Listeria monocytogenes infection. *Advances in immunology* **113**, 119-134 (2012).
114. Jia, T., Leiner, I., Dorothee, G., Brandl, K. & Pamer, E.G. MyD88 and Type I interferon receptor-mediated chemokine induction and monocyte recruitment during Listeria monocytogenes infection. *J Immunol* **183**, 1271-1278 (2009).
115. Serbina, N.V., *et al.* Sequential MyD88-independent and -dependent activation of innate immune responses to intracellular bacterial infection. *Immunity* **19**, 891-901 (2003).
116. Bishop, D.K. & Hinrichs, D.J. Adoptive transfer of immunity to Listeria monocytogenes. The influence of in vitro stimulation on lymphocyte subset requirements. *J Immunol* **139**, 2005-2009 (1987).

117. Brundage, R.A., Smith, G.A., Camilli, A., Theriot, J.A. & Portnoy, D.A. Expression and phosphorylation of the Listeria monocytogenes ActA protein in mammalian cells. *Proceedings of the National Academy of Sciences of the United States of America* **90**, 11890-11894 (1993).
118. Wollert, T., *et al.* Extending the host range of Listeria monocytogenes by rational protein design. *Cell* **129**, 891-902 (2007).
119. Li, M., *et al.* Evolution of virulence in epidemic community-associated methicillin-resistant Staphylococcus aureus. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 5883-5888 (2009).
120. Kinkel, T.L. & McIver, K.S. CcpA-mediated repression of streptolysin S expression and virulence in the group A streptococcus. *Infection and immunity* **76**, 3451-3463 (2008).
121. Laboratories, A. MATERIAL SAFETY DATA SHEET- PROPOFLO. (Abbot Laboratories, Abbot Laboratories, North Chicago, IL, 2005).
122. Pharmaceuticals, J. MATERIAL SAFETY DATA SHEET- KETALAR. (ed. Pharmaceuticals, J.) (JHP Pharmaceuticals, Parsippany, NJ, 2011).
123. McCurnin, D., and JM Bassert. *Clinical Textbook for Veterinary Technicians, 5th ed.*, (Saunders, Philadelphia, PA, 2002).
124. Felzemburgh, V.A., Cettolin Qda, C., Machado, K.M. & Campos, J.H. Comparison between the anesthetic induction times in the first and second surgery in rabbits. *Acta cirurgica brasileira / Sociedade Brasileira para Desenvolvimento Pesquisa em Cirurgia* **27**, 482-486 (2012).
125. Pharmaceuticals, A. Nembutal® Sodium Solution (pentobarbital sodium injection, USP). (ed. Pharmaceuticals, A.) (Akorn Pharmaceuticals, Lake Forest, IL, 2012).
126. Ohki, K. & Nagayama, A. Establishment and characterization of factor-dependent macrophage cell lines. *Journal of leukocyte biology* **44**, 465-473 (1988).
127. Sun, A.N., Camilli, A. & Portnoy, D.A. Isolation of Listeria monocytogenes small-plaque mutants defective for intracellular growth and cell-to-cell spread. *Infection and immunity* **58**, 3770-3778 (1990).
128. Steinberg, T.H., Newman, A.S., Swanson, J.A. & Silverstein, S.C. Macrophages possess probenecid-inhibitable organic anion transporters that remove fluorescent dyes from the cytoplasmic matrix. *The Journal of cell biology* **105**, 2695-2702 (1987).
129. Bhatt, A.S., *et al.* Coordinate expression and functional profiling identify an extracellular proteolytic signaling pathway. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 5771-5776 (2007).
130. Alonzo, F., 3rd, McMullen, P.D. & Freitag, N.E. Actin polymerization drives septation of Listeria monocytogenes namA hydrolase mutants, demonstrating host correction of a bacterial defect. *Infection and immunity* **79**, 1458-1470 (2011).
131. Shi, C., *et al.* Ly6G+ neutrophils are dispensable for defense against systemic Listeria monocytogenes infection. *J Immunol* **187**, 5293-5298 (2011).
132. Edwards, J.R., *et al.* National Healthcare Safety Network (NHSN) report: data summary for 2006 through 2008, issued December 2009. *American journal of infection control* **37**, 783-805 (2009).
133. Venkatachalan, S.P. & Czajkowski, C. A structural link between the GABAA receptor's agonist binding site and inner beta-sheet governs channel activation and allosteric drug modulation. *The Journal of biological chemistry* (2012).

