Sickness Behavior and the Metabolic Demand of Immunity: Insight from a Live Bacterial Infection Model.

by

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Abstract

Life-history theory states that animals have access to a finite amount of resources over their lifespan. These resources are allocated between growth, reproduction, and maintenance, and how these resources are allocated will affect the overall fitness of an organism. A fundamental assumption of this theory is that once a resource is utilized by a trait, it cannot be used by another trait. Thus, when the demand for resources for one trait increases, it will come at the cost of the other traits. The host's immune system is responsible for survival from pathogenic invasions. Therefore, it is a critical part of maintenance. Studies have examined the trade-offs induced by an immune response.

The current knowledge on trade-offs incurred during an immune response come from the use of pathogen-associated molecular patterns (PAMPs), non-pathogenic antigens such as sheep red blood cells (SRBCs), or keyhole limpet hemocyanin (KLH). These studies have observed trade-offs with metabolism and weight. Additionally, some of these studies have observed decreased activity, increased fatigue, loss of appetite, and fever. Collectively, these symptoms are knowns as sickness behavior. In recent years, the field of science investigating the cellular metabolism of immune cells termed immunometabolism has observed a rapid growth. However, the knowledge gained from these studies relies on PAMPs or non-specific activation of cells of the adaptive immune system. Thus, the understanding of trade-offs and immunometabolism to a pathogen remains poorly characterized.

Our present study aims to longitudinally characterize the trade-off to life-history traits, induced sickness behavior, and immunometabolism to a well-characterized model *Listeria monocytogenes.* During a primary immune response, we observed trade-offs and

sickness behavior that corresponded to the timing of the innate immune response. Additionally, during this time, we observed a shift in cellular metabolism towards aerobic glycolysis in cells of the innate immune system. During the time of maximal cost of adaptive immunity, clonal expansion, we observed the resolution trade-offs and sickness behavior. Additionally, the cell's immunometabolism resembled cells from control. Thus, during a primary immune response, the innate immune system likely is the driver of trade-offs. During a secondary response, we observed trade-offs that coincided with the timing of reactivation of cells of the adaptive immune system. Thus, reactivation of the adaptive immune response is likely to cause trade-offs.

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2-DG	2-Deoxyglucose
APCs	Antigen-Presenting Cells
BBB	Blood-Brain Barrier
BMR	Basal Metabolic Rate
CD	Cluster of Differentiation
CNS	Central Nervous System
COX2	Cyclooxygenase 2
FAO	Fatty Acid Oxidation
Glut-1	Glucose Transporter-1
HIF-1α	Hypoxia-Induced Factor-1α
IFN-γ	Interferon-γ
IL	Interleukin
KLH	Keyhole Limpet Hemocyanin
LPS	Lipopolysaccharide
NK	Natural Killer
OXPHOS	Oxidative Phosphorylation
PAMP	Pathogen Associated Molecular Patter
PGD_2	Prostaglandin D_2
PGE_2	Prostaglandin E_2
PHA	Phytohemagglutinin
PMN	Polymorphic Neutrophils
PRR	Pathogen Recognition Receptor

RMR	Resting Metabolic Rate
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen Species
SRBCs	Sheep Red Blood Cells
TCA	Tricarboxylic Acid
TCR	T cell Receptor
TipDCs	TNF-α/Inducible nitric oxide synthase
TNF-α	Tumor Necrosis Factor-α
VLPO	Ventrolateral Preoptic Nucleus

Chapter 1

Literature Review

Life-history Theory and Sickness Behavior

In nature, organisms strive to obtain energy and nutrients in the effort to grow, survive, and reproduce. An organism may experience events in which it has limited access to resources; these events drive the allocation of acquired resources between fitness traits. The evolutionary physiological and behavioral strategies an organism uses to partition these resources over its lifespan is explained within the life-history theory (1-3). A fundamental assumption of the life-history theory is that a resource can only be allocated to one trait. For example, the same resources could not be allocated to both locomotion and immunity; thus, trade-offs among life-history traits are inevitable. The immune system is responsible for immunocompetence, which is defined as the ability of an organism to mount an effective immune response to a pathogen (4). This is an essential component of self-maintenance and host survival. To survive, organisms will allocate resources to the immune system in trade-offs with other traits (2-5). Norris, *et al.* established the three requirements to demonstrate the trade-off between life-history traits 1) immunity must compete with other life-history traits for access to limited resources 2) increased investment in a particular life-history trait must reduce immunity 3) a reduction in immunity must cause a reduction in fitness (4). Using these requirements, studies have investigated what traits immunocompetence induce trade-offs with.

Several studies have investigated the impact of immunocompetence on life-history traits in birds, rodents, and bats through the use of immune stimulators including: lipopolysaccharide (LPS), keyhole limpet hemocyanin (KLH), phytohemagglutinin (PHA), or sheep red blood cells (SRBCs). After subjecting these individuals to an immune stimulator, multiple parameters to determine overall fitness were recorded, and included parameters such as: weight loss, an increase in basal metabolic rate (BMR) or resting metabolic rate (RMR), decreased activity, decreased energy expenditure, and decreased fecundity (1, 6-14). LPS, specifically, induced observed symptoms of lethargy, anorexia, fever, sleepiness, and reclusiveness. Collectively, the symptoms are known as sickness behavior (15-19). This behavior has been observed across in vertebrates; thus, it is likely a conserved adaptive evolutionary strategy to direct resources towards immunocompetence (15, 17, 20). Because behavior is ultimately organized and controlled by the brain, there must be either an interaction between the brain and pathogen or the brain and immune system. Since most pathogens do not infect the brain, it is more likely the interaction between the immune system and the brain that causes sickness behavior (15).

Initiation of sickness behavior is linked to activation of the innate immune response. Cells of the innate immune system are the first line of defense, and responsible for the initial recognition of a pathogen. Upon pathogen detection, cells of the innate immune system become activated and produce pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β (21, 22). These systemic pro-inflammatory cytokines can interact with the central nervous system (CNS) either through being actively transported into the brain by endothelial cells of the blood-brain barrier (BBB) or through direct interaction on the

brain at circumventricular organs, which lacking a BBB (18, 19, 23, 24). The interaction of cytokines on the brain leads to the production of prostaglandins (PG). Cyclooxygenase 2 (COX2) is expressed in the brain and the enzyme responsible for converting arachidonic acid into prostaglandin H_2. Prostaglandin H_2 can be further converted into prostaglandin E_2 (PGE_2) by microsomal PGE synthase 1 or prostaglandin D_2 (PGD_2) by lipocalin PGD synthase. PGE_2 and PGD_2 are implicated in the induction of fever, sleepiness, and anorexia, symptoms of sickness behavior (25). Ushikubi, *et al.* established that LPS-induced fever is due to PGE_2 interaction with the EP3 receptor while Lazarus, *et al.* established is was the EP3 receptors in the medial preoptic nucleus within the hypothalamus (26, 27). The PGE_2 is produced by endothelial cells of the brain (28-31). Ueno, *et al.* identified PGD_2 as the significant sleep-promoting prostaglandin (32). Several studies helped to identify the ventrolateral preoptic nucleus (VLPO) within the hypothalamus, which leads to the production of PGD_2 (32-34). Many of the LPS-induced responses in the hypothalamus are driven by the cytokine TNFα. However, other cytokines produced by the innate immune response also aid in driving symptoms of sickness behavior. For example, IL-1β interacts with the paraventricular nucleus of the hypothalamus (PVH) leading to the production of PGE_2 which interacts with the EP4 receptor and drives anorexia (35, 36). These studies have used either pathogen associated molecular patterns (PAMPs), non-pathogenic antigen, or mitogen to determine the demand of immunocompetence to an organism. Yet, to truly understand the demand of immunocompetence, an immune response to an infectious pathogen must be determined.

Primary Immune Response

A primary immune response to a pathogen relies on the innate and adaptive immune system. Cells of the innate immune system can be grouped into granulocytes, antigen-presenting cells (APCs), or innate lymphoid cells. Granulocytes include polymorphic neutrophils (PMNs), eosinophils, mast cells and basophils and are characterize by granules within their cytoplasm. APCs include monocytes, macrophages, and DCs and are capable of processing and presenting antigen to T cells, cells of the adaptive immune system. Innate lymphocytes include natural killer (NK) cells and innate lymphocytes (not discussed here). NK cells detect intracellular host cells and lyse these cells to assist with pathogen clearance. Cells of the innate immune system detect microbes through Pattern Recognition Receptors (PRRs) that bind Pathogen Associated Molecular Patterns (PAMPs), such as LPS, which are expressed by a variety of microbes (37, 38). Upon PRR:PAMP interaction, cells of the innate immune system become activated, leading to increased production of pro-inflammatory cytokines and chemokines (21, 22, 39, 40). These pro-inflammatory cytokines help to orchestrate the local immune response (21, 22). Chemokines are responsible for cell recruitment from the bone marrow and bloodstream to the site of infection (41-44). DCs are the link between the innate and adaptive immune system. Activated DCs leave the site of infection and travel to secondary lymphoid organs where they activate cells of the adaptive immune system, specifically T cells.

T lymphocytes are cells of the adaptive immune system and go through four distinct phases during an immune response: activation, clonal expansion, contraction, and memory. Activation occurs within minutes of T cell receptor (TCR) engagement. T cells then increase in size over the next 24 hours in preparation for the first round of division.

After this period, T cells will go through their first round of clonal expansion, with each successive round of expansion occurring about every 10 hours (45, 46). Each T cell can go through a minimum of 9 rounds of replication (46, 47). Taken together, one activated antigen-specific T cell can increase 10,000-fold during the clonal expansion phase (48-50). During the contraction phase, 90-95% of these cells die off (48, 51), and the remaining 5-10% of cells will be retained as memory T cells. Memory T cells possess an immunological memory, which allows for a rapid immune response to previously encountered antigens upon subsequent infections (48, 49, 52).

Secondary Immune Response

During a subsequent infection, memory cells of the adaptive immune system "orchestrate the show" compared to a primary immune response where cells of the innate immune system initiate the immune response (53). Memory cells can be divided into central memory T cells and effector memory T cells. Central memory T cells hone to secondary lymphoid organs, and effector memory T cells survey peripheral tissues. Central memory T cells are better equipped to proliferate in response to antigen stimulation (51, 53). Upon stimulation, memory cells will again undergo activation, expansion, contraction, and reestablishment of memory but at a much faster rate. After activation, the first round of clonal expansion occurs with 4-6 hours, and the clonal expansion is about 5-fold higher than a primary immune response (54, 55). During the contraction phase, 20-40% of the cells remain to reestablish memory (50, 56). Effector memory T cells are poised to be the first line of defense to a subsequent infection in the periphery. Upon activation, these cells secrete effector cytokines (51, 53). Thus, the

adaptive immune response in a secondary response is more rapid, greater in magnitude, and more proficient. The characterization of the mammalian immune system has come from the use of a well-characterized pathogen, one of these model pathogens being *Listeria monocytogenes*.

Listeria monocytogenes

Listeria monocytogenes is a Gram-positive, facultative intracellular, food-borne pathogen that causes gastroenteritis in healthy individuals. In immunocompromised individuals, such as pregnant women, elderly, or children it can cause more severe disease. In pregnant women, *Listeria* can cause spontaneous abortion, and the elder and young can experience meningoencephalitis (57-60). Immunologists have characterized the primary and secondary immune response to *Listeria.* Thus, it is a great model of host-pathogen interactions.

During a primary infection, the early immune response (< 3 days) to *Listeria* is characterized by an innate immune response (57-60). Residential macrophages are the first cells to combat the infection. Unfortunately, *Listeria* can easily escape the phagosome and replicate within the cytosol (61, 62). Thus, initially, these cells serve as an intracellular growth niche for *Listeria*. However, IFN-γ activated macrophages kill *Listeria* through reactive nitrogen species; the primary source of early IFN-γ is NK cells (63, 64). The first infiltrating cell to encounter *Listeria* is PMNs. Liu *et al.*, observed liver PMN infiltration within minutes of systemic infection with the peak recruitment occurring 4 hours after infection (65). PMNs kill Listeria through degranulation and ROS (66-68). Hours after infection, inflammatory monocytes are recruited to the spleen.

Serbina *et al.* demonstrated that inflammatory monocytes are recruited to the spleen in a CCR2 dependent manner (42) following the chemokines CCL2 and CCL3 (41). Serbina *et al.* established the differentiation of these cells into TNF-α/Inducible nitric oxide synthase dendritic cells (TipDCs) within the spleen (69). Through ROS and RNS, these cells kill *Listeria* (43, 44, 70). DCs can traffic to the white pulp of the spleen or T cell zone of lymph nodes. The trafficking to the white pulp of the spleen is a critical step for colonization in the spleen. Edelson *et al.* demonstrated that mice lacking conventional DCs prevented *Listeria's* movement to the white pulp of the spleen (71). Starting at day 3 post infection, the adaptive immune response begins to take control. Since *Listeria* is an intracellular pathogen, the T cell response is the most appropriate adaptive immune response.

The adaptive immune response is critical for Listeria clearance. For example, mice lacking an adaptive immune system initially are more resistant to a L*isteria* infection; however, these mice eventually succumb to the infection (72-74). Recall that cells of the adaptive immune system go through four distinct phases: activation, expansion, contraction, and memory. Mercado *et al.* established the first 24 hours of infects as the time frame for T activation. They observed mice treated with antibiotics to stop the *Listeria* infection before 24 hours had an impaired T cell response (75). Although clonal expansion occurs 24 hours after activation, through the use of IFN-γ intracellular cytokine staining, the T cell expansion is readily detectable within 3 to 4 days, and the peak of T cell response typically occurs 7 to 8 days post-infection. This expansion of effector T cells allows for clearance of the pathogen. As the infection

subsides, a majority of these cells die off, and the remaining cells develop long-lasting memory and are poised to respond to subsequent infection (50, 56, 76).

During a subsequent infection with *Listeria*, the adaptive immune system orchestrates the immune response compared to a primary where innate immune cells orchestrated the immune response. Memory T cells rapidly begin to control the infection within 6 hours (77). The proliferation of effector cells occurs more rapidly, with the peak occurring 3 to 5 days post-infection. Due to the resolution of the infection, these effector cells die off by day 7 post-infection, leaving memory T cells (50, 56, 76, 78-81). Thus a secondary response relies on an adaptive immune response, and this response is more rapid and robust compared to a primary provided an excellent opportunity to study the impact adaptive immunity has during a pathogenic response.

While the mammalian immune response to *Listeria* has been well characterized, one aspect that remains to be explored is immunometabolism, the cellular metabolism of immune cells, upon infection.

Immunometabolism

The need to produce ATP to provide energy for cellular function is essential in both quiescent and activated cells. Glucose can be used to fuel this process through two integrated pathways. The first is glycolysis, which converts glucose to pyruvate producing energy through substrate-level phosphorylation. The second is the tricarboxylic acid (TCA) cycle, which produces energy through oxidative phosphorylation (OXPHOS). These two processes can be integrated by the breakdown of pyruvate to acetyl-CoA, which enters the TCA cycle. Through anaplerotic reactions, cells

can use fatty acid via β-oxidation (FAO) or glutamine via glutaminolysis to replenish the TCA cycle to fuel OXPHOS. Within recent years, our understanding of the cellular metabolism of the immune system has dramatically evolved. It is well established that cells of the immune system differ in cellular metabolism based on their activation state (39, 40, 82).

Quiescent cells of the innate immune system rely on oxidative phosphorylation (OXPHOS) for energy. Upon PRR activation, cells of the innate immune system shift cellular metabolism towards aerobic glycolysis (83-86). Hallmarks of aerobic glycolysis are increased glucose uptake through the glucose transporter (Glut-1) and increased lactate production. Chen, *et al.,* demonstrated that hypoxia-induced factor-1α (HIF-1α) binds to the *glut1* promoter, which leads to the upregulation of Glut-1 (87). Aerobic glycolysis is essential for all activated cells of the innate immune system (86, 88, 89). Everts, *et al*., demonstrated the importance of aerobic glycolysis for effector function using 2-deoxyglucose (2-DG). They observed that LPS induced activation of DCs was significantly impaired in the presence of 2-DG (90). Since these cells are not using the TCA to generate energy, cataplerosis of TCA intermediates occurs. For example, succinate can stabilize hypoxia-induced factor-1α (HIF-1α) which binds to the IL-1β promote inducing the production of pro-IL-1β (91, 92). Additionally, citrate is used for fatty acid synthesis, which in turns is used for membrane biogenesis. For example, DCs rely on membrane production to support antigen presentation to T cells. (86, 90). Activated neutrophils and macrophages rely on the oxidative burst to kill pathogens. The commitment to glycolysis supports these cells respiratory burst through the production of NADPH by two distinct pathways. First, the PPP pathway of glucose catabolism

generates NADPH. Second, these cells use glutaminolysis which uses TCA intermediates and the malate-aspartate shuttle, these cells degrade glutamine to malate. Malate is further oxidized to pyruvate through a $NADP^+$ reaction. Thus, glycolysis is essential for pathogen killing by these cells. Stimulated macrophages and DCs increases the expression of iNOS, which generates nitric oxide (NO). Unfortunately NO through nitrosylation of iron-sulfur clusters in the electron transport chain inhibits OXPHOS requiring these cells to use glycolysis for energy (93, 94). The immunometabolism of cells of the adaptive immune system is complex because quiescence cells upon activation proliferate and differentiate into effector or memory cells.