134. Brown, E.N., Purdon, P.L. & Van Dort, C.J. General anesthesia and altered states of arousal: a systems neuroscience analysis. *Annual review of neuroscience* **34**, 601-628 (2011).
135. Hong, D.M., *et al.* Interactions of midazolam and propofol on alpha1beta2gamma2L and alpha1beta2gamma2S gamma aminobutyric acid type a receptors expressed in human embryonic kidney cells. *Anesthesia and analgesia* **112**, 1096-1102 (2011).
136. FDA. Guidance for Industry: Estimating the Maximum Safe Starting Dose in Initial Clinical Trials for Therapeutics in Adult Healthy Volunteers. (ed. Toxicology, P.a.) 1-30 (Office of Training and Communications
Division of Drug Information, HFD-240
Center for Drug Evaluation and Research
Food and Drug Administration, Rockville, MD, 2005).
137. Nikitas, G., *et al.* Transcytosis of Listeria monocytogenes across the intestinal barrier upon specific targeting of goblet cell accessible E-cadherin. *The Journal of experimental medicine* **208**, 2263-2277 (2011).
138. Ebbes, M., *et al.* Fold and function of the InlB B-repeat. *The Journal of biological chemistry* **286**, 15496-15506 (2011).
139. Bonazzi, M., Lecuit, M. & Cossart, P. Listeria monocytogenes internalin and E-cadherin: from structure to pathogenesis. *Cellular microbiology* **11**, 693-702 (2009).
140. Qiu, J., *et al.* Intranasal vaccination with the recombinant Listeria monocytogenes DeltaactA prfA* mutant elicits robust systemic and pulmonary cellular responses and secretory mucosal IgA. *Clinical and vaccine immunology : CVI* **18**, 640-646 (2011).
141. Drevets, D.A., Jelinek, T.A. & Freitag, N.E. Listeria monocytogenes-infected phagocytes can initiate central nervous system infection in mice. *Infection and immunity* **69**, 1344-1350 (2001).
142. Chang, J., *et al.* Evaluation of the effects of thiopental, propofol, and etomidate on glomerular filtration rate measured by the use of dynamic computed tomography in dogs. *American journal of veterinary research* **72**, 146-151 (2011).
143. Favetta, P., *et al.* Propofol metabolites in man following propofol induction and maintenance. *British journal of anaesthesia* **88**, 653-658 (2002).
144. Liu, Y., Zheng, Y., Gu, X. & Ma, Z. The Efficacy of NMDA receptor antagonists for preventing remifentanil-induced increase in postoperative pain and analgesic requirement: A Meta-Analysis. *Minerva anestesiologica* (2012).
145. Curd-Sneed, C.D. & Stewart, J.J. Oral absorption of sodium pentobarbital and effects on gastrointestinal function. *Pharmacology & toxicology* **64**, 23-27 (1989).
146. Czuprynski, C.J., Theisen, C. & Brown, J.F. Treatment with the antigranulocyte monoclonal antibody RB6-8C5 impairs resistance of mice to gastrointestinal infection with Listeria monocytogenes. *Infection and immunity* **64**, 3946-3949 (1996).
147. Czuprynski, C.J. & Faith, N.G. Sodium bicarbonate enhances the severity of infection in neutropenic mice orally inoculated with Listeria monocytogenes EGD. *Clinical and diagnostic laboratory immunology* **9**, 477-481 (2002).
148. Czuprynski, C.J., Faith, N.G. & Steinberg, H. Ability of the Listeria monocytogenes strain Scott A to cause systemic infection in mice infected by the intragastric route. *Applied and environmental microbiology* **68**, 2893-2900 (2002).

149. Jo, Y.Y., Jun, N.H., Kim, E.J., Choi, E.K. & Kil, H.K. Optimal dose of propofol for intubation after sevoflurane inhalation without neuromuscular blocking agent in children. *Acta anaesthesiologica Scandinavica* **55**, 332-336 (2011).
150. Lucendo, A.J., *et al.* Nonanesthesiologist-administered propofol sedation for colonoscopy is safe and effective: a prospective Spanish study over 1000 consecutive exams. *European journal of gastroenterology & hepatology* **24**, 787-792 (2012).
151. rxlist.com. Diprivan (Propofol Injectable Emulsion). in *Drugs A-Z*, Vol. 2012 (Cerner Multum, Inc, 2010).
152. Clark, M.A. & Jepson, M.A. Intestinal M cells and their role in bacterial infection. *International journal of medical microbiology : IJMM* **293**, 17-39 (2003).
153. Corr, S., Hill, C. & Gahan, C.G. An in vitro cell-culture model demonstrates internalin- and hemolysin-independent translocation of Listeria monocytogenes across M cells. *Microbial pathogenesis* **41**, 241-250 (2006).
154. Kalliomaki, M.A. & Walker, W.A. Physiologic and pathologic interactions of bacteria with gastrointestinal epithelium. *Gastroenterology clinics of North America* **34**, 383-399, vii (2005).
155. Lu, L.a.W., A. Pathologic and physiologic interactions of bacteria with the gastrointestinal epithelium. in *Experimental Biology 2000* (San Diego, CA, 2000).
156. Chiba, S., *et al.* Listerial invasion protein internalin B promotes entry into ileal Peyer's patches in vivo. *Microbiology and immunology* **55**, 123-129 (2011).
157. Di, Y.M., Chow, V.D., Yang, L.P. & Zhou, S.F. Structure, function, regulation and polymorphism of human cytochrome P450 2A6. *Current drug metabolism* **10**, 754-780 (2009).
158. Vree, T.B., Lagerwerf, A.J., Bleeker, C.P. & de Grood, P.M. Direct high-performance liquid chromatography determination of propofol and its metabolite quinol with their glucuronide conjugates and preliminary pharmacokinetics in plasma and urine of man. *Journal of chromatography. B, Biomedical sciences and applications* **721**, 217-228 (1999).
159. Bryson, H.M., Fulton, B.R. & Faulds, D. Propofol. An update of its use in anaesthesia and conscious sedation. *Drugs* **50**, 513-559 (1995).
160. Simons, P.J., *et al.* Disposition in male volunteers of a subanaesthetic intravenous dose of an oil in water emulsion of 14C-propofol. *Xenobiotica; the fate of foreign compounds in biological systems* **18**, 429-440 (1988).
161. Mendu, S.K., Bhandage, A., Jin, Z. & Birnir, B. Different Subtypes of GABA-A Receptors Are Expressed in Human, Mouse and Rat T Lymphocytes. *PloS one* **7**, e42959 (2012).
162. Nigam, R., El-Nour, H., Amatya, B. & Nordlind, K. GABA and GABA(A) receptor expression on immune cells in psoriasis: a pathophysiological role. *Archives of dermatological research* **302**, 507-515 (2010).
163. Bhat, R., *et al.* Inhibitory role for GABA in autoimmune inflammation. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 2580-2585 (2010).
164. Graham, A.C., *et al.* IL-22 production is regulated by IL-23 during Listeria monocytogenes infection but is not required for bacterial clearance or tissue protection. *PloS one* **6**, e17171 (2011).

165. Kursar, M., *et al.* Organ-specific CD4+ T cell response during Listeria monocytogenes infection. *J Immunol* **168**, 6382-6387 (2002).
166. Lara-Tejero, M. & Pamer, E.G. T cell responses to Listeria monocytogenes. *Current opinion in microbiology* **7**, 45-50 (2004).
167. Mittrucker, H.W., *et al.* Inducible costimulator protein controls the protective T cell response against Listeria monocytogenes. *J Immunol* **169**, 5813-5817 (2002).
168. Nakane, A., *et al.* Endogenous interleukin-4, but not interleukin-10, is involved in suppression of host resistance against Listeria monocytogenes infection in interferon-depleted mice. *Infection and immunity* **64**, 1252-1258 (1996).
169. Rayamajhi, M., Humann, J., Penheiter, K., Andreasen, K. & Lenz, L.L. Induction of IFN-alphabeta enables Listeria monocytogenes to suppress macrophage activation by IFN-gamma. *The Journal of experimental medicine* **207**, 327-337 (2010).
170. Rutledge, B.J., *et al.* High level monocyte chemoattractant protein-1 expression in transgenic mice increases their susceptibility to intracellular pathogens. *J Immunol* **155**, 4838-4843 (1995).
171. Woodward, J.J., Iavarone, A.T. & Portnoy, D.A. c-di-AMP secreted by intracellular Listeria monocytogenes activates a host type I interferon response. *Science* **328**, 1703-1705 (2010).
172. Zhong, M.X., Kuziel, W.A., Pamer, E.G. & Serbina, N.V. Chemokine receptor 5 is dispensable for innate and adaptive immune responses to Listeria monocytogenes infection. *Infection and immunity* **72**, 1057-1064 (2004).
173. Kernbauer, E., *et al.* Conditional Stat1 Ablation Reveals the Importance of Interferon Signaling for Immunity to Listeria monocytogenes Infection. *PLoS pathogens* **8**, e1002763 (2012).
174. Jung, S., *et al.* In vivo depletion of CD11c+ dendritic cells abrogates priming of CD8+ T cells by exogenous cell-associated antigens. *Immunity* **17**, 211-220 (2002).
175. Drevets, D.A., Schawang, J.E., Mandava, V.K., Dillon, M.J. & Leenen, P.J. Severe Listeria monocytogenes infection induces development of monocytes with distinct phenotypic and functional features. *J Immunol* **185**, 2432-2441 (2010).
176. Busch, D.H., Kerksiek, K. & Pamer, E.G. Processing of Listeria monocytogenes antigens and the in vivo T-cell response to bacterial infection. *Immunological reviews* **172**, 163-169 (1999).
177. Busch, D.H. & Pamer, E.G. T cell affinity maturation by selective expansion during infection. *The Journal of experimental medicine* **189**, 701-710 (1999).
178. Shi, C., *et al.* Bone marrow mesenchymal stem and progenitor cells induce monocyte emigration in response to circulating toll-like receptor ligands. *Immunity* **34**, 590-601 (2011).
179. Tam, M.A. & Wick, M.J. Dendritic cells and immunity to Listeria: TipDCs are a new recruit. *Trends in immunology* **25**, 335-339 (2004).
180. Tam, M.A. & Wick, M.J. Differential expansion, activation and effector functions of conventional and plasmacytoid dendritic cells in mouse tissues transiently infected with Listeria monocytogenes. *Cellular microbiology* **8**, 1172-1187 (2006).
181. Serbina, N.V., Hohl, T.M., Cherny, M. & Pamer, E.G. Selective expansion of the monocytic lineage directed by bacterial infection. *J Immunol* **183**, 1900-1910 (2009).
182. Cesta, M.F. Normal structure, function, and histology of mucosa-associated lymphoid tissue. *Toxicologic pathology* **34**, 599-608 (2006).