The energy requirements of naïve T cells are satisfied through FAO and OXPHOS (95, 96). Activation of naïve T cell requires both costimulatory and TCR engagement, and each leads to the activation of different intracellular metabolic pathways. For example, stimulation through the costimulatory receptor CD28 leads to increased aerobic glycolysis in a similar manner observed in innate immune cells (97, 98). Carr, *et al.,* demonstrated that TCR engagement leads to increased glutaminolysis (99). Since both glucose and glutamine are only partially oxidized to generate ATP, these molecules likely do not provide the cell with energy but instead provide T cells with the necessary precursors for macromolecule synthesis required for cellular proliferation (100-102). Chang, *et al.,* completed the understanding of T cell metabolism by demonstrated that activated T cells use OXPHOS for energy (103). Thus, activated T cells rely on glycolysis, glutaminolysis, and OXPHOS for proper function. Effector T cells rely on the increased glycolysis to produce cytokines (82, 98, 101, 104). A hallmark of a successful immune response is the generation of memory cells. Pearce, *et al.*,

through the manipulation of FAO was able to enhance T cell memory (105). Van der Windt, *et al*,. followed up this study and demonstrated that IL-15 was essential for mitochondrial biogenesis, which increases mitochondrial respiratory capacity (106). Thus, memory T cells are dependent on FAO mitochondrial oxidative metabolism (105, 106). Upon reactivation, memory T cells have an initial glycolytic switch, which is required for cytokine production (107).

Excluding the investigation of memory T cells, our understanding of immunometabolism comes from studies that have used either LPS, a PAMP, or non-specific activation of T cells. Our understand of immunometabolism in the context of a pathogen is extremely limited. Thus, the immunometabolism induced by a pathogen remains poorly characterized.

Hypothesis and Goals

The overall hypothesis of this project states that immunocompetence will induce trade-offs among life-history traits (growth, reproduction, and maintenance), and these trade-offs will be driven by the innate immune system rather than the adaptive immune system. Our current understanding of immunocompetence and immunometabolism come from studies that utilized PAMPs, model antigens, or non-specific activation of T cells. Because of this, the metabolism of immune cells using a live pathogen has yet to be well characterized and can add much-needed resolution to preliminary publications mentioned above. Thus, the goal of this dissertation was to determine the immunocompetence and immunometabolism to a live pathogen. To achieve this goal, we longitudinally monitored

the metabolic phenotype, systemic metabolism, and cellular metabolism of mice infected with well-characterized pathogen *Listeria monocytogenes*.

References

1. Lochmiller, R. L., M. R. Vestey, and J. C. Boren. 1993. Relationship Between Protein Nutritional Status and Immunocompetence in Northern Bobwhite Chicks. *The Auk* 110: 503-510.

2. Zuk, M., and A. M. Stoehr. 2002. Immune defense and host life history. *The American naturalist* 160 Suppl 4: S9-s22.

3. Rauw, W. M. 2012. Immune response from a resource allocation perspective. *Frontiers in Genetics* 3.

4. Norris, K. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behavioral Ecology* 11: 19-26.

5. Lochmiller Robert, L., and C. Deerenberg. 2003. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88: 87-98.

6. Demas, G. E., V. Chefer, M. I. Talan, and R. J. Nelson. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 273: R1631-R1637.

7. Otálora-Ardila, A., L. G. Herrera M, J. J. Flores-Martínez, and K. C. Welch. 2016. Metabolic Cost of the Activation of Immune Response in the Fish-Eating Myotis (Myotis vivesi): The Effects of Inflammation and the Acute Phase Response. *PLOS ONE* 11: e0164938.

8. Ots, I., A. B. Kerimov, E. V. Ivankina, T. A. Ilyina, and P. Hõrak. 2001. Immune challenge affects basal metabolic activity in wintering great tits. *Proceedings of the Royal Society of London B: Biological Sciences* 268: 1175-1181.

9. Nilsson, J.-Å., M. Granbom, and L. Råberg. 2007. Does the Strength of an Immune Response Reflect Its Energetic Cost? *Journal of Avian Biology* 38: 488-494.

10. Martin, L. B., A. Scheuerlein, and M. Wikelski. 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270: 153-158.

11. Amat, J. A., E. Aguilera, and G. H. Visser. 2006. Energetic and developmental costs of mounting an immune response in greenfinches (Carduelis chloris. *Ecol Res* 22: 282-287.

12. Bonneaud, C., J. Mazuc, G. Gonzalez, C. Haussy, O. Chastel, B. Faivre, and G. Sorci. 2003. Assessing the Cost of Mounting an Immune Response. *The American naturalist* 161: 367-379.

13. Merlo, J. L., A. P. Cutrera, F. Luna, and R. R. Zenuto. 2014. PHA-induced inflammation is not energetically costly in the subterranean rodent Ctenomys talarum (tuco-tucos). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 175: 90-95.

14. Stockmaier, S., D. I. Bolnick, R. A. Page, and G. G. Carter. 2018. An immune challenge reduces social grooming in vampire bats. *Animal Behaviour* 140: 141-149.

15. Johnson, R. W. 2002. The concept of sickness behavior: a brief chronological account of four key discoveries. *Veterinary Immunology and Immunopathology* 87: 443-450.

16. Dantzer, R. 2001. Cytokine-Induced Sickness Behavior: Where Do We Stand? *Brain, Behavior, and Immunity* 15: 7-24.

17. Aubert, A. 1999. Sickness and behaviour in animals: a motivational perspective. *Neuroscience & Biobehavioral Reviews* 23: 1029-1036.

18. Dantzer, R., and K. W. Kelley. 2007. Twenty years of research on cytokine-induced sickness behavior. *Brain, Behavior, and Immunity* 21: 153-160.

19. Adelman, J. S., and L. B. Martin. 2009. Vertebrate sickness behaviors: Adaptive and integrated neuroendocrine immune responses. *Integr. Comp. Biol.* 49: 202-214.

20. Hart, B. L. 1988. Biological basis of the behavior of sick animals. *Neuroscience & Biobehavioral Reviews* 12: 123-137.

21. Wright, T. M. 1997. Cytokines in acute and chronic inflammation. *Frontiers in Bioscience* 2: d12-26.

22. Arango Duque, G., and A. Descoteaux. 2014. Macrophage cytokines: involvement in immunity and infectious diseases. *Frontiers in immunology* 5: 491-491.

23. Hennessy, M. B., T. Deak, and P. A. Schiml. 2014. Sociality and sickness: Have cytokines evolved to serve social functions beyond times of pathogen exposure? *Brain, Behavior, and Immunity* 37: 15-20.

24. Dantzer, R., C. J. Heijnen, A. Kavelaars, S. Laye, and L. Capuron. 2014. The neuroimmune basis of fatigue. *Trends in Neurosciences* 37: 39-46.

25. Saper, C. B., A. A. Romanovsky, and T. E. Scammell. 2012. Neural circuitry engaged by prostaglandins during the sickness syndrome. *Nature neuroscience* 15: 1088-1095.

26. Ushikubi, F., E. Segi, Y. Sugimoto, T. Murata, T. Matsuoka, T. Kobayashi, H. Hizaki, K. Tuboi, M. Katsuyama, A. Ichikawa, T. Tanaka, N. Yoshida, and S. Narumiya. 1998. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 395: 281-284.

27. Lazarus, M., K. Yoshida, R. Coppari, C. E. Bass, T. Mochizuki, B. B. Lowell, and C. B. Saper. 2007. EP3 prostaglandin receptors in the median preoptic nucleus are critical for fever responses. 10: 1131-1133.

28. Matsumura, K., C. Cao, M. Ozaki, H. Morii, K. Nakadate, and Y. Watanabe. 1998. Brain Endothelial Cells Express Cyclooxygenase-2 during Lipopolysaccharide-Induced Fever: Light and Electron Microscopic Immunocytochemical Studies. *The Journal of Neuroscience* 18: 6279-6289.

29. Schiltz, J. C., and P. E. Sawchenko. 2002. Distinct Brain Vascular Cell Types Manifest Inducible Cyclooxygenase Expression as a Function of the Strength and Nature of Immune Insults. *The Journal of Neuroscience* 22: 5606-5618.

30. Breder, C. D., and C. B. Saper. 1996. Expression of inducible cyclooxygenase mRNA in the mouse brain after systemic administration of bacterial lipopolysaccharide. 713: 64-69.

31. Steiner, A. A., A. I. Ivanov, J. Serrats, H. Hosokawa, A. N. Phayre, J. R. Robbins, J. L. Roberts, S. Kobayashi, K. Matsumura, P. E. Sawchenko, and A. A. Romanovsky. 2006. Cellular and Molecular Bases of the Initiation of Fever. 4: e284.

32. Ueno, R., S. Narumiya, T. Ogorochi, T. Nakayama, Y. Ishikawa, and O. Hayaishi. 1982. Role of prostaglandin D2 in the hypothermia of rats caused by bacterial lipopolysaccharide. *Proceedings of the National Academy of Sciences* 79: 6093-6097.

33. Terao, A., H. Matsumura, H. Yoneda, and M. Saito. 1998. Enhancement of slow-wave sleep by tumor necrosis factor-α is mediated by cyclooxygenase-2 in rats. *NeuroReport* 9: 3791-3796.

34. Matsumura, H., T. Nakajima, T. Osaka, S. Satoh, K. Kawase, E. Kubo, S. S. Kantha, K. Kasahara, and O. Hayaishi. 1994. Prostaglandin D2-sensitive, sleep-promoting zone defined in the ventral surface of the rostral basal forebrain. *Proceedings of the National Academy of Sciences* 91: 11998.

35. Pecchi, E. 2006. Involvement of central microsomal prostaglandin E synthase-1 in IL-1 -induced anorexia. 25: 485-492.

36. Ohinata, K., K. Suetsugu, Y. Fujiwara, and M. Yoshikawa. 2006. Activation of prostaglandin E receptor EP4 subtype suppresses food intake in mice. *Prostaglandins & Other Lipid Mediators* 81: 31-36.

37. Takeuchi, O., and S. Akira. 2010. Pattern Recognition Receptors and Inflammation. *Cell* 140: 805-820.

38. Brubaker, S. W., K. S. Bonham, I. Zanoni, and J. C. Kagan. 2015. Innate Immune Pattern Recognition: A Cell Biological Perspective. *Annual Review of Immunology* 33: 257-290.

39. O'Neill, L. A. J., R. J. Kishton, and J. Rathmell. 2016. A guide to immunometabolism for immunologists. *Nature Reviews Immunology* 16: 553-565.

40. Pearce, E. L., and E. J. Pearce. 2013. Metabolic Pathways in Immune Cell Activation and Quiescence. *Immunity* 38: 633-643.

41. Jia, T., N. V. Serbina, K. Brandl, M. X. Zhong, I. M. Leiner, I. F. Charo, and E. G. Pamer. 2008. Additive Roles for MCP-1 and MCP-3 in CCR2-mediated Recruitment of Inflammatory Monocytes During Listeria monocytogenes Infection. *Journal of immunology (Baltimore, Md. : 1950)* 180: 6846-6853.

42. Serbina, N. V., and E. G. Pamer. 2006. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol* 7: 311-317.

43. Serbina, N. V., T. Jia, T. M. Hohl, and E. G. Pamer. 2008. Monocyte-Mediated Defense Against Microbial Pathogens. *Annual review of immunology* 26: 421-452.

44. Serbina, N. V., C. Shi, and E. G. Pamer. 2012. Monocyte-Mediated Immune Defense Against Murine Listeria monocytogenes Infection. *Advances in immunology* 113: 119-134.

45. Veiga-Fernandes, H., U. Walter, C. Bourgeois, A. McLean, and B. Rocha. 2000. Response of naïve and memory CD8+ T cells to antigen stimulation in vivo. *Nat. Immunol.* 1: 47-53.

46. Homann, D., L. Teyton, and M. B. A. Oldstone. 2001. Differential regulation of antiviral T-cell immunity results in stable CD8+ but declining CD4+ T-cell memory. *Nature Medicine* 7: 913-919.

47. Murali-Krishna, K., J. D. Altman, M. Suresh, D. J. D. Sourdive, A. J. Zajac, J. D. Miller, J. Slansky, and R. Ahmed. 1998. Counting Antigen-Specific CD8 T Cells: A Reevaluation of Bystander Activation during Viral Infection. *Immunity* 8: 177-187.

48. Haring, J. S., V. P. Badovinac, and J. T. Harty. 2006. Inflaming the CD8+ T Cell Response. *Immunity* 25: 19-29.

49. Kaech, S. M., E. J. Wherry, and R. Ahmed. 2002. Effector and memory T-cell differentiation: implications for vaccine development. *Nature Reviews Immunology* 2: 251-262.

50. Badovinac, V. P., B. B. Porter, and J. T. Harty. 2002. Programmed contraction of CD8+ T cells after infection. *Nat. Immunol.* 3: 619-626.

51. Sprent, J., and C. D. Surh. 2002. T CELLMEMORY. *Annual Review of Immunology* 20: 551-579.

52. Masopust, D., and R. Ahmed. 2004. Reflections on CD8 T-Cell Activation and Memory. *Immunologic Research* 29: 151-160.

53. Pennock, N. D., J. T. White, E. W. Cross, E. E. Cheney, B. A. Tamburini, and R. M. Kedl. 2013. T cell responses: naive to memory and everything in between. *Adv Physiol Educ* 37: 273-283.

54. Opferman, J. T. 1999. Linear Differentiation of Cytotoxic Effectors into Memory T Lymphocytes. *Science* 283: 1745-1748.

55. Lakkis, F. G. 2003. Memory T Cells: A Hurdle to Immunologic Tolerance. *Journal of the American Society of Nephrology* 14: 2402-2410.

56. Corbin, G. A., and J. T. Harty. 2004. Duration of Infection and Antigen Display Have Minimal Influence on the Kinetics of the CD4+ T Cell Response to Listeria monocytogenes Infection. *The Journal of Immunology* 173: 5679-5687.

57. D'Orazio, S. E. F. 2019. Innate and Adaptive Immune Responses during Listeria monocytogenes Infection. *Microbiol Spectr* 7.

58. Pamer, E. G. 2004. Immune responses to Listeria monocytogenes. *Nature Reviews Immunology* 4: 812-823.

59. Zenewicz, L. A., and H. Shen. 2007. Innate and adaptive immune responses to Listeria monocytogenes: a short overview. *Microbes and Infection* 9: 1208-1215.

60. Radoshevich, L., and P. Cossart. 2017. Listeria monocytogenes: towards a complete picture of its physiology and pathogenesis. *Nature Reviews Microbiology* 16: 32.

61. Birmingham, C. L., V. Canadien, N. A. Kaniuk, B. E. Steinberg, D. E. Higgins, and J. H. Brumell. 2008. Listeriolysin O allows Listeria monocytogenes replication in macrophage vacuoles. *Nature* 451: 350-354.

62. Portnoy, D. A., P. S. Jacks, and D. J. Hinrichs. 1988. Role of hemolysin for the intracellular growth of Listeria monocytogenes. *The Journal of Experimental Medicine* 167: 1459-1471.

63. Andersson, Å., W. J. Dai, J. P. Di Santo, and F. Brombacher. 1998. Early IFN-γ Production and Innate Immunity During Listeria monocytogenes Infection in the Absence of NK Cells. *The Journal of Immunology* 161: 5600-5606.

64. Humann, J., and L. L. Lenz. 2010. Activation of naive NK cells in response to Listeria monocytogenes requires IL-18 and contact with infected dendritic cells. *J Immunol* 184: 5172-5178.

65. Liu, M., K. Chen, T. Yoshimura, Y. Liu, W. Gong, A. Wang, J.-L. Gao, P. M. Murphy, and J. M. Wang. 2012. Formylpeptide receptors are critical for rapid neutrophil mobilization in host defense against Listeria monocytogenes. *Scientific Reports* 2.

66. Rogers, H. W., and E. R. Unanue. 1993. Neutrophils are involved in acute, nonspecific resistance to Listeria monocytogenes in mice. *Infection and Immunity* 61: 5090-5096.

67. Guleria, I., and J. W. Pollard. 2001. Aberrant macrophage and neutrophil population dynamics and impaired Th1 response to Listeria monocytogenes in colony-stimulating factor 1-deficient mice. *Infect Immun* 69: 1795-1807.