183. Warren, S.E., Mao, D.P., Rodriguez, A.E., Miao, E.A. & Aderem, A. Multiple Nod-like receptors activate caspase 1 during Listeria monocytogenes infection. *J Immunol* **180**, 7558-7564 (2008).
184. van Riet, E., *et al.* Combined TLR2 and TLR4 ligation in the context of bacterial or helminth extracts in human monocyte derived dendritic cells: molecular correlates for Th1/Th2 polarization. *BMC immunology* **10**, 9 (2009).
185. Carrero, J.A. & Unanue, E.R. Mechanisms and immunological effects of apoptosis caused by Listeria monocytogenes. *Advances in immunology* **113**, 157-174 (2012).
186. Kubota, K. & Kadoya, Y. Innate IFN-gamma-Producing Cells in the Spleen of Mice Early after Listeria monocytogenes Infection: Importance of Microenvironment of the Cells Involved in the Production of Innate IFN-gamma. *Frontiers in immunology* **2**, 26 (2011).
187. Cope, A., Le Friec, G., Cardone, J. & Kemper, C. The Th1 life cycle: molecular control of IFN-gamma to IL-10 switching. *Trends in immunology* **32**, 278-286 (2011).
188. Narni-Mancinelli, E., *et al.* Memory CD8+ T cells mediate antibacterial immunity via CCL3 activation of TNF/ROI+ phagocytes. *The Journal of experimental medicine* **204**, 2075-2087 (2007).
189. Carr, K.D., *et al.* Specific depletion reveals a novel role for neutrophil-mediated protection in the liver during Listeria monocytogenes infection. *European journal of immunology* **41**, 2666-2676 (2011).
190. Auffray, C., Sieweke, M.H. & Geissmann, F. Blood monocytes: development, heterogeneity, and relationship with dendritic cells. *Annual review of immunology* **27**, 669-692 (2009).
191. Solovjov, D.A., Pluskota, E. & Plow, E.F. Distinct roles for the alpha and beta subunits in the functions of integrin alphaMbeta2. *The Journal of biological chemistry* **280**, 1336-1345 (2005).
192. Chung, N.P., Chen, Y., Chan, V.S., Tam, P.K. & Lin, C.L. Dendritic cells: sentinels against pathogens. *Histology and histopathology* **19**, 317-324 (2004).
193. Hu, X.L., Tang, H.H., Zhou, Z.G., Yin, F. & Liu, W.J. [The effect of sevoflurane inhalation anesthesia only and propofol total intravenous anesthesia on perioperative cytokine balance in lung cancer patients]. *Xi bao yu fen zi mian yi xue za zhi = Chinese journal of cellular and molecular immunology* **27**, 659-661 (2011).
194. Scholing, M., Schneeberger, P.M., van den Dries, P. & Drenth, J.P. Clinical features of liver involvement in adult patients with listeriosis. Review of the literature. *Infection* **35**, 212-218 (2007).
195. Conlan, J.W. Early pathogenesis of Listeria monocytogenes infection in the mouse spleen. *Journal of medical microbiology* **44**, 295-302 (1996).
196. Pohlers, D., *et al.* TGF-beta and fibrosis in different organs - molecular pathway imprints. *Biochimica et biophysica acta* **1792**, 746-756 (2009).
197. Dinarello, C.A. Immunological and inflammatory functions of the interleukin-1 family. *Annual review of immunology* **27**, 519-550 (2009).
198. Mocci, S., Dalrymple, S.A., Nishinakamura, R. & Murray, R. The cytokine stew and innate resistance to L. monocytogenes. *Immunological reviews* **158**, 107-114 (1997).
199. Dalrymple, S.A., *et al.* Interleukin-6-deficient mice are highly susceptible to Listeria monocytogenes infection: correlation with inefficient neutrophilia. *Infection and immunity* **63**, 2262-2268 (1995).

200. Indramohan, M., Sieve, A.N., Break, T.J. & Berg, R.E. Inflammatory monocyte recruitment is regulated by IL-23 during systemic bacterial infection. *Infection and immunity* (2012).
201. Abreu, C., *et al.* Listeria infection in patients on anti-TNF treatment: Report of two cases and review of the literature. *Journal of Crohn's & colitis* (2012).
202. Lorente, J.A. & Marshall, J.C. Neutralization of tumor necrosis factor in preclinical models of sepsis. *Shock* **24 Suppl 1**, 107-119 (2005).
203. Drevets, D.A., Dillon, M.J., Schawang, J.E., Stoner, J.A. & Leenen, P.J. IFN-gamma triggers CCR2-independent monocyte entry into the brain during systemic infection by virulent Listeria monocytogenes. *Brain, behavior, and immunity* **24**, 919-929 (2010).
204. Nishikawa, S., Miura, T., Sasaki, S. & Nakane, A. The protective role of endogenous cytokines in host resistance against an intragastric infection with Listeria monocytogenes in mice. *FEMS immunology and medical microbiology* **16**, 291-298 (1996).
205. Rot, A. & von Andrian, U.H. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. *Annual review of immunology* **22**, 891-928 (2004).
206. Hashimoto, S., *et al.* Macrophage-derived chemokine (MDC)/CCL22 produced by monocyte derived dendritic cells reflects the disease activity in patients with atopic dermatitis. *Journal of dermatological science* **44**, 93-99 (2006).
207. Chong, S.Z., *et al.* Human CD8(+) T cells drive Th1 responses through the differentiation of TNF/iNOS-producing dendritic cells. *European journal of immunology* **41**, 1639-1651 (2011).
208. Shortman, K. & Naik, S.H. Steady-state and inflammatory dendritic-cell development. *Nature reviews. Immunology* **7**, 19-30 (2007).
209. Nakane, A., *et al.* Transforming growth factor beta is protective in host resistance against Listeria monocytogenes infection in mice. *Infection and immunity* **64**, 3901-3904 (1996).
210. DePaolo, R.W., *et al.* Co-adjuvant effects of retinoic acid and IL-15 induce inflammatory immunity to dietary antigens. *Nature* **471**, 220-224 (2011).
211. Obar, J.J., *et al.* Pathogen-induced inflammatory environment controls effector and memory CD8+ T cell differentiation. *J Immunol* **187**, 4967-4978 (2011).
212. Sheridan, B.S. & Lefrancois, L. Regional and mucosal memory T cells. *Nature immunology* **12**, 485-491 (2011).
213. Izcue, A., Coombes, J.L. & Powrie, F. Regulatory lymphocytes and intestinal inflammation. *Annual review of immunology* **27**, 313-338 (2009).
214. Schmetterer, K.G., Neunkirchner, A. & Pickl, W.F. Naturally occurring regulatory T cells: markers, mechanisms, and manipulation. *The FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **26**, 2253-2276 (2012).
215. Cao, Q., *et al.* IL-10/TGF-beta-modified macrophages induce regulatory T cells and protect against adriamycin nephrosis. *Journal of the American Society of Nephrology : JASN* **21**, 933-942 (2010).
216. Ooi, J.D., *et al.* Endogenous foxp3(+) T-regulatory cells suppress anti-glomerular basement membrane nephritis. *Kidney international* **79**, 977-986 (2011).
217. Atsukawa, M., *et al.* Ribavirin downmodulates inducible costimulator on CD4+ T cells and their interleukin-10 secretion to assist in hepatitis C virus clearance. *Journal of gastroenterology and hepatology* **27**, 823-831 (2012).

218. Kobayashi, T., *et al.* Ribavirin modulates the conversion of human CD4(+) CD25(-) T-cell to CD4(+) CD25(+) FOXP3(+) T-cell via suppressing Interleukin-10 producing T-regulatory cell. *Immunology* (2012).
219. Galli, S.J., Borregaard, N. & Wynn, T.A. Phenotypic and functional plasticity of cells of innate immunity: macrophages, mast cells and neutrophils. *Nature immunology* **12**, 1035-1044 (2011).
220. Todar, K. Todar's Online Textbook of Bacteriology. (ed. Todar, K.) (University of Wisconsin, Madison, WI, 2008).
221. Andrews-Polymenis, H.L., Baumler, A.J., McCormick, B.A. & Fang, F.C. Taming the elephant: Salmonella biology, pathogenesis, and prevention. *Infection and immunity* **78**, 2356-2369 (2010).
222. Swearingen, M.C., Porwollik, S., Desai, P.T., McClelland, M. & Ahmer, B.M. Virulence of 32 Salmonella strains in mice. *PloS one* **7**, e36043 (2012).
223. Loetscher, Y., *et al.* Salmonella transiently reside in luminal neutrophils in the inflamed gut. *PloS one* **7**, e34812 (2012).
224. Mair, S.M., *et al.* Nifedipine affects the course of Salmonella enterica serovar Typhimurium infection by modulating macrophage iron homeostasis. *The Journal of infectious diseases* **204**, 685-694 (2011).
225. Nairz, M., *et al.* Absence of functional Hfe protects mice from invasive Salmonella enterica serovar Typhimurium infection via induction of lipocalin-2. *Blood* **114**, 3642-3651 (2009).
226. Karlinsey, J.E., Maguire, M.E., Becker, L.A., Crouch, M.L. & Fang, F.C. The phage shock protein PspA facilitates divalent metal transport and is required for virulence of Salmonella enterica sv. Typhimurium. *Molecular microbiology* **78**, 669-685 (2010).
227. Richardson, A.R., *et al.* Multiple targets of nitric oxide in the tricarboxylic acid cycle of Salmonella enterica serovar typhimurium. *Cell host & microbe* **10**, 33-43 (2011).
228. Jones, B.D. & Falkow, S. Salmonellosis: host immune responses and bacterial virulence determinants. *Annual review of immunology* **14**, 533-561 (1996).
229. Torres, D., *et al.* Toll-like receptor 2 is required for optimal control of Listeria monocytogenes infection. *Infection and immunity* **72**, 2131-2139 (2004).
230. Arpaia, N., *et al.* TLR signaling is required for Salmonella typhimurium virulence. *Cell* **144**, 675-688 (2011).
231. Chang, J.C., LaSarre, B., Jimenez, J.C., Aggarwal, C. & Federle, M.J. Two group A streptococcal peptide pheromones act through opposing Rgg regulators to control biofilm development. *PLoS pathogens* **7**, e1002190 (2011).
232. Chiappini, N., *et al.* Streptococcus pyogenes SpyCEP Influences Host-Pathogen Interactions during Infection in a Murine Air Pouch Model. *PloS one* **7**, e40411 (2012).
233. Sultan, H.Y., Boyle, A.A. & Sheppard, N. Necrotising fasciitis. *BMJ* **345**, e4274 (2012).
234. Fisher, M., *et al.* Shr is a broad-spectrum surface receptor that contributes to adherence and virulence in group A streptococcus. *Infection and immunity* **76**, 5006-5015 (2008).
235. Nakata, M., *et al.* Mode of expression and functional characterization of FCT-3 pilus region-encoded proteins in Streptococcus pyogenes serotype M49. *Infection and immunity* **77**, 32-44 (2009).
236. Oehmcke, S., *et al.* Treatment of invasive streptococcal infection with a peptide derived from human high-molecular weight kininogen. *Blood* **114**, 444-451 (2009).