68. Southgate, E. L., R. L. He, J. L. Gao, P. M. Murphy, M. Nanamori, and R. D. Ye. 2008. Identification of formyl peptides from Listeria monocytogenes and Staphylococcus aureus as potent chemoattractants for mouse neutrophils. *J Immunol* 181: 1429-1437.

69. Serbina, N. V., T. P. Salazar-Mather, C. A. Biron, W. A. Kuziel, and E. G. Pamer. 2003. TNF/iNOS-Producing Dendritic Cells Mediate Innate Immune Defense against Bacterial Infection. *Immunity* 19: 59-70.

70. Serbina, N. V., W. Kuziel, R. Flavell, S. Akira, B. Rollins, and E. G. Pamer. 2003. Sequential MyD88-Independent and -Dependent Activation of Innate Immune Responses to Intracellular Bacterial Infection. *Immunity* 19: 891-901.

71. Edelson, Brian T., Tara R. Bradstreet, K. Hildner, Javier A. Carrero, Katherine E. Frederick, W. Kc, R. Belizaire, T. Aoshi, Robert D. Schreiber, Mark J. Miller, Theresa L. Murphy, Emil R. Unanue, and Kenneth M. Murphy. 2011. CD8α+ Dendritic Cells Are an Obligate Cellular Entry Point for Productive Infection by Listeria monocytogenes. *Immunity* 35: 236-248.

72. Newborg, M. F., and R. J. North. 1980. On the mechanism of T cell-independent anti-Listeria resistance in nude mice. *The Journal of Immunology* 124: 571.

73. Lee, C.-C., and J. T. Kung. 2012. Marginal Zone B Cell Is a Major Source of Il-10 in Listeria monocytogenes Susceptibility. *The Journal of Immunology* 189: 3319.

74. Bhardwaj, V., O. Kanagawa, P. E. Swanson, and E. R. Unanue. 1998. Chronic Listeria Infection in SCID Mice: Requirements for the Carrier State and the Dual Role of T Cells in Transferring Protection or Suppression. *The Journal of Immunology* 160: 376.

75. Mercado, R., S. Vijh, S. E. Allen, K. Kerksiek, I. M. Pilip, and E. G. Pamer. 2000. Early Programming of T Cell Populations Responding to Bacterial Infection. *The Journal of Immunology* 165: 6833-6839.

76. Williams, M. A., and M. J. Bevan. 2004. Shortening the Infectious Period Does Not Alter Expansion of CD8 T Cells but Diminishes Their Capacity to Differentiate into Memory Cells. *The Journal of Immunology* 173: 6694-6702.

77. Bajénoff, M., E. Narni-Mancinelli, F. Brau, and G. Lauvau. 2010. Visualizing early splenic memory CD8+ T cells reactivation against intracellular bacteria in the mouse. *PloS one* 5: e11524-e11524.

78. Wirth, T. C., J. T. Harty, and V. P. Badovinac. 2010. Modulating numbers and phenotype of CD8+ T cells in secondary immune responses. 40: 1916-1926.

79. Harty, J. T., and M. J. Bevant. 1995. Specific immunity to listeria monocytogenes in the absence of IFNγ. *Immunity* 3: 109-117.

80. Kägi, D., B. Ledermann, K. Bürki, H. Hengartner, and R. M. Zinkernagel. 1994. CD8+ T cell-mediated protection against an intracellular bacterium by perforin-dependent cytotoxicity. *European Journal of Immunology* 24: 3068-3072.

81. Tripp, C. S., O. Kanagawa, and E. R. Unanue. 1995. Secondary response to Listeria infection requires IFN-gamma but is partially independent of IL-12. *The Journal of Immunology* 155: 3427.

82. Ganeshan, K., and A. Chawla. 2014. Metabolic Regulation of Immune Responses. *Annual Review of Immunology* 32: 609-634.

83. Everts, B., E. Amiel, G. J. W. van der Windt, T. C. Freitas, R. Chott, K. E. Yarasheski, E. L. Pearce, and E. J. Pearce. 2012. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. *Blood* 120: 1422.

84. Krawczyk, C. M., T. Holowka, J. Sun, J. Blagih, E. Amiel, R. J. DeBerardinis, J. R. Cross, E. Jung, C. B. Thompson, R. G. Jones, and E. J. Pearce. 2010. Toll-like receptor–induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 115: 4742.

85. Greiner, E. F., M. Guppy, and K. Brand. 1994. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. *J Biol Chem* 269: 31484-31490.

86. Kelly, B., and L. A. J. O'Neill. 2015. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res* 25: 771-784.

87. Chen, C., N. Pore, A. Behrooz, F. Ismail-Beigi, and A. Maity. 2001. Regulation ofglut1mRNA by Hypoxia-inducible Factor-1. *Journal of Biological Chemistry* 276: 9519-9525.

88. Thwe, P. M., L. Pelgrom, R. Cooper, S. Beauchamp, J. A. Reisz, A. D'Alessandro, B. Everts, and E. Amiel. Cell-Intrinsic Glycogen Metabolism Supports Early Glycolytic Reprogramming Required for Dendritic Cell Immune Responses. *Cell Metabolism* 26: 558-567.e555.

89. Raulien, N., K. Friedrich, S. Strobel, S. Rubner, S. Baumann, M. von Bergen, A. Körner, M. Krueger, M. Rossol, and U. Wagner. 2017. Fatty Acid Oxidation Compensates for Lipopolysaccharide-Induced Warburg Effect in Glucose-Deprived Monocytes. *Frontiers in Immunology* 8: 609.

90. Everts, B., E. Amiel, S. C.-C. Huang, A. M. Smith, C.-H. Chang, W. Y. Lam, V. Redmann, T. C. Freitas, J. Blagih, G. J. W. van der Windt, M. N. Artyomov, R. G. Jones, E. L. Pearce, and E. J. Pearce. 2014. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat. Immunol.* 15: 323-332.

91. Palsson-McDermott, Eva M., Anne M. Curtis, G. Goel, Mario A. R. Lauterbach, Frederick J. Sheedy, Laura E. Gleeson, Mirjam W. M. van den Bosch, Susan R. Quinn, R. Domingo-Fernandez, Daniel G. W. Johnston, J.-k. Jiang, William J. Israelsen, J. Keane, C. Thomas, C. Clish, M. Vander Heiden, Ramnik J. Xavier, and Luke A. J. O'Neill. 2015. Pyruvate Kinase M2 Regulates Hif-1α Activity and IL-1β Induction and Is a Critical Determinant of the Warburg Effect in LPS-Activated Macrophages. *Cell Metabolism* 21: 65-80.

92. Tannahill, G. M., A. M. Curtis, J. Adamik, E. M. Palsson-McDermott, A. F. McGettrick, G. Goel, C. Frezza, N. J. Bernard, B. Kelly, N. H. Foley, L. Zheng,

A. Gardet, Z. Tong, S. S. Jany, S. C. Corr, M. Haneklaus, B. E. Caffrey, K. Pierce, S. Walmsley, F. C. Beasley, E. Cummins, V. Nizet, M. Whyte, C. T. Taylor, H. Lin, S. L. Masters, E. Gottlieb, V. P. Kelly, C. Clish, P. E. Auron, R. J. Xavier, and L. A. O'Neill. 2013. Succinate is an inflammatory signal that induces IL-1beta through HIF-1alpha. *Nature* 496: 238-242.

93. Clementi, E., G. C. Brown, M. Feelisch, and S. Moncada. 1998. Persistent inhibition of cell respiration by nitric oxide: Crucial role of S-nitrosylation of mitochondrial complex I and protective action of glutathione. *Proceedings of the National Academy of Sciences* 95: 7631.

94. Cleeter, M. W. J., J. M. Cooper, V. M. Darley-Usmar, S. Moncada, and A. H. V. Schapira. 1994. Reversible inhibition of cytochrome c oxidase, the terminal enzyme of the mitochondrial respiratory chain, by nitric oxide. *FEBS Letters* 345: 50-54.

95. Maciver, N. J., R. D. Michalek, and J. C. Rathmell. 2013. Metabolic Regulation of T Lymphocytes. *Annual Review of Immunology* 31: 259-283.

96. Rathmell, J. C., M. G. V. Heiden, M. H. Harris, K. A. Frauwirth, and C. B. Thompson. 2000. In the Absence of Extrinsic Signals, Nutrient Utilization by Lymphocytes Is Insufficient to Maintain Either Cell Size or Viability. *Molecular Cell* 6: 683-692.

97. Frauwirth, K. A., J. L. Riley, M. H. Harris, R. V. Parry, J. C. Rathmell, D. R. Plas, R. L. Elstrom, C. H. June, and C. B. Thompson. 2002. The CD28 Signaling Pathway Regulates Glucose Metabolism. *Immunity* 16: 769-777.

98. Jacobs, S. R., C. E. Herman, N. J. MacIver, J. A. Wofford, H. L. Wieman, J. J. Hammen, and J. C. Rathmell. 2008. Glucose Uptake Is Limiting in T Cell Activation and Requires CD28-Mediated Akt-Dependent and Independent Pathways. *The Journal of Immunology* 180: 4476.

99. Carr, E. L., A. Kelman, G. S. Wu, R. Gopaul, E. Senkevitch, A. Aghvanyan, A. M. Turay, and K. A. Frauwirth. 2010. Glutamine Uptake and Metabolism Are Coordinately Regulated by ERK/MAPK during T Lymphocyte Activation. *The Journal of Immunology* 185: 1037.

100. Vander Heiden, M. G., L. C. Cantley, and C. B. Thompson. 2009. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. *Science (New York, N.Y.)* 324: 1029-1033.

101. Pollizzi, K. N., and J. D. Powell. 2014. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. *Nature Reviews Immunology* 14: 435-446.

102. DuPage, M., and J. A. Bluestone. 2016. Harnessing the plasticity of CD4+ T cells to treat immune-mediated disease. *Nature Reviews Immunology* 16: 149-163.

103. Chang, C.-H., Jonathan, Leonard, B. Faubert, Alejandro, D. O'Sullivan, Stanley, Gerritje, J. Blagih, J. Qiu, Jason, Edward, Russell, and Erika. 2013. Posttranscriptional Control of T Cell Effector Function by Aerobic Glycolysis. *Cell* 153: 1239-1251.

104. Varanasi, S. K., D. Donohoe, U. Jaggi, and B. T. Rouse. 2017. Manipulating Glucose Metabolism during Different Stages of Viral Pathogenesis Can Have either Detrimental or Beneficial Effects. *The Journal of Immunology*.

105. Pearce, E. L., M. C. Walsh, P. J. Cejas, G. M. Harms, H. Shen, L.-S. Wang, R. G. Jones, and Y. Choi. 2009. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* 460: 103.

106. van der Windt, G. J., B. Everts, C. H. Chang, J. D. Curtis, T. C. Freitas, E. Amiel, E. J. Pearce, and E. L. Pearce. 2012. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* 36: 68-78.

107. Gubser, P. M., G. R. Bantug, L. Razik, M. Fischer, S. Dimeloe, G. Hoenger, B. Durovic, A. Jauch, and C. Hess. 2013. Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat. Immunol.* 14: 1064-1072.

Chapter 2

The Systemic and Cellular Metabolic Phenotype of Infection and Immune Response to *Listeria monocytogenes*

Abstract

It is widely accepted that infection and immune response incur significant metabolic demands, yet the respective demands of the innate vs. adaptive immune responses have not been well delineated. It has also been established that cellular metabolic pathways are altered in leukocytes upon activation. However, most studies that have demonstrated this metabolic demand at the systemic or cellular level have utilized pathogen associated molecular patterns (PAMPs) or model antigens at isolated time points. Thus, the dynamics of pathogenesis and immune response to a live infection remain largely undocumented. To better quantitate the metabolic demands induced by infection, we utilized a live pathogenic infection model. Mice infected with *Listeria monocytogenes* were monitored longitudinally over the course of infection through clearance. We measured systemic metabolic phenotype, bacterial load, innate and adaptive immune responses, and cellular metabolic pathways. To further delineate the role of innate vs. adaptive immunity in the metabolic phenotype, we utilized two doses of bacteria, one that induced both sickness behavior and protective immunity, and the other protective immunity alone. We determined that the greatest impact to systemic metabolism occurred during the early immune response. Additionally, this time corresponded to the greatest shift in cellular metabolism of cells of the innate immune

system. During the time of maximal adaptive immune response (T cell expansion) we observed the return to resting state in systemic metabolism. Taken together, our findings implicate the innate immune system as more metabolically demanding than the adaptive immune response.

Introduction

One of the central postulates of life history theory is that certain finite resources must be allocated between growth, reproduction and maintenance over an animal's lifespan. (1, 2). With limited resources, competitive trade-offs will occur when the demand for one trait is greater than the others (1, 2). Several studies have identified such trade-offs that occur between immunity, growth, and reproduction (1, 2). In one study, house sparrows challenged with systemic Lipopolysaccharide (LPS) exhibited a decrease in weight, activity, and reproduction (3). In another study, investigators observed increased basal metabolic rate ($V0_2$) following keyhole limpet hemocyanin (KLH) challenge (4). While many such studies have highlighted systemic trade-offs that occur during model immune responses, we still have much to learn about such tradeoffs during live infection. First, the use of pathogen associated molecular patterns (PAMPs), model antigens, or mitogens cannot replicate the stages of live infection (invasion, replication, spread, infection-induced pathology). Secondly, these studies model systemic infections such as sepsis, and thus fail to represent localized infection and inflammation. Finally, many of the metabolic measurements have been performed at selected timepoints (removing animals from home caging for metabolic measurements, inducing stress), not longitudinally throughout the infection and immune response. To better elucidate the metabolic demands and trade-offs that occur from initial infection through clearance, the systemic metabolism of the host should be monitored longitudinally during live pathogen infection and clearance.

Listeria monocytogenes is a Gram positive intracellular foodborne pathogen. It's pathogenicity and immune response have been well documented (5-7). In a dose range

from $2x10^3$- $2x10^4$ (8, 9), protective T cell responses develop, with little observable sickness at the low end while measurable, but non-life-threatening illness is observed at the high end (8, 9). During the early phase following infection, a robust innate immune response is observed consisting of inflammatory cytokine production and recruitment of neutrophils and monocytes to the liver and spleen (5, 10-16). Bacterial colonization in the spleen and liver also peaks during this phase (13, 14, 17). On the heels of the innate response, the adaptive immune response (predominantly T cells) undergoes rapid expansion (5, 8, 18, 19). The *Listeria*-specific T cell response peaks around day 7-9 then undergoes contraction, establishing a memory population by day 14 (8, 18-20). While *L. monocytogenes* infection has been well characterized, the metabolic status of cells responding to this infection remains to be determined.

Under resting conditions, cells primarily use oxidative phosphorylation (OXPHOS) for energy when oxygen is available. Under anerobic conditions, cells utilize fermentation for energy. However, in 1924 Dr. Otto Warburg observed unique metabolic patterns in cancer cells. He determined that cancerous cells use lactic acid fermentation for energy production in the presence or absence of oxygen (21, 22), a phenomenon termed the "Warburg" effect (23). In recent years, there has been a surge in the study of cellular metabolic status of immune cells, a field now known as immunometabolism (24, 25). Several studies have now established that cells of the immune system differ in metabolic processes based on their activation state (24, 25). Quiescent cells of the innate immune system primarily use OXPHOS for energy, however, upon Toll Like Receptor activation, these cells shift to glycolysis (26-28). Cells of the adaptive immune system are more complex. Naïve cells primarily utilize OXPHOS for energy production. Upon

activation, if the cell becomes an effector cell, it shifts towards glycolysis for energy (29, 30). However, if the cell becomes a memory cell, it will primarily use fatty acid oxidation (31, 32). While these changes in cellular metabolism of the immune system have now been well documented, most of these studies used either PAMPs or non-specific T cell activation, thus, the immunometabolic changes during live infection remain poorly understood.

Our present study aims to provide an integrated examination of systemic and cellular metabolism over the time course of a pathogenic infection with *L. monocytogenes.* We determined that changes in systemic metabolism occurred only above a threshold level of infection, and these changes occurred simultaneously with the innate immune response. We also observed a shift in cellular metabolism in cells of the innate immune system during this time. Thus, it is likely that the innate immune response is the most metabolically impactful on systemic and cellular metabolic phenotype. Additionally, in our infection model, the adaptive immune response did not cause detectable changes in host behavior or systemic metabolism yet induced protective immunity.

Materials and Methods

Mice

C57BL/6J mice were obtained from The Jackson Laboratory. The mice used in these studies were between 8 and 12 weeks old and were age-matched for each experiment. All mice were maintained in a specific pathogen-free (SPF) facility and in full compliance

with the Institutional Care and Use Committee of Auburn University regarding the use of animals.