237. Crotty Alexander, L.E., *et al.* M1T1 group A streptococcal pili promote epithelial colonization but diminish systemic virulence through neutrophil extracellular entrapment. *J Mol Med (Berl)* **88**, 371-381 (2010).
238. Cole, J.N., Barnett, T.C., Nizet, V. & Walker, M.J. Molecular insight into invasive group A streptococcal disease. *Nature reviews. Microbiology* **9**, 724-736 (2011).
239. Zhang, S., *et al.* Streptococcal M1 protein-provoked CXC chemokine formation, neutrophil recruitment and lung damage are regulated by Rho-kinase signaling. *Journal of innate immunity* **4**, 399-408 (2012).
240. Dileepan, T., *et al.* Robust antigen specific th17 T cell response to group A Streptococcus is dependent on IL-6 and intranasal route of infection. *PLoS pathogens* **7**, e1002252 (2011).
241. Wang, B., *et al.* Induction of TGF-beta1 and TGF-beta1-dependent predominant Th17 differentiation by group A streptococcal infection. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 5937-5942 (2010).
242. Rigby, K.M. & DeLeo, F.R. Neutrophils in innate host defense against Staphylococcus aureus infections. *Seminars in immunopathology* **34**, 237-259 (2012).
243. Storch, G.A., Radcliff, J.L., Meyer, P.L. & Hinrichs, J.H. Methicillin-resistant Staphylococcus aureus in a nursing home. *Infection control : IC* **8**, 24-29 (1987).
244. Matouskova, I. & Janout, V. Current knowledge of methicillin-resistant Staphylococcus aureus and community-associated methicillin-resistant Staphylococcus aureus. *Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia* **152**, 191-202 (2008).
245. Miller, L.S. & Cho, J.S. Immunity against Staphylococcus aureus cutaneous infections. *Nature reviews. Immunology* **11**, 505-518 (2011).
246. Casey, J.A., Cosgrove, S.E., Stewart, W.F., Pollak, J. & Schwartz, B.S. A population-based study of the epidemiology and clinical features of methicillin-resistant Staphylococcus aureus infection in Pennsylvania, 2001-2010. *Epidemiology and infection*, 1-14 (2012).
247. Kennedy, A.D., *et al.* Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. *The Journal of infectious diseases* **202**, 1050-1058 (2010).
248. Kim, H.K., *et al.* IsdA and IsdB antibodies protect mice against Staphylococcus aureus abscess formation and lethal challenge. *Vaccine* **28**, 6382-6392 (2010).
249. Spellberg, B., *et al.* The antifungal vaccine derived from the recombinant N terminus of Als3p protects mice against the bacterium Staphylococcus aureus. *Infection and immunity* **76**, 4574-4580 (2008).
250. Thammavongsa, V., Kern, J.W., Missiakas, D.M. & Schneewind, O. Staphylococcus aureus synthesizes adenosine to escape host immune responses. *The Journal of experimental medicine* **206**, 2417-2427 (2009).
251. Cheng, A.G., *et al.* Contribution of coagulases towards Staphylococcus aureus disease and protective immunity. *PLoS pathogens* **6**, e1001036 (2010).
252. Tanaka, M., *et al.* Photodynamic Therapy Can Induce a Protective Innate Immune Response against Murine Bacterial Arthritis via Neutrophil Accumulation. *PloS one* **7**, e39823 (2012).

253. Schindler, D., *et al.* Dendritic Cells Are Central Coordinators of the Host Immune Response to Staphylococcus aureus Bloodstream Infection. *The American journal of pathology* (2012).
254. Farthing, M.J. Novel targets for the pharmacotherapy of diarrhoea: a view for the millennium. *Journal of gastroenterology and hepatology* **15 Suppl**, G38-45 (2000).
255. Nathan, C. Neutrophils and immunity: challenges and opportunities. *Nature reviews. Immunology* **6**, 173-182 (2006).
256. Brinkmann, V. & Zychlinsky, A. Beneficial suicide: why neutrophils die to make NETs. *Nature reviews. Microbiology* **5**, 577-582 (2007).
257. Menegazzi, R., Decleva, E. & Dri, P. Killing by neutrophil extracellular traps: fact or folklore? *Blood* **119**, 1214-1216 (2012).
258. Schorn, C., *et al.* Monosodium urate crystals induce extracellular DNA traps in neutrophils, eosinophils, and basophils but not in mononuclear cells. *Frontiers in immunology* **3**, 277 (2012).
259. Berends, E.T., *et al.* Nuclease expression by Staphylococcus aureus facilitates escape from neutrophil extracellular traps. *Journal of innate immunity* **2**, 576-586 (2010).
260. Agbor, T.A. & McCormick, B.A. Salmonella effectors: important players modulating host cell function during infection. *Cellular microbiology* **13**, 1858-1869 (2011).
261. Bueno, S.M., Riquelme, S., Riedel, C.A. & Kalergis, A.M. Mechanisms used by virulent Salmonella to impair dendritic cell function and evade adaptive immunity. *Immunology* **137**, 28-36 (2012).
262. Fink, S.L. & Cookson, B.T. Pyroptosis and host cell death responses during Salmonella infection. *Cellular microbiology* **9**, 2562-2570 (2007).
263. Green, D.R. Overview: apoptotic signaling pathways in the immune system. *Immunological reviews* **193**, 5-9 (2003).
264. Tart, A.H., Walker, M.J. & Musser, J.M. New understanding of the group A Streptococcus pathogenesis cycle. *Trends in microbiology* **15**, 318-325 (2007).
265. Kreikemeyer, B., McIver, K.S. & Podbielski, A. Virulence factor regulation and regulatory networks in Streptococcus pyogenes and their impact on pathogen-host interactions. *Trends in microbiology* **11**, 224-232 (2003).
266. Nakagawa, I., *et al.* Autophagy defends cells against invading group A Streptococcus. *Science* **306**, 1037-1040 (2004).
267. Yoshimori, T. & Amano, A. Group a Streptococcus: a loser in the battle with autophagy. *Current topics in microbiology and immunology* **335**, 217-226 (2009).
268. Sakurai, A., *et al.* Specific behavior of intracellular Streptococcus pyogenes that has undergone autophagic degradation is associated with bacterial streptolysin O and host small G proteins Rab5 and Rab7. *The Journal of biological chemistry* **285**, 22666-22675 (2010).
269. Aldridge, J.R., Jr., *et al.* TNF/iNOS-producing dendritic cells are the necessary evil of lethal influenza virus infection. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 5306-5311 (2009).
270. Hasegawa, T., *et al.* Characterization of a virulence-associated and cell-wall-located DNase of Streptococcus pyogenes. *Microbiology* **156**, 184-190 (2010).
271. Chang, A., Khemlani, A., Kang, H. & Proft, T. Functional analysis of Streptococcus pyogenes nuclease A (SpnA), a novel group A streptococcal virulence factor. *Molecular microbiology* **79**, 1629-1642 (2011).

272. McQuaid, K.R. & Laine, L. A systematic review and meta-analysis of randomized, controlled trials of moderate sedation for routine endoscopic procedures. *Gastrointestinal endoscopy* **67**, 910-923 (2008).
273. Miner, J.R., Danahy, M., Moch, A. & Biros, M. Randomized clinical trial of etomidate versus propofol for procedural sedation in the emergency department. *Annals of emergency medicine* **49**, 15-22 (2007).
274. Tringali, G., *et al.* Pharmacokinetic profile of Oncofid-S after intraperitoneal and intravenous administration in the rat. *The Journal of pharmacy and pharmacology* **64**, 360-365 (2012).
275. Cole, C., Thomas, S., Filak, H., Henson, P.M. & Lenz, L.L. Nitric oxide increases susceptibility of Toll-like receptor-activated macrophages to spreading Listeria monocytogenes. *Immunity* **36**, 807-820 (2012).
276. Weiman, S., *et al.* O-Acetylation of sialic acid on Group B Streptococcus inhibits neutrophil suppression and virulence. *The Biochemical journal* **428**, 163-168 (2010).
277. Doisne, J.M., *et al.* Cutting edge: crucial role of IL-1 and IL-23 in the innate IL-17 response of peripheral lymph node NK1.1- invariant NKT cells to bacteria. *J Immunol* **186**, 662-666 (2011).
278. Azuma, M., *et al.* The peptide sequence of diacyl lipopeptides determines dendritic cell TLR2-mediated NK activation. *PloS one* **5**(2010).
279. Small, C.L., *et al.* Influenza infection leads to increased susceptibility to subsequent bacterial superinfection by impairing NK cell responses in the lung. *J Immunol* **184**, 2048-2056 (2010).