Listeria monocytogenes I*nfection and Bacterial Enumeration*

Wild type *Listeria monocytogenes* (Lm-10403s) was grown in brain-heart infusion (BHI) broth overnight at 37°C to an OD_{600} of 1.0. The overnight culture (1ml) was centrifuged, resuspended in PBS, and washed twice in PBS. Mice were injected intraperitoneally with either $2x10^4$ CFU/mouse (High Dose) or $1x10^4$ CFU/mouse (Low Dose) or an equal volume of PBS diluent (uninfected control). The infectious dose was confirmed by plating dilutions of the inoculum on BHI agar, and colonies were counted after incubation at 37°C for 18-24 h. Spleens and livers were homogenized and lysed in sterile dH_2O, serial dilutions of the homogenates were plated on BHI agar, and colonies were counted after incubation at 37°C for 18-24 h.

Metabolic Phenotyping, Food, and Water Intake

To assess metabolic phenotype, Promethion metabolic cages (Sable Systems, Las Vegas, NV) were used as previously described (33, 34). Briefly, animals were individually housed in the metabolic cages throughout the 12-day experiment. Activity was measured by Promethion XYZ Beambreak Activity Monitors and was determined by consecutive adjacent beam breaks in the X, Y and Z planes. Quiet bouts were defined as no engagement in locomotion, eating, drinking, or grooming for 40 seconds, while sleep was determined as a quiet bout lasting for greater than 40 seconds.

Food, water and body mass were measured by Promethion MM-1 Load Cell sensors. The amount, frequency, duration and rate at which food and water were withdrawn from the hoppers were measured and analyzed. The body mass monitors were plastic tubes that also functioned as in-cage enrichment and nesting devices.

Respiratory gases were measured by the Promethion GA-3 gas-analyzer which measured water vapor, CO_2 and O_2 in mL/min. Energy expenditure was calculated using the Weir equation (35): kcal/h = $60 \times (0.003941 \times \dot{V}O_2 + 0.001106 \times \dot{V}CO_2)$. Respiratory Exchange Ratio (RER) was calculated as the ratio of $\dot{V}CO_2/\dot{V}O_2$ where a RER of about 0.7 indicates pure lipid utilization and a RER of about 1.0 indicates pure carbohydrate utilization. Data acquisition and system control were coordinated using MetaScreen v. 2.2.8, and the obtained raw data were processed using ExpeData v. 1.9.14 (Sable Systems) and Universal Macro Collection v. 10.1.11.

Listeria-Specific T Cell Enumeration

Bone marrow derived dendritic cells (BMDCs) were generated as previously described (36). Day 7 BMDCs were used as antigen presenting cells in the T cell activation assay. BMDCs were infected with *Lm*-10403s at a MOI of 1, and after 1 hour, 20 μg/mL gentamicin (VWR) was added to the culture to inhibit bacterial replication. The cells were incubated for 24 hours at 37℃ with 5% CO_2. Splenocytes were cocultured with DCs at a ratio of 10:1 for 5 hours in the presence of GolgiStop (monensin) (BD Biosciences). Cells were washed in FACS buffer (PBS supplemented with 3% FBS) and were incubated with AF488-anti-CD3 at 4℃ for 10 min. The cells were washed in FACS buffer twice then fixed and permeabilized (BD CytoFix/CytoPerm) by incubating for 20

min at 4°C. The cells were washed in Perm/Wash buffer and incubated with PE-anti-IFN-γ for 30 min at 4°C. Cells were washed twice in Perm/Wash buffer and resuspended in FACS buffer before flow cytometric analysis.

Glucose Transporter-1 Detection

Splenocytes were washed twice in FACS buffer and were incubated with PE-Cy7-anti-Ly6C, AF488-anti-CD3, and AF647-anti-Glut-1 at 4°C for 10 min. Cells were washed twice in FACS buffer and resuspended in FACS buffer before flow cytometric analysis. The level of fluorescence was determined by flow cytometry using a BD Accuri™ C6 flow cytometer and analyzed using FlowJo® software.

Glucose Uptake

The fluorescently-labeled glucose analog, 2-N-(7-nitro-benz-2-oxa-1, 3-diazol-4-yl) amino)-2 deoxyglucose, (2-NBDG) (VWR) was used as a proxy of glucose uptake. Splenocytes were washed twice in RPMI-1640 medium and treated with 2-NBDG at 37°C with 5% CO_2 for 30 min. Cells were washed twice in FACS buffer, incubated with PeCy-7 anti-CD11b at 4°C for 10 min., washed twice in FACS buffer and resuspended in FACS buffer for cytometric analysis. The level of fluorescence was determined by flow cytometry using a BD Accuri™ C6 flow cytometer and analyzed using FlowJo® software.

Statistical Analysis

Statistical analyses were performed using Prism Software, version 8 (GraphPad). Results are presented as mean +/- SD, and significance was determined using a one-way ANOVA followed by a Dunnett's post hoc test. Asterisks denote level of statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, and ****$p < 0.001$). Linear regressions were calculated in R studio. The regression of Activity and VO_2 included the variables Group and Time. Control and High Dose activity and VO_2 were used to generate the graphs in Prism Software, version 8 (GraphPad).

Antibodies

Antibodies	Source	Identifier
PE-Cy7-anti-Ly6C	BioLegend	Catalog: 128018 Clone: HK1.4
AF488-anti-CD3	BD Biosciences	Catalog: 557666 Clone: 145-2C11
AF647-anti-Glut-1	Novus Biologicals	Catalog: NB110-39113AF647
PECy7-anti-CD11b	BioLegend	Catalog: 101216 Clone: M1/70
PE-anti-IFN-γ	BioLegend	Catalog: 505808 Clone: XMG1.2

Results

Metabolic Phenotype

To evaluate the impact of bacterial infection and immune response on the systemic metabolic phenotype of the host, we infected mice with one of two doses of *Lm* and compared their metabolic phenotype to that of uninfected mice. Previous studies have observed that a high bacterial dose ($2x10^4$ CFU/mouse) induces a moderate illness that resolves within 4-5 days, ultimately conferring protective immunity (8, 9), while a lower dose (10^4 CFU/mouse) also confers protective immunity, but without overt signs of illness. For the infections in the current study, we used a wild type strain of *Listeria*

monocytogenes, 10403s, and monitored the mice for 12 days in Promethion® metabolic cages to assess multiple physiological and behavioral parameters, collectively known as metabolic phenotype. These parameters included body mass, activity, sleep, VO_2, VCO_2, Respiratory Exchange Rate (RER), and Energy Expenditure (EE). The metabolic data were averaged for each group of mice over each 12-hour period, corresponding to either the dark cycle (active period), or the light cycle (inactive period).

We first examined changes in body mass induced by infection. Mice infected with the higher bacterial dose lost significant weight beginning at day 1 and continued to lose weight until day 4, ultimately losing a total of ~11% of their body mass (Fig. 2.1). Mice infected with the higher dose of *Lm* did not recover to their initial weight until day 7.5 and even then, gained weight more slowly than their uninfected and lower-dose infected counterparts. In fact, mice infected with the higher dose of *Lm* demonstrated significant differences in weight vs. uninfected mice from days 1 through 12 (Fig. 2.1). The duration and magnitude of weight loss in the mice infected with the lower dose of *Lm* was significantly less than those infected with the higher dose. Lower dose-infected mice lost only ~2% of their body weight, with maximal loss around day 2, and recovered to their starting weight by day 3.5. The average body mass of this group was significantly different from uninfected mice only from days 2-3.5. Thus, we observed a dose-dependent loss in body mass in mice infected with *Lm* and a sustained slowing of body mass recovery in mice infected with the higher dose.

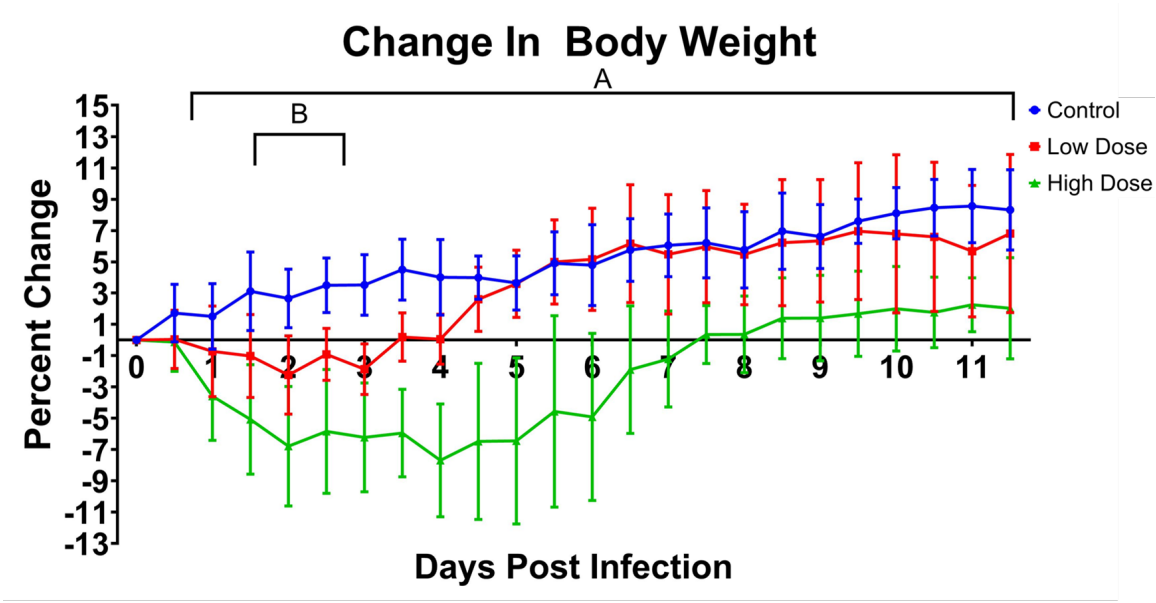

Figure 2.1: Infection induced change in body weight.
Analysis of change in body weight for a 12-hour light dark cycle over the 12-day experimental period. Significance was assessed using one-way ANOVA followe. A indicates a significant between High Dose - Control and B indicates a difference between Low Dose - Control at a p =/< 0.05.

The next parameters examined were activity and sleep (Fig. 2.2). Activity was measured in total meters, averaged across each group over each 12h period. As expected, uninfected mice were much more active during the dark cycle than the light, establishing a control level of activity. We also observed no differences in activity at any time point between uninfected control animals and mice infected with the lower dose of *Lm* (Fig. 2.2A). However, there was a marked reduction in the activity of mice infected with the higher dose of *Lm* (~ 40-50% of control) during the active cycles of days 1, 2, and 3 as well as in the inactive cycle of day 4. Activity returned to control levels by the active cycle of day 5 in these mice (Fig. 2.2A).

Conversely, mice infected with the higher dose of bacteria demonstrated increased time spent in quiet/sleep compared to control mice (Fig. 2.2B). These mice slept significantly more than uninfected or low dose-infected mice during the active periods of days 1, 2, 3, and 4, but resumed normal levels of sleep by day 5. Again, no significant differences were observed between uninfected and low dose infected mice in sleep at any time point (Fig. 2.2B). Thus, in addition to weight loss, mice infected with the higher dose of *Lm* experienced markedly reduced activity and more time spent in sleep, typical symptoms of illness or sickness behavior (37, 38). However, even though the lower dose of *Listeria* is known to induce a strong T cell response and protective immunity (Fig. 2.6) this level of infection induced no detectable changes in activity or sleep at any time.

We next wanted to determine whether infection impacted systemic metabolic parameters including the exchange rates of O_2 and CO_2 under each condition (Fig. 2.3). We observed a significant drop in both VO_2 and VCO_2 in the high dose-infected group

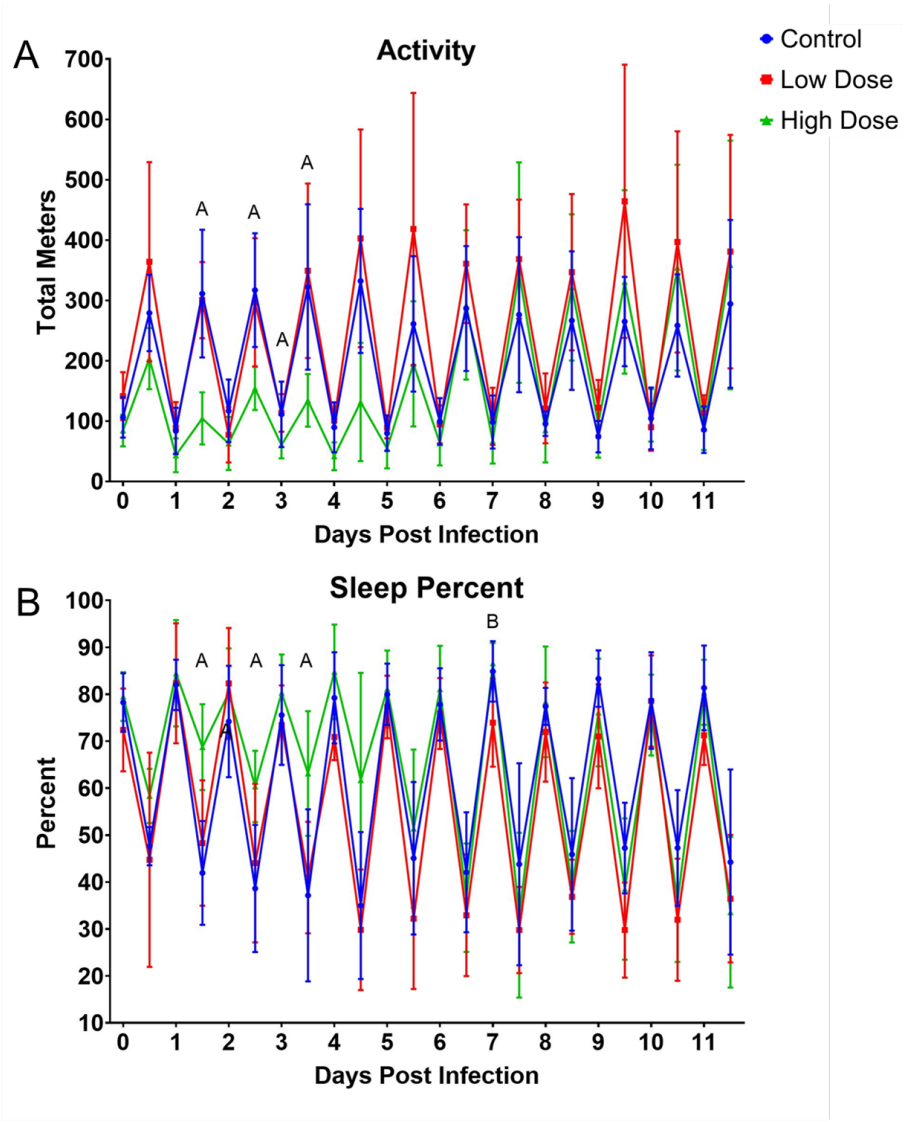

Figure 2.2: Infection induced lethargy.
Analysis of change in activity **(A)** or sleep **(B)** for a 12-hour light dark cycle over the 12-day experimental period. Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD. A indicates a significant between High Dose - Control and B indicates a difference between Low Dose - Control at a p =/< 0.05.

vs. control beginning during the active period of day 1 and continuing through the active period of day 4 (Fig. 2.3 A & B). Interestingly, the drops observed in VO_2 and VCO_2 in the high dose-infected group were observed even during several inactive periods in this group between days 3 and 8. Notably, there were no significant differences in VO_2 or VCO_2 at any time point between mice infected with the lower dose of *Lm* and control mice (Fig. 2.3 A & B).

To determine if infection and immune response altered carbon substrate utilization and/or overall energy expenditure, we determined the Respiratory Exchange Rate (RER, VCO_2/VO_2) and calculated energy expenditure using the Weir equation, adjusted for body mass (Fig. 2.3 C & D). RER was significantly reduced in mice infected with the high dose of *Lm* from day 1.5 to day 2, indicating a shift toward increased lipid utilization during this time (Fig. 2.3C). A similar shift toward enhanced lipid utilization was also observed at day 2 in the low-dose infected mice. There was also a significant decrease in overall energy expenditure in mice infected with the higher *Lm* dose, beginning on day 0.5 and continuing through day 8. This reduced energy expenditure was significant during both active and inactive periods (Fig. 2.3D). There were no significant differences in energy expenditure between mice infected with low dose *Lm* and uninfected controls (Fig. 2.3D).

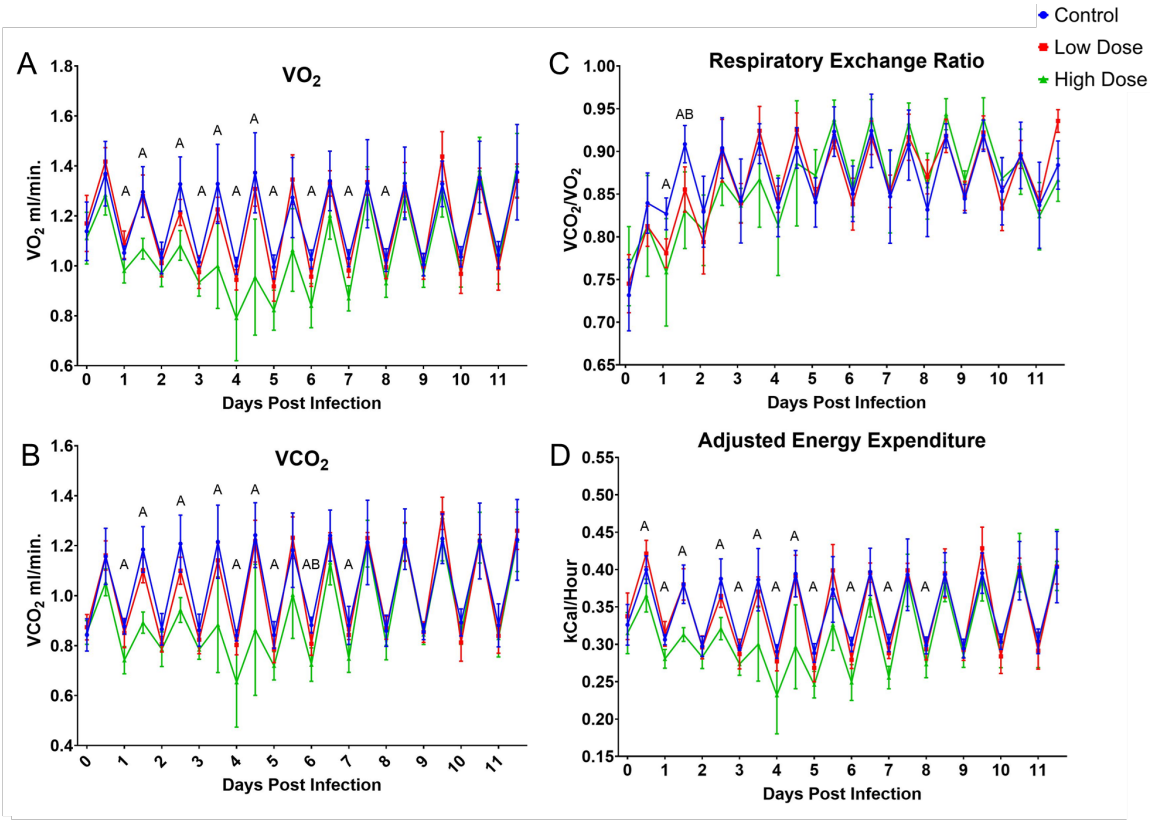

Figure 2.3: Infection induced changes in respiratory gases and Energy Expenditure.
Analysis of change in **(A)** metabolic rate, **(B)** VCO_2, **(C)** Respiratory Exchange Ration (VCO_2/VO_2), **(D)** Energy Expenditure (EE) for a 12-hour light dark cycle over the 12-day experimental period. The Weir Equation was used to calculate EE (kcal/h = $60 \times (0.003941 \times VO_2 + 0.001106 \times VCO_2)$), and an ANCOVA was utilized to adjust EE for bodyweight. Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. A indicates a significant between High Dose - Control and B indicates a difference between Low Dose - Control at a p =/< 0.05.

Metabolic Rate and Activity

Infection and sickness have been shown to raise basal metabolic rate in numerous studies (4, 39). However, our results demonstrated an overall decrease in energy expenditure in infected animals, likely due to greatly decreased activity. Thus, we sought to examine the relationship between activity and $V0_2$ in infected and uninfected animals using a linear model. Since the most pronounced differences in systemic metabolic profile between infected and uninfected groups occurred during the active (night) cycle we used these values to examine the relationship between activity and $V0_2$.

Prior to infection, a positive relationship between activity and $V0_2$ was observed in the control and pre-infected mice, with no significant difference between groups (Fig. 2.4A & Table 2.1). In contrast, we observed a significant difference in metabolic rate between the groups at 4 days post infection in the night (active period) when the greatest differences in metabolic rates over the course of the infection were observed between the control and infected mice. (Fig. 2.4B & Table 2.1). Beginning at night 5, the systemic metabolic rate between control and infected mice began to resolve; and we no longer observed a group effect (Fig. 2.4C & Table 2.1). Finally, by night 6 when the metabolic rate of infected mice returned to control level, so did the relationship between activity and metabolic rate (Fig. 2.4D & Table 2.1). Over the course of the infection, the relationship between activity and metabolic rate changed. During the period of greatest differences observed in metabolic rate between control and infected mice (night 4), we observed a trend of increased slope in the infected mice. These results indicate that increased activity of infected mice was more metabolically demanding compared to control.

Table 2.1: Results of Linear Regression

	Pre-Infection			Night 4			Night 5			Night 6		
Coefficients	Estimate	Std. Error	p-value	Estimate	Std. Error	p-value	Estimate	Std. Error	p-value	Estimate	Std. Error	p-value
Activity	0.0011104	0.0002726	0.00473	0.0010835	0.0002839	0.00657	0.0011884	0.0004142	0.024024	0.0013347	0.0004340	0.017932
Group	0.3094273	0.2393017	0.23703	-0.4101615	0.1710334	0.04760	-0.2900857	0.1702388	0.132159	-0.0570628	0.1663601	0.741663
Interaction	-0.0007672	0.0005806	0.22787	0.0021209	0.0010396	0.08070	0.0008401	0.0007003	0.269327	-0.0003321	0.0006810	0.640658

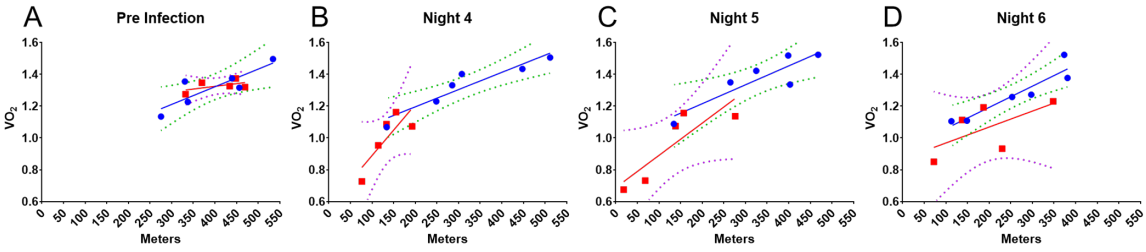

Figure 2.4: Linear Regression of Activity and VO_2 during the course of the infection.
Linear Regression at **(A)** Pre-infection, **(B)** Night 4 post infection, (**C**) Night 5 post infection, & (**D**) Night 6 post infection. Dotted regions represents the 95% Confidence interval. Control (n=6), Infected (n=5)

Kinetics of Bacterial Colonization and T Cell Response

To determine how the kinetics of systemic metabolism corresponded to bacterial burden and to the anti-*Listeria* immune response, groups of conventionally housed mice were infected (simultaneously with those housed in metabolic cages), again with either $1x10^4$ CFU/mouse or $2x10^4$ CFU/mouse of *Lm*-10403s, in addition to the uninfected controls. These animals were sacrificed over the course of fourteen days post infection to monitor bacterial burden in the spleen and liver as well as the *Listeria*-specific T cell response in the spleen (Fig. 2.5 & 2.6).

The bacterial burden was maximal for both infection groups at day 3 in the spleen and day 5 in the liver (Fig. 2.5 A & B). Yet, the level of colonization was dramatically higher in the high dose-infected group vs. the low dose group. The mice infected with the low dose showed clearance of bacteria from the spleen after day 5 and after day 7 in the liver. The kinetics of clearance were slightly slower for the high dose-infected group with evidence of colonization at day 7 in the spleen and out to day 10, and 14 in the liver (in one animal per group). Thus, though the inoculating doses were only different by two-fold, the colonization was dramatically higher and took longer to clear in the high dose group. Perhaps the higher dose exceeded a threshold of control, requiring more time and perhaps more immune mechanisms for clearance.

We also examined the *Listeria*-specific T cell response to determine if the kinetics and magnitude of this response were significantly different at the two infectious doses (Fig. 2.6). Splenocytes were cultured in the presence of syngeneic *Listeria*-infected DC to measure the *Listeria*-specific T cell response in the form of IFN-γ production.

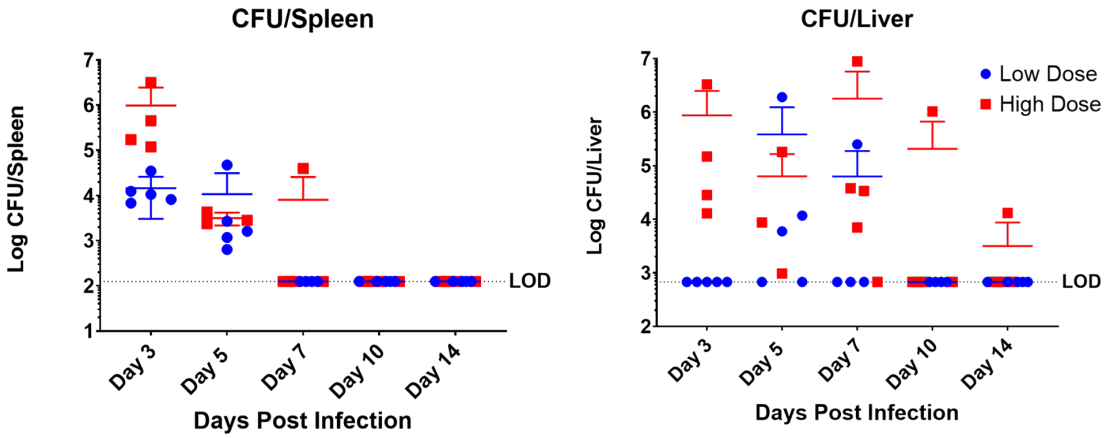

Figure 2.5: Bacterial Enumeration in the Spleen and Liver.
Bacterial burden at various time points following a *Listeria* infection in the spleen (**A**) and liver (**B**). LOD indicated limit of detection. Data are represented as mean ± SD.

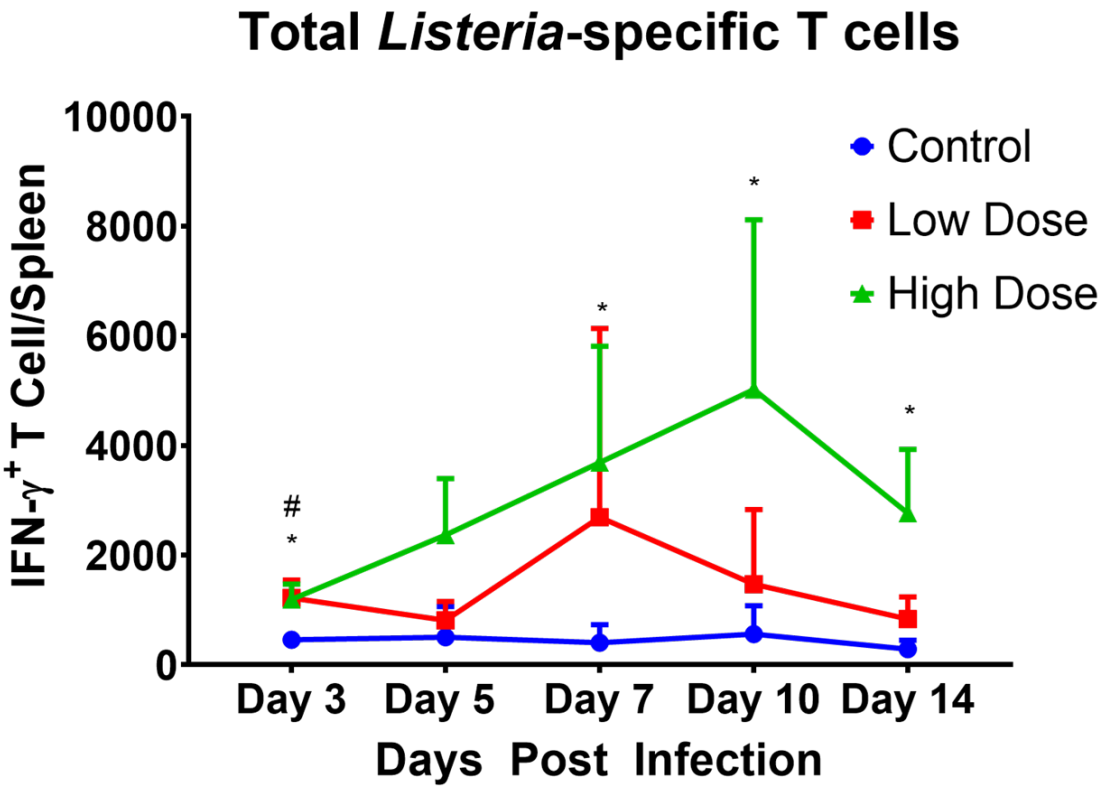

Figure 2.6: Enumeration of *Listeria*-specific T cells in the Spleen. *Listeria*-specific IFN-γ producing T cells were enumerated at various time points post infection in the spleen following a *Listeria* infection. Statistical analysis was performed by a Mixed-effect analysis followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD. (* $p < 0.05$, High dose compared to control # $p < 0.05$, Low doe compared to control)

Measurement of IFN-γ production was enabled by intracellular cytokine staining and flow cytometry compared to splenocytes from control (uninfected) mice.

The observed peak of the *Lm*-specific T cell response was at day 7 in the low dose infected group and day 10 in the high dose group (Fig. 2.6). While the peak responses occurred on different days, and there was a trend toward higher numbers of *Lm*-specific T cells in high dose infected group, there were no significant differences between the infected groups in the number of *Lm*-specific T cell over the course of the infection.

Changes in Cellular Metabolic Status upon Infection

To determine whether changes in systemic metabolic phenotype correspond to changes in immune cellular metabolism over the course of infection, we measured the expression of the glucose transporter (Glut-1) on myeloid cells and T cells. These cell types were identified by Ly6C or CD3-costaining. The Glut-1 transporter is up-regulated on many cell types after activation and is used as an indicator of cells shifting into a state of aerobic glycolysis (27, 29). Given that the high dose infection group displayed the most significantly different metabolic phenotype (systemic metabolism), we monitored the cellular metabolic status of this group compared to the uninfected controls.

There was a dramatic increase in the percentage of $Ly6C^+$ cells in the spleen of infected mice at day 3 and 5 post infection (Fig 2.7A). While the percentage decreased from day 5 to day 10, it remained above control before returning to control level by day 14 (Fig. 2.7A). The percentage of $Ly6C^+$ cells expressing Glut-1 peaked on day 3, decreased on day 5, and return to control level on day 7 post infection (Fig 2.7B).

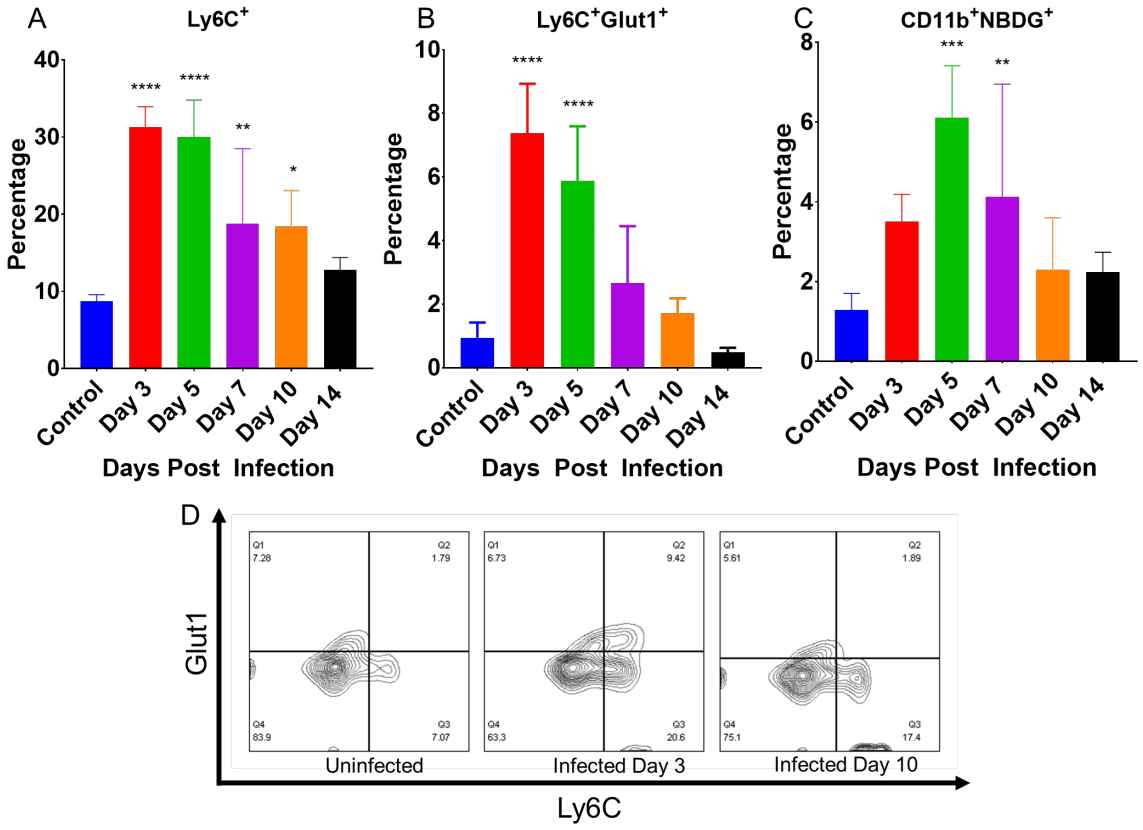

Figure 2.7: Innate immune cell recruitment and shifts in cellular metabolism by cells of the innate immune system. **(A)** Ly6C$^+$ expression over the course of the infection **(B)** Glut1$^+$ expression on Ly6C$^+$ over the course of the infection **(C)** Glucose uptake by CD11b$^+$ cells over the course of the infection. **(D)** Representative contour flowcytometric plot of the expression of Glut1 on Ly6c$^+$ cells. Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.0001$.)

The extent of these changes can be visualized in Fig. 2.7D in the flow cytometric contour plots. Fig. 2.7D shows an increase in $Ly6C^+$ cells in infected mice (bottom right quadrant) and the increased expression in Glut-1 by these cells (top right quadrant). This change in metabolic status of myeloid cells coincides with the majority of changes in systemic metabolic phenotype. To further confirm the shift in cellular metabolism of myeloid cells, we measured glucose uptake by myeloid cells though the use of a fluorescent glucose analog 2-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)Amino)-2-Deoxyglucose (2-NBDG). The uptake of 2-NBDG by myeloid cells peaked on day 3 and decreased over the course of the infection and finally returned to control level by day 14 (Fig. 2.7D). Taken together, these findings highlight the recruitment of myeloid cells to the spleen during *Lm* infection and demonstrate that these cells utilize aerobic glycolysis during this response, based on an increase in glucose transporter expression and increased glucose uptake (Fig. 2.7 B&C).

We next examined the metabolic status of T cells by measuring expression of Glut-1 (Fig. 2.8). Fig. 2.8A depicts the percentage of T cells ($CD3^+$) over the course of the infection. We initially observed a decrease in the percentage of T cells at day 3 continuing until day 5 post infection (Fig. 2.8A). This is expected as *Listeria* infection is known to induced T cell apoptosis (40, 41). By Day 7 post infection, the percentage of T cells began to increase (Fig. 2.8A). On day 10 post infection, the percentage of T cell returned to control (Fig. 2.8A). Day 14 infected mice had a greater percentage of T cell compared to control (Fig. 2.8A). Fig. 8C depicts the expression of Glut-1 on $CD3^+$ T cells while Fig. 2.8B is representative of several compiled, independent experiments.

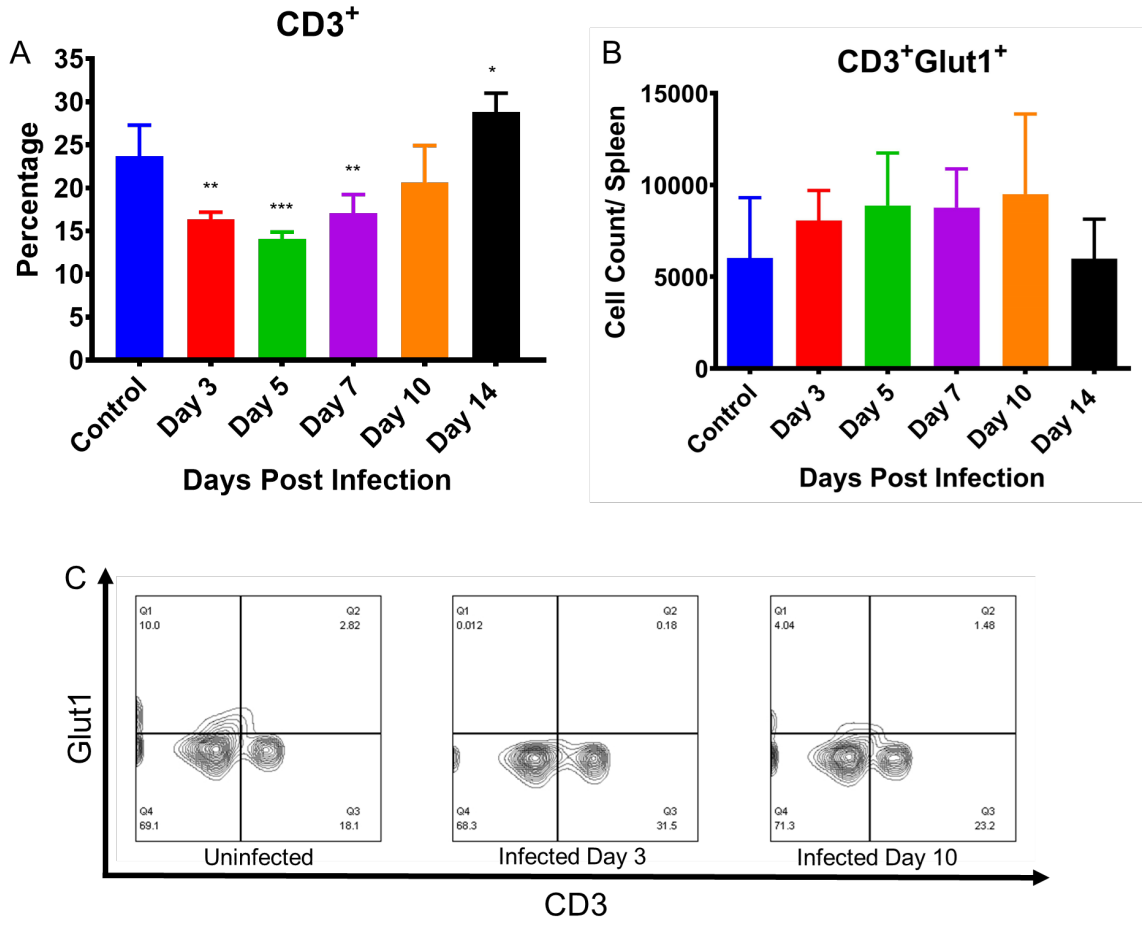

Figure 2.8: Glut1 Expression on the cells of the adaptive immune system.
(A) T cell ($CD3^+$) expression over the course of the infection. **(B)** Glut1 expressing on $CD3^+$ (T cells) over the course of the infection. **(C)** Representative contour flowcytometric plot of the expression of Glut1 on T cells. Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD. (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$.)

We observed little change in the number of T cells expressing Glut-1 and only a modest increase in Glut-1 expression on T cells over the course of the infection, none of which were significantly different when compared to control (Fig. 2.8B).

Discussion

To our knowledge, our study is the first to simultaneously monitor the systemic and cellular metabolic phenotype in a live infection using a low and high bacterial burden. The longitudinal analysis of metabolic phenotype over the entire course of infection and primary immune response also provided a detailed assessment of the metabolic changes when compared to previous studies in which metabolic rates were measured as single time points. These combined analyses provided new insights into the role of live infection modulating behavior and metabolism. Our findings lead us to conclude that for *Lm* infection, the innate immune response induces significant metabolic changes at the systemic and cellular levels, leading to decreased activity and energy expenditure, likely the driver of trade-offs. Additionally, we discovered that the adaptive immune response to this intracellular bacterium, while advantageous, does not present a detectable metabolic burden to the host.

Mice infected with the high dose of *Listeria* exhibited sickness behavior characterized by weight loss and lethargy starting as early as 1 day after infection (Fig. 2.1&2). Coinciding with this, we observed a decrease in RER on day one and two post infection, indicating preferential lipid utilization (Fig. 2.3C). This corresponds directly to the well-established timing of inflammatory cytokine production including TNF-α, IL-1 and IL-6 (10-16). TNF-α has been shown to induce insulin resistance (42, 43). We

observed a modest insulin resistance in infected mice at day 3 post infection (data not shown). It is possible that the mice were insulin resistant before day three, and this insulin resistance could cause infected mice to preferentially utilize lipids as an energy source since carbohydrate utilization was reduced (Fig. 2.3C). Thus, it is possible that the systemic pro-inflammatory cytokines produced initially after a *Listeria* infection are the driving force behind the whole-body insulin resistance, causing a preferential use of lipids over carbohydrates (10-16).

Our study comprehensively assesses the cost of immunity by monitoring behavioral and metabolic changes (metabolic phenotype) longitudinally over the course of infection and immune response to a live pathogen. Three phases of the infection and response emerge from our metabolic and immune measurements: early (day 0-4), intermediate (day 5-8), and late stages (day 9-14). The early stage consists of innate immunity only. During the intermediate stage innate immune response is returning to baseline and adaptive immunity is expanding and becoming detectable. Finally, the late stage is characterized by adaptive immunity and return to baseline for this response as well.

In the early phase, the mice infected with the higher bacterial dose had decreased activity accompanied by increased sleep (Fig. 2.2). Additionally, we observed a decrease in their systemic metabolism an energy expenditure (Fig. 2.3 A&D). Since we observed significant differences between the mice infected with higher bacterial dose and control, we wanted to investigate their immune response. We observed monocyte recruitment to the spleen and their activation through the upregulation of Glut-1 transporter and increased uptake of glucose (Fig. 2.7 B-D). This was likely due to their interaction with

Listeria or the inflammatory environment induced by infection. It was surprising that such different responses were observed in the two groups given that the inoculating doses were only two-fold different. However, when we examined the relative bacterial loads at day 3, we observed that the mice were not able to control the higher dose of *Listeria* and colonization increased by several-fold (Fig. 2.5A). We observed minimal changes to the behavior and systemic metabolism of mice infected with the low dose of *Listeria*, suggesting that perhaps below a certain threshold of infection, no signs of sickness behavior (hypometabolic state, anorexia and lethargy) are demonstrated. The higher dose of *Listeria* caused trade-offs to occur likely to provide energy for immunity.

During the intermediate stage, the mice had generally cleared the infection (Fig. 2.5). The mice infected with the high dose of *Listeria* continued to exhibit a hypometabolic state (decreased VO_2), but no longer showed sickness behaviors of anorexia and lethargy (Fig. 2.1, 2.2, & 2.3A). We observed the peak uptake of glucose in innate immune cells and the waning of Glut-1 expression on myeloid cells during this phase (Fig 2.7 B&C). We observed a *Listeria*-specific T cell response in both low and high dose-infected mice which peaked at day 7 or 10; however, we did not observe detectable Glut-1 upregulation on T cells at any timepoint at the high infectious dose (Fig. 2.6 & 2.8A). The persistent hypometabolic state may facilitate shifting energy to cells of the innate immune system in aerobic glycolysis since they have a higher demand for energy (24, 25).

The final stage of *Listeria* infection, days 9-14, is characterized by the contraction of adaptive immune response and the return to homeostasis (5-7). During this stage the effector antigen-specific T cells go through contraction to establish a level of protective

immunity for future challenges (8, 18, 19). In our study, we observed cells of the innate immune system return to control Glut-1 expression and glucose uptake levels (Fig. 2.7 B&C). The *Listeria*-specific T cells went through contraction (Fig. 2.6). Based on the return of animals to normal metabolic parameters during this phase, we conclude that this phase of the immune response carries little metabolic demand (Fig. 2.2 & 2.3A). This data further supports the idea of the innate immune system being the driver of sickness behavior and hypometabolic state due to the lack of observable differences in metabolic phenotype between infected and control when innate immunity is quiescent or conversely, when adaptive immunity is active.

An interesting finding that occurred in the high-dose infection group was a sustained slower rate of weight gain (Fig. 2.1). By the end of this study, these mice only returned to pre-infection weight instead of catching up to control animals. A study of infectious colitis in mice demonstrated that infection caused growth failure (44). DeBoer, *et al.* observed, weight loss, linear-growth failure and lower levels of insulin-like growth factor-1 (IGF-1). They determined that this failure to grow was due to reduced levels of IGF-1. Our study further supports the notion that a severe infection at a young age may have the potential to impair growth rate over a longer term.

Infection has been shown to raise basal metabolic rate (BMR) which is an animal's metabolic rate while resting and fasting at thermoneutrality (4, 39). However, these studies investigating BMR have limitations such as animal restraint (likely causing stress) and temperatures outside of thermoneutral (4, 39). We were unable to directly monitor BMR in our study because the metabolic cages resemble conventional housing, allowing for locomotion. Our system does allow for monitoring of EE during the 30

minutes with the lowest activity score over the 12-hour cycle which is as close to BMR as can be achieved in these cages. This "low activity EE" followed the same pattern as average daily EE (data not shown). We were also able to monitor average daily metabolic rate. Average daily metabolic rate is defined as the metabolic rate of a free-living animal that may or may not be in a thermoneutral zone (45, 46) This is likely a better parameter to measure since it resembles an animal's natural environment by allowing for activity. In contrast to previous studies, we observed a decrease in metabolic rate during infection (Fig. 2.3A). To address the discrepancy between our study and previous studies, we wanted to better understand the relationship between activity and metabolic rate over the course of infection using a linear regression model. We observed that the relationship between activity and metabolic rate differed over the course of the infection (Fig. 2.4). During the period with greatest differences in metabolic phenotype (VO_2, bodyweight, activity & sleep), we observed an increase in the slope for infected mice (Fig. 2.4B). Thus, for each additional step taken by infected mice, their metabolic rate increased more compared to control. We therefore postulate that the reduced metabolic rates we observed under high dose infection conditions were likely primarily driven by decreased activity, which would not have been captured in previous approaches. This highlights the importance of decreased activity in shaping the outcomes of sickness behavior (4, 39).

Our study is consistent with the established hypothesis that immunity does induce trade-offs (1, 2). However, our study extends previous knowledge by demonstrating that the innate response likely drives this trade-off with little contribution from adaptive immunity. Additionally, *In vitro* studies have demonstrated when cells of the innate immune system become activated these cells shift cellular metabolic pathways away from

oxidative phosphorylation (OXPHOS) toward aerobic glycolysis to allow for the increased demand of cytokine production (24, 25) Our study supports previous findings *in vitro* with *in vivo* evidence and extends these findings. The *in vitro* studies have only observed these results up to hours after pathogen recognition receptor activation, however, our study demonstrates that this upregulation of Glut-1 and increased glucose uptake is sustained for days (Fig. 2.7). Perhaps, with increasing bacterial burden and failure to control bacterial numbers early, there was likely a greater production of pro-inflammatory cytokines (8, 18, 47). These cytokines are known to act on the brain inducing prostaglandin synthesis (48). These prostaglandins are known to cause decreased activity, increased sleep, induce anorexia, and induce fever (49-51). Thus, the increased production of pro-inflammatory cytokines could be the driver of trade-offs (1, 2, 48).

Both the low ($1x10^4$ CFU/mouse) and high ($2x10^4$ CFU/mouse) doses of *Listeria* are known to induce protective immunity in mice, yet only the high dose causes overt disease (8, 9). While both groups of mice are protected from future *Listeria* infection, the mice infected with the high dose of *Listeria* exhibited sickness behavior and a hypometabolic state. Thus, the threshold to induce protective immunity for this infection must be below the threshold for trade-offs between growth, reproduction, and maintenance. This phenomenon is used to great advantage in vaccination. Vaccines provide protective immunity through the development of strong adaptive immune responses without inducing disease symptoms including sickness behavior.

References

1. Zuk, M., and A. M. Stoehr. 2002. Immune defense and host life history. *The American naturalist* 160 Suppl 4: S9-s22.

2. Lochmiller, R. L., and C. Deerenberg. 2000. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88: 87-98.

3. Bonneaud, C., J. Mazuc, G. Gonzalez, C. Haussy, O. Chastel, B. Faivre, and G. Sorci. 2003. Assessing the Cost of Mounting an Immune Response. *The American naturalist* 161: 367-379.

4. Demas, G. E., V. Chefer, M. I. Talan, and R. J. Nelson. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 273: R1631-R1637.

5. Pamer, E. G. 2004. Immune responses to Listeria monocytogenes. *Nature Reviews Immunology* 4: 812-823.

6. Zenewicz, L. A., and H. Shen. 2007. Innate and adaptive immune responses to Listeria monocytogenes: a short overview. *Microbes and Infection* 9: 1208-1215.

7. Radoshevich, L., and P. Cossart. 2017. Listeria monocytogenes: towards a complete picture of its physiology and pathogenesis. *Nature Reviews Microbiology* 16: 32.

8. Corbin, G. A., and J. T. Harty. 2004. Duration of Infection and Antigen Display Have Minimal Influence on the Kinetics of the CD4+ T Cell Response to Listeria monocytogenes Infection. *The Journal of Immunology* 173: 5679-5687.

9. Czuprynski, C. J., and J. F. Brown. 1986. The relative difference in anti-Listeria resistance of C57BL/6 and A/J mice is not eliminated by active immunization or by transfer of Listeria-immune T cells. *Immunology* 58: 437-443.

10. Ohga, S., K. Ueda, Y. Yoshikai, Y. Takeda, K. Hiromatsu, and K. Nomoto. 1991. Kinetics of fever and its related cytokines in mice after intraperitoneal infection with listeria monocytogenes. *Journal of Thermal Biology* 16: 103-107.

11. Wang, A., S. C. Huen, H. H. Luan, S. Yu, C. Zhang, J.-D. Gallezot, C. J. Booth, and R. Medzhitov. Opposing Effects of Fasting Metabolism on Tissue Tolerance in Bacterial and Viral Inflammation. *Cell* 166: 1512-1525.e1512.

12. Ganeshan, K., J. Nikkanen, K. Man, Y. A. Leong, Y. Sogawa, J. A. Maschek, T. Van Ry, D. N. Chagwedera, J. E. Cox, and A. Chawla. 2019. Energetic Trade-

Offs and Hypometabolic States Promote Disease Tolerance. *Cell* 177: 399-413.e312.

13. Jia, T., N. V. Serbina, K. Brandl, M. X. Zhong, I. M. Leiner, I. F. Charo, and E. G. Pamer. 2008. Additive Roles for MCP-1 and MCP-3 in CCR2-mediated Recruitment of Inflammatory Monocytes During Listeria monocytogenes Infection. *Journal of immunology (Baltimore, Md. : 1950)* 180: 6846-6853.

14. Serbina, N. V., and E. G. Pamer. 2006. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol* 7: 311-317.

15. Seki, E., H. Tsutsui, N. M. Tsuji, N. Hayashi, K. Adachi, H. Nakano, S. Futatsugi-Yumikura, O. Takeuchi, K. Hoshino, S. Akira, J. Fujimoto, and K. Nakanishi. 2002. Critical Roles of Myeloid Differentiation Factor 88-Dependent Proinflammatory Cytokine Release in Early Phase Clearance of Listeria monocytogenes in Mice. *The Journal of Immunology* 169: 3863.

16. Dyatlov, V. A., and D. A. Lawrence. 2002. Neonatal Lead Exposure Potentiates Sickness Behavior Induced by Listeria monocytogenes Infection of Mice. *Brain, Behavior, and Immunity* 16: 477-492.

17. Serbina, N. V., T. P. Salazar-Mather, C. A. Biron, W. A. Kuziel, and E. G. Pamer. 2003. TNF/iNOS-Producing Dendritic Cells Mediate Innate Immune Defense against Bacterial Infection. *Immunity* 19: 59-70.

18. Badovinac, V. P., B. B. Porter, and J. T. Harty. 2002. Programmed contraction of CD8+ T cells after infection. *Nat. Immunol.* 3: 619-626.

19. Williams, M. A., and M. J. Bevan. 2004. Shortening the Infectious Period Does Not Alter Expansion of CD8 T Cells but Diminishes Their Capacity to Differentiate into Memory Cells. *The Journal of Immunology* 173: 6694-6702.

20. Haring, J. S., V. P. Badovinac, and J. T. Harty. 2006. Inflaming the CD8+ T Cell Response. *Immunity* 25: 19-29.

21. Warburg, O., K. Posener, and E. Negelein. 1924. The metabolism of cancer cells. *Biochem Z* 152: 319-344.

22. Warburg, O. 1956. On the origin of cancer cells. *Science* 123: 309-314.

23. Vander Heiden, M. G., L. C. Cantley, and C. B. Thompson. 2009. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. *Science (New York, N.Y.)* 324: 1029-1033.

24. O'Neill, L. A. J., R. J. Kishton, and J. Rathmell. 2016. A guide to immunometabolism for immunologists. *Nature Reviews Immunology* 16: 553-565.

25. Ganeshan, K., and A. Chawla. 2014. Metabolic Regulation of Immune Responses. *Annual Review of Immunology* 32: 609-634.

26. Everts, B., E. Amiel, G. J. W. van der Windt, T. C. Freitas, R. Chott, K. E. Yarasheski, E. L. Pearce, and E. J. Pearce. 2012. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. *Blood* 120: 1422.

27. Krawczyk, C. M., T. Holowka, J. Sun, J. Blagih, E. Amiel, R. J. DeBerardinis, J. R. Cross, E. Jung, C. B. Thompson, R. G. Jones, and E. J. Pearce. 2010. Toll-like receptor–induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 115: 4742.

28. Greiner, E. F., M. Guppy, and K. Brand. 1994. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. *J Biol Chem* 269: 31484-31490.

29. Frauwirth, K. A., J. L. Riley, M. H. Harris, R. V. Parry, J. C. Rathmell, D. R. Plas, R. L. Elstrom, C. H. June, and C. B. Thompson. 2002. The CD28 Signaling Pathway Regulates Glucose Metabolism. *Immunity* 16: 769-777.

30. Jacobs, S. R., C. E. Herman, N. J. MacIver, J. A. Wofford, H. L. Wieman, J. J. Hammen, and J. C. Rathmell. 2008. Glucose Uptake Is Limiting in T Cell Activation and Requires CD28-Mediated Akt-Dependent and Independent Pathways. *The Journal of Immunology* 180: 4476.

31. Pearce, E. L., M. C. Walsh, P. J. Cejas, G. M. Harms, H. Shen, L.-S. Wang, R. G. Jones, and Y. Choi. 2009. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* 460: 103.

32. van der Windt, G. J., B. Everts, C. H. Chang, J. D. Curtis, T. C. Freitas, E. Amiel, E. J. Pearce, and E. L. Pearce. 2012. Mitochondrial respiratory capacity is a critical regulator of CD8+ T cell memory development. *Immunity* 36: 68-78.

33. Woodie, L. N., Y. Luo, M. J. Wayne, E. C. Graff, B. Ahmed, A. M. O'Neill, and M. W. Greene. 2018. Restricted feeding for 9h in the active period partially abrogates the detrimental metabolic effects of a Western diet with liquid sugar consumption in mice. *Metabolism* 82: 1-13.

34. Luo, Y., C. M. Burrington, E. C. Graff, J. Zhang, R. L. Judd, P. Suksaranjit, Q. Kaewpoowat, S. K. Davenport, A. M. O'Neill, and M. W. Greene. 2016. Metabolic phenotype and adipose and liver features in a high-fat Western diet-

induced mouse model of obesity-linked NAFLD. *American Journal of Physiology - Endocrinology and Metabolism* 310: E418-E439.

35. Weir, J. B. d. V. 1949. New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology* 109: 1-9.

36. Brzoza, K. L., A. B. Rockel, and E. M. Hiltbold. 2004. Cytoplasmic Entry of Listeria monocytogenes Enhances Dendritic Cell Maturation and T Cell Differentiation and Function. *The Journal of Immunology* 173: 2641-2651.

37. McCusker, R. H., and K. W. Kelley. 2013. Immune–neural connections: how the immune system's response to infectious agents influences behavior. *The Journal of Experimental Biology* 216: 84.

38. Dantzer, R. 2001. Cytokine-Induced Sickness Behavior: Where Do We Stand? *Brain, Behavior, and Immunity* 15: 7-24.

39. Ots, I., A. B. Kerimov, E. V. Ivankina, T. A. Ilyina, and P. Hõrak. 2001. Immune challenge affects basal metabolic activity in wintering great tits. *Proceedings of the Royal Society of London B: Biological Sciences* 268: 1175-1181.

40. Merrick, J. C., B. T. Edelson, V. Bhardwaj, P. E. Swanson, and E. R. Unanue. 1997. Lymphocyte apoptosis during early phase of Listeria infection in mice. *The American Journal of Pathology* 151: 785-792.

41. Carrero, J. A., B. Calderon, and E. R. Unanue. 2004. Listeriolysin O from Listeria monocytogenes is a lymphocyte apoptogenic molecule. *J Immunol* 172: 4866-4874.

42. Nieto-Vazquez, I., S. Fernández-Veledo, D. K. Krämer, R. Vila-Bedmar, L. Garcia-Guerra, and M. Lorenzo. 2008. Insulin resistance associated to obesity: the link TNF-alpha. *Archives of Physiology and Biochemistry* 114: 183-194.

43. Hotamisligil, G. S., D. L. Murray, L. N. Choy, and B. M. Spiegelman. 1994. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proceedings of the National Academy of Sciences* 91: 4854-4858.

44. DeBoer, M. D., V. Vijayakumar, M. Gong, J. L. Fowlkes, R. M. Smith, F. Ruiz-Perez, and J. P. Nataro. 2017. Mice with infectious colitis exhibit linear growth failure and subsequent catch-up growth related to systemic inflammation and IGF-1. *Nutr Res* 39: 34-42.

45. Nagy, K. A. 1987. Field Metabolic Rate and Food Requirement Scaling in Mammals and Birds. *Ecological Monographs* 57: 112-128.

46. Lifson, N., and R. McClintock. 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. *Journal of Theoretical Biology* 12: 46-74.

47. Kumar, N. P., K. Moideen, V. V. Banurekha, D. Nair, and S. Babu. 2019. Plasma Proinflammatory Cytokines Are Markers of Disease Severity and Bacterial Burden in Pulmonary Tuberculosis. *Open Forum Infect Dis* 6: ofz257-ofz257.

48. Saper, C. B., A. A. Romanovsky, and T. E. Scammell. 2012. Neural circuitry engaged by prostaglandins during the sickness syndrome. *Nature neuroscience* 15: 1088-1095.

49. Chai, Z., S. Gatti, C. Toniatti, V. Poli, and T. Bartfai. 1996. Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. *J Exp Med* 183: 311-316.

50. Terao, A., H. Matsumura, H. Yoneda, and M. Saito. 1998. Enhancement of slow-wave sleep by tumor necrosis factor-α is mediated by cyclooxygenase-2 in rats. *NeuroReport* 9: 3791-3796.

51. Pecchi, E. 2006. Involvement of central microsomal prostaglandin E synthase-1 in IL-1 -induced anorexia. 25: 485-492.

Chapter 3

Comparing the Metabolic Phenotype of a Primary and a Secondary Immune Response to *Listeria monocytogenes*

Abstract

Numerous studies have examined the trade-offs to life-history traits using non-specific stimuli such as LPS or phytohemagglutinin. Upon activation, cells of the adaptive immune system go through clonal expansion, and while most of these cells die off during contraction, 5-10% of the cells remain and establish memory. During a subsequent infection, these memory cells respond rapidly and go through clonal expansion with greater magnitude compared to a primary response. Thus, if the adaptive immune response to a pathogen does indeed induces trade-offs, a secondary response would have the greatest potential to induce such trade-offs as it has the highest potential demand. To better quantitate the demand and trade-offs induced by adaptive immunity, we longitudinally monitored the primary and secondary immune response to *Listeria monocytogenes*, a live pathogenic infection model. We measured the systemic metabolic phenotype and adaptive immune response. We utilized two doses of bacteria in the primary immune response to further explore the role of T cell expansion in the metabolic phenotype of a secondary response. We determined that the greatest impact to systemic metabolism occurred early during a secondary response. During a secondary response, the innate immune response would be similar for both groups of immunized mice, but their adaptive immune response would be different. Because this impact was observed by

only one group of immunized mice, it was likely not innate immunity, but adaptive immunity driving these changes. Our finding implicates that activation of memory T cells is metabolically taxing to the host.

Introduction

Animals must allocate limited resources between the life-history traits: growth, reproduction, and maintenance (1-3). Life-history theory aims to explain how the allocation of these resources impacts an animal's fitness (1-3). Once a resource is used by a trait it cannot be used by other traits. Thus, trade-offs between traits are inevitable (1-3). The host's immune system is responsible for immune defense to a pathogen. Since it regulates host survival, it is an essential part of host's maintenance, and could induce trade-offs. Through the use of phytohemagglutinin (PHA), keyhole limpet hemocyanin (KLH), or sheep red blood cells (SRBCs), studies have determined the trade-offs induced by activated cells of the adaptive immune system (4-8).

PHA crosslinks the T cell receptor triggering an intracellular signaling pathway which leads to non-specific T cell activation (9, 10), while KLH and SRBCs non-specifically induce antibody production by B cells. In one study utilizing PHA with house sparrows, Martin, *et. al* observed a significantly elevated resting metabolic rate (RMR) and calculated the cost to be equivalent to the production of half of an egg (4). When Nilsson, *et. al* injected birds, with PHA, they observed a 5% increase in resting metabolic rate but did not observe a dose-dependent energetic cost (5). Ots, *et. al* also injected birds with SRBCs and observed a 9% higher basal metabolic rate (BMR) and the loss of about 3% body mass (6). These studies demonstrate that birds injected with non-specific stimuli of cells of the adaptive immune system elevates metabolic rate at the cost of reproduction or growth. In addition to birds, the demand of non-specific stimuli to T and B cells have been evaluated in mice. Derting, *et. al* observed a significant energetic cost in mice injected with SRBCs and PHA (7), while Nelson, *et. al* observed an increase in metabolic

rate in mice injected with KLH. (8) Overall, mice and birds increase metabolic rate when injected with non-specific stimuli of cells of the adaptive immune system; however, in birds, trade-offs were observed with growth or reproduction. These studies only provide insight into the trade-offs induced by the "primary" response of the adaptive immune system; however, to our knowledge, there are no studies that examine the trade-offs induced by primary vs. secondary responses to a pathogen.

When cells of the immune system become activated, there are intracellular changes that are potentially taxing to the host (11-15). Quiescent cells of the immune system primarily rely on oxidative phosphorylation (OXPHOS) for energy. While this process provided ATP, it fails to provide biosynthetic precursors that are essential for growth, proliferation, and effector function. Activated cells of the immune system primarily utilizes aerobic glycolysis which provides biosynthetic precursors. Aerobic glycolysis is the conversion of glucose to pyruvate, and then, the reduction of pyruvate to lactic acid. This allows TCA intermediates to enter biosynthetic pathways (16-20). To produce cytokines, cells must increase their protein synthesis. To meet the increase demand of protein synthesis, the endoplasmic reticulum will increase size which requires lipid synthesis. In addition to cytokine production, cells of the adaptive immune system have an increase demand for nutrients and energy due to clonal expansion or proliferation. During a primary immune response, antigen-specific T cells can increase as much as 10,000-fold, and during a subsequent response, memory antigen-specific T cells can increase about 5-fold over the primary response. Additionally, each proliferating cell would need nutrients and energy to carry out their effector functions. Thus, during an immune response, cells of the immune system have an increased demand for

carbohydrates, triglycerides, and amino acids for the synthesis of nucleic acids, lipids, and proteins, respectively, and for the biosynthesis of these macromolecules. It is possible activation, proliferation, and or effector functions could be the drivers of trade-offs among life history traits.

A primary immune response relies on an innate and adaptive immune response. APC such as (dendritic cells and monocytes) are cells of innate immune system that are responsible for activating naïve T cells (21, 22). T cell response can be divided into four distinct phases: activation, expansion, contraction, and establishing immunological memory (23-25). During clonal expansion, antigen-specific T cell can increase 10,000-fold, while during the contraction phase, roughly 90%-95% of the effector T cells are eliminated (23, 26-28). After contraction, the remaining 5%-10% of T cells are memory T cells, which are responsible for establishing immunological memory (23, 27). Immunological memory is the ability of cells of the adaptive immune system to rapidly respond explicitly to previously encountered antigens upon subsequent infections (29).

A secondary response is predominantly characterized by an adaptive immune response (26, 30). However, there are several key differences between a primary and secondary adaptive immune response. One, memory T cells have a lower threshold for activation, and are surveying periphery, so these cells are poised to be the “first responders” to prevent subsequent infections. Additionally, these cells have a lower threshold for activation. (26, 30). Thus, the adaptive immune response to a secondary response is more rapid compared to a primary response. The magnitude of T cell expansion is also approximately 5-fold greater than a primary response (31, 32). Two, the magnitude of clonal expansion of antigen-specific T cells is greater than a primary

response. (27). Three, at the conclusion of a secondary response, the number of memory T cells is greater than the previously established level (33). With the magnitude of T cell expansion greater in subsequent infections, it has the capacity to be more metabolically taxing on the host than a primary immune response. Our understanding of primary and secondary immune responses originates from studying the host's immune response to a model pathogen such as *Listeria monocytogenes*.

Listeria monocytogenes pathogenesis, and the subsequent host immune response, have been well characterized in the literature (34-37). The primary immune response to *Listeria* relies on an innate and adaptive immune response. Initially following infection, a robust innate immune response is observed consisting of inflammatory cytokine production and recruitment of cells of the innate immune system to site of infection which controls the infection until around day three post-infection (34, 38-44). On the heels of the innate immune response, the adaptive immune response occurs and is responsible for pathogen clearance. Because *Listeria* is an intracellular pathogen, the adaptive immune response primarily involves T cells. Within minutes of TCR engagement, T cell activation occurs (19, 20). The first round of clonal expansion occurs about 24 hours after TCR engagement (27, 45, 46). However, through the detection of IFN-γ, T cell clonal expansion is readily detectible from about day 5 to day 7 post-infection (28, 47, 48). The contraction phase ends around day 10 post-infection, and by day 14 post-infection, memory begins to be established and fully established by day 30 post-infection (28, 47, 48).

The secondary response to *Listeria* is predominantly characterized by a T cell response (26, 30). The *Listeria*-specific T cell kinetics of a secondary response occurs more

rapidly due to immunological memory. The expansion phase is readily detected in the form of IFN-γ production from about day 3 until day 5 post-infection (28, 47-52). The contraction phase generally is complete by day 7 post-infection where a new level of memory is established (28, 47-49).

The adaptive immune response to *Listeria* involves helper T cells ($CD4^+$) and cytotoxic T lymphocytes (CTLs, $CD8^+$). MHC tetramer staining in conjunction with intracellular cytokine staining has allowed for *ex vivo* enumeration of $CD4^+$ and $CD8^+$ T cells. Generally, the enumeration of helper T cells *ex vivo* has been difficult because most pathogen-derived $CD4^+$ epitopes are subdominant. However, C57Bl/6 mice generate a robust CD4 response to *Listeria* that can be readily enumerated *ex vivo* (53-55). C57Bl/6 mice generate a $CD8^+$ response to *Listeria*. However, the frequency of these cells is around 1-2% making the enumeration *ex vivo* difficult (53-55). For this reason, it makes the direct comparison of the $CD4^+$ and $CD8^+$ T cell kinetics to *Listeria* challenging. To address this issue, a recombinant *Lm* expressing the model antigen ovalbumin (r*Lm*-OVA) was generated (56, 57). C57Bl/6 mice generate $CD4^+$ and $CD8^+$ OVA-specific T cells with higher frequency allowing for direct comparison *ex vivo* of $CD4^+$ and $CD8^+$ T cells (33, 47, 48).

Our present study aimed to determine if an adaptive immune response to a pathogen induces trade-offs in a mouse model. To achieve this, we longitudinally monitored the systemic metabolic phenotype throughout a primary and secondary immune response to r*Lm*-OVA. We observed a delay in growth only in mice during the primary immune response, and it overlapped with the timing of the innate immune response. During the secondary response, we observed more systemic changes than the

primary immune response. While these changes overlapped with the timing of the innate immune system, innate immunity was likely not the cause since this was only observed with one of the two challenged groups. Instead, it is likely due to the activation of memory T cells.

Materials and Methods

Mice and Listeria monocytogenes Infection

C57BL/6J mice were obtained from The Jackson Laboratory. The mice used in these studies were between 8 and 12 weeks old and were age-matched for each experiment. All mice were maintained in a specific pathogen-free (SPF) facility and in full compliance with the Institutional Care and Use Committee of Auburn University regarding the use of animals. Recombinant *L. monocytogenes* expressing ovalbumin (r*Lm*-OVA) was obtained from Dr. Hao Shen (University of Pennsylvania). r*Lm*-OVA was grown in brain-heart infusion (BHI) broth overnight at 37℃ to an OD_{600} of 1.0. The overnight culture (1ml) was centrifuged, resuspended, and washed twice in PBS. Mice were injected intraperitoneally with either $2x10^3$ CFU/mouse (Low Dose) or $1x10^4$ CFU/mouse (High Dose) or an equal volume of PBS diluent (uninfected control). For the challenge experiment, previously infected mice were injected with, 10xLD50, $1x10^6$ CFU/mouse of r*Lm*OVA. Uninfected mice were again injected with PBS since this dose is lethal to naïve mice. The infectious doses were confirmed by plating dilutions of the inoculum on BHI agar, and colonies were counted after incubation at 37℃ for 18-24 hours.

Metabolic Phenotyping, Food, and Water Intake

To assess metabolic phenotype, Promethion metabolic cages (Sable Systems, Las Vegas, NV) were used as previously described (58, 59). Briefly, animals were individually housed in the metabolic cages throughout the 12-day experiment. Activity was measured by Promethion XYZ Beambreak. Activity was determined by consecutive adjacent beam breaks in the X, Y and Z planes. Quiet bouts were defined as no engagement in locomotion, eating, drinking, or grooming for 40 seconds, while sleep was determined as a quiet bout lasting for greater than 40 seconds.

Food, water, and body mass were measured by Promethion MM-1 Load Cell sensors. The amount, frequency, duration, and rate at which food and water were withdrawn from the hoppers were measured and analyzed. The body mass monitors were plastic tubes that also functioned as in-cage enrichment and nesting devices.

Respiratory gases were measured by the Promethion GA-3 gas-analyzer, which measured water vapor, CO_2, and O_2 in mL/min. Energy expenditure was calculated using the Weir equation (60): kcal/h = $60 \times (0.003941 \times \dot{V}O_2 + 0.001106 \times \dot{V}CO_2)$. Respiratory Exchange Ratio (RER) was calculated as the ratio of $\dot{V}CO_2/\dot{V}O_2$ where a RER of about 0.7 indicates pure lipid utilization and a RER of about 1.0 indicates pure carbohydrate utilization. Data acquisition and system control were coordinated using MetaScreen v. 2.2.8, and the obtained raw data were processed using ExpeData v. 1.9.14 (Sable Systems) and Universal Macro Collection v. 10.1.11.

OVA-specific T cell enumeration

Splenocytes were incubated with 1 ng/mL of $OVA_{257-264}$ peptide for one hour. Cells were then incubated in the presence of GolgiStop (BD Biosciences) for four hours. Cells were

washed in FACS buffer (PBS supplemented with 3% FBS) and were incubated with AF488-anti-CD8 and PE-anti-CD4 on ice for 30 min. The cells were washed in FACS buffer twice, then fixed and permeabilized (BD CytoFix/CytoPerm) by incubating for 20 min at 4°C. The cells were washed in Perm/Wash buffer and incubated with APC-anti-IFN-γ for 30 min at 4°C. Cells were washed twice in Perm/Wash buffer and resuspended in FACS buffer before flow cytometric analysis.

Statistical Analysis

Statistical analyses were performed using Prism Software, version 8 (GraphPad). Results are presented as mean +/- SD, and significance was determined using a one-way ANOVA followed by Dunnett's post hoc test. Asterisks denote level of statistical significance (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, and ****$p < 0.001$).

Results

Metabolic Phenotype of Primary Immune Response

We wanted to evaluate the systemic metabolic impact of a primary immune response to a live pathogen. To accomplish this, we infected mice with one of two doses, either a low ($2x10^3$ CFU/mouse) or high dose ($1x10^4$ CFU/mouse), of r*Lm*-OVA and compared their systemic metabolism to that of uninfected mice. To monitor the systemic metabolic phenotype of mice, we utilized the Promethion® metabolic cages to assess body mass, metabolic rate (VO_2), energy expenditure (EE), activity, and sleep. Collectively, these parameters are known as the metabolic phenotype. The metabolic data were collected over the course of the 14-day experiment. The metabolic data was the

average of a 12-hour light (inactive period) /dark (active period) cycle for each group of mice.

First, we examined changes in body mass over the course of the infection (Fig 1). Mice infected with the high dose did not lose significant weight after infection. Instead, they demonstrated a delay in weight gain until night three after infection (Fig. 1). Control mice gained ~5% more bodyweight before the high dose group started to gain weight. By day three of the infection, the mice infected with the high dose returned to control weight. Weight loss could not be attributed to decreased food consumption because there was no difference in food consumption between infected mice and control (data not shown). The low dose of infection did not affect weight. Thus, we observed a dose-dependent growth delay in mice infected with *Listeria.*

Next, we examined the behavioral parameters of activity and sleep (Fig. 2). The control mice exhibited a diurnal pattern of activity as expected. They were more active during the dark cycle and less active during the light cycle, thus establishing a circadian rhythm of activity. During this primary infection, we failed to observe infection affecting activity or sleep (Fig 2).

To determine if infection altered systemic metabolic parameters, we examined metabolic rate (VO_2) and energy expenditure (Fig. 3). The amount of O_2 consumed can be used as a proxy for metabolic rate. Since these mice are unrestrained, we monitored the average daily metabolic rate, which accounts for locomotion and thermogenesis. There were no differences in metabolic rate at any time point between infected mice and control (Fig 3B). Energy expenditure was calculated using the Weir equation (60). Again, there were no differences in energy expenditure between infected mice and control (Fig

3A). Activity influences metabolic rate which in turn will affect energy expenditure. Since we did not observe any changes in activity, it is not surprising that we did not observe differences in energy expenditure or metabolic rate of infected mice (Fig. 2 & 3).

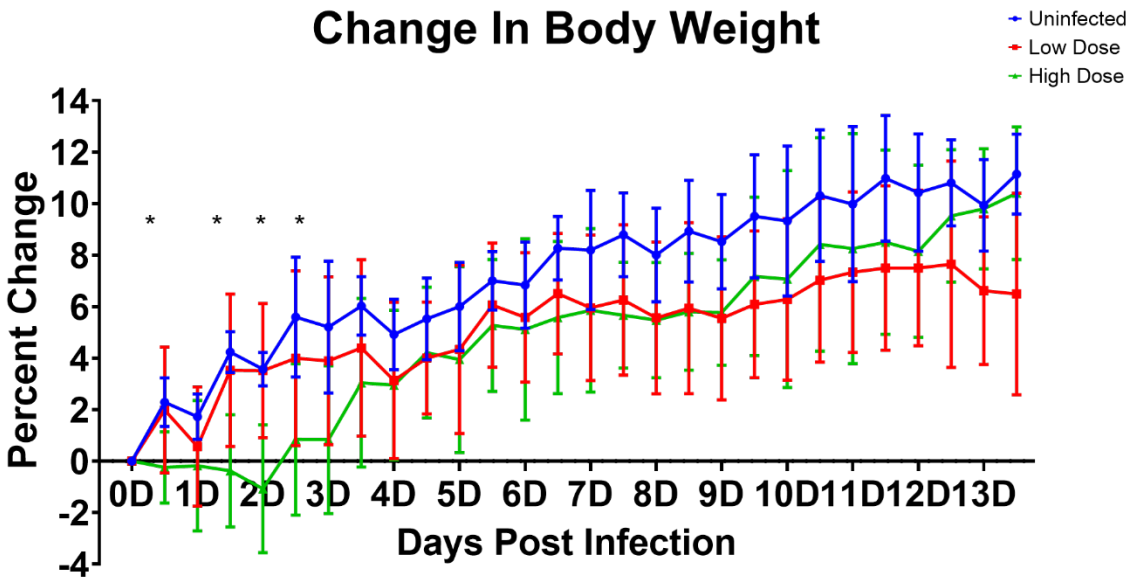

Figure 3.1: Primary response induced change in body weight.
Analysis of change in body weight for a 12-hour light dark cycle over the 14-day experimental period. Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD. (* $p < 0.05$, ** $p < 0.01$, High dose compared to control)

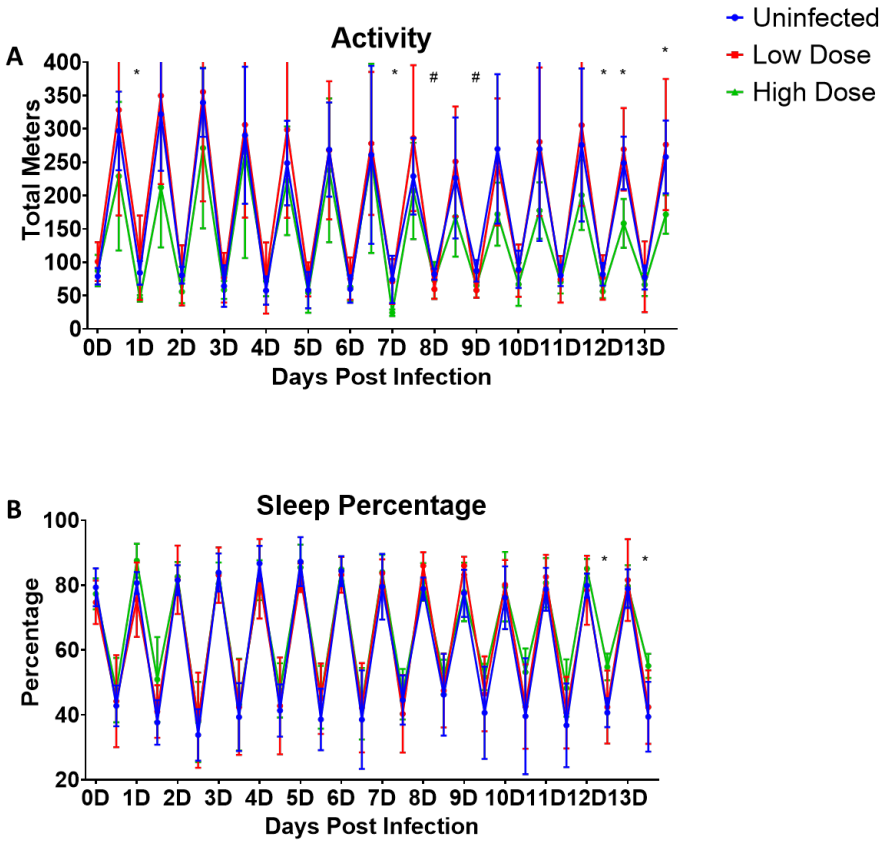

Figure 3.2: Primary response induced lethargy.
Analysis of change in **(A)** Activity or **(B)** Sleep for a 12-hour light dark cycle over the 14-day experimental period. Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD. (* $p < 0.05$, High dose compared to control # $p < 0.05$, Low doe compared to control)

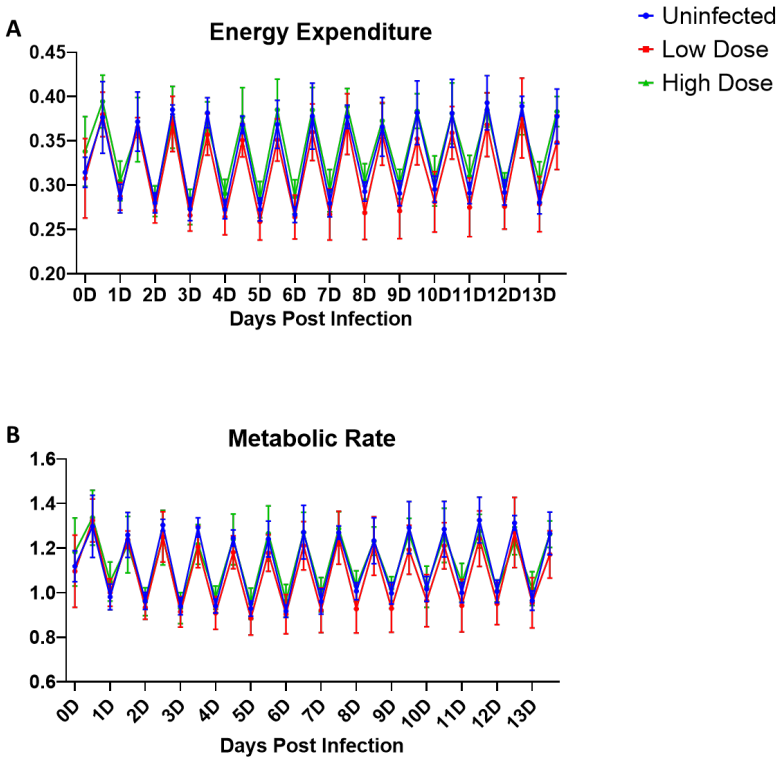

Figure 3.3: Primary response induced changes in Energy Expenditure and Metabolic rate . Analysis of change in **(A)** Energy Expenditure or **(B)** Metabolic Rate for a 12-hour light dark cycle over the 14-day experimental period. The Weir Equation was used to calculate EE (kcal/h = 60 × (0.003941 × $\dot{V}O_2$ + 0.001106 × $\dot{V}CO_2$)). Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD.

Metabolic Phenotype of an Immune Challenge

A secondary immune response heavily relies on immunological memory by cells of the adaptive immune system. The secondary immune response has a more considerable clonal expansion of antigen-specific T cells compared to a primary immune response. Thus, to determine if such a strong proliferative adaptive immune response induces trade-offs, we elicited a secondary response by challenging the animals with a high dose of *Lm*-OVA. We challenged the previously infected animals from the primary study 30 days after the primary infection with $1x10^6$ CFU/mouse of r*Lm*-OVA. In unimmunized mice, the LD50 of *Lm*-OVA is $1x10^5$ CFU (49, 61, 62). Since this would be a lethal dose in unimmunized mice, such animals were not used as controls. Instead, previously uninfected mice were injected with PBS. We monitored the metabolic phenotype of control and challenged mice over the course of an immune challenge.

Listeria challenge induced only a modest decrease