Lavanya Visvabharathy
2346 W Taylor St, #3R

Chicago, IL 60612
Phone: 773.715.7064
Email: lvisva1@uic.edu

EDUCATION:

The University of Illinois at Chicago, B.Sc., 5/2006

Major: *Biological Sciences, concentration Neuroscience*
Minors: *Chemistry & Russian*

The University of Illinois at Chicago, Ph.D candidate, 2007- 2012

Degree sought- Ph.D, Microbiology/Immunology
Concentrations: Molecular Immunology, Microbiology
Relevant coursework: Cell Biology, Advanced Immunology, Virology, Structural Biology, Neuroscience

RESEARCH EXPERIENCE:

Ph.D work: Effects of propofol anesthesia on innate immune parameters of the host in the context of bacterial infection. Mentor: Dr. Nancy Freitag, Ph.D. University of Illinois at Chicago.

Undergraduate research: Molecular mechanisms of pain perception and opioid addiction in rats. Mentor: Zaije Jim Wang, Ph.D. University of Illinois at Chicago.

Undergraduate research: Mechanisms of memory deficiency in mice with Fragile X syndrome. Mentor: John Larson, Ph.D. University of Illinois at Chicago.

HONORS/AWARDS:

- UIC College of Medicine Research Forum: Poster Presentation, Honorable mention. Fall, 2011. Title of poster: Propofol Anesthesia Increases Host Susceptibility to Bacterial Infection.
- Chancellor's Graduate Research Fellowship, UIC. January-August, 2012.

PUBLICATIONS:

Visvabharathy L, Xayarath B, Weinberg G, and Freitag NE. 'The commonly used anesthetic propofol dramatically increases host susceptibility to microbial infection'. Manuscript submitted to Nature Medicine.

Visvabharathy L and Freitag, NE. 'Functional comparisons of the effects of anesthetic agents on host immunity to microbial infection'. Manuscript in preparation.

MEETINGS ATTENDED:

- 17th annual Midwest Microbial Pathogenesis Meeting, St. Louis, MO. Sept. 2010
- 18th annual Midwest Microbial Pathogenesis Meeting, Ann Arbor, MI. Oct. 2011

- Keystone Conference on Innate Immunity: Sensing the Microbes and Damage Signals, Keystone Resort, CO. Mar. 2012
- 19th annual Midwest Microbial Pathogenesis Meeting, Milwaukee, WI. Sept. 2012
- 4th annual International Conference on Gram Positive Pathogens, Omaha, NE. Oct. 2012

PRESENTATIONS AND ABSTRACTS:

Visvabharathy, L and Freitag, NE. "The commonly used anesthetic propofol dramatically increases host susceptibility to microbial infection". International Conference on Gram Positive Pathogens, Omaha, NE, October 2012. *Oral presentation*.

Visvabharathy, L, Weinberg, G, and Freitag, NE. "Propofol anesthesia increases host susceptibility to bacterial infection". Keystone Conference on Innate Immunity: Sensing the Microbes and Damage Signals, Keystone Resort, CO, March 2012. *Poster Presentation.*

Visvabharathy, L and Freitag, NE. "Propofol anesthesia increases host susceptibility to bacterial infection with *L. monocytogenes*". 18th Annual Midwest Microbial Pathogenesis Meeting. University of Michigan, Ann Arbor, MI. October 2011. *Poster Presentation.*

Bruno, J, **Visvabharathy L**, and Freitag, NE. "Constitutive activation of PrfA tilts the balance of *Listeria monocytogenes* fitness towards life within the host versus environmental survival". 17th Annual Midwest Microbial Pathogenesis Meeting. Washington University. St. Louis, MO. Septermber 2010. *Poster Presentation*.

TEACHING:

North Central College Dept. of Psychology (Mar 2008- Jun 2008)
Instructor: Drugs and Behavior

University of Illinois at Chicago Dept. of Biological Sciences (Spring and Fall, 2010)
TA: Genetics, Neuroscience, and Neuroethology

RELEVENT WORK EXPERIENCE:

NYU School of Medicine (Jul 2006 through Nov 2006), *Research/Medical Assistant*
NYU Environmental Medicine- Center for AIDS Research

Department of Medicine, University of Illinois at Chicago (Aug 2005 – Jun 2006), *Lab Assistant*
Merck STEP HIV vaccine trial.

Walgreens Pharmacy (Sept 2000 – Sept 2007), *Certified Pharmacy Technician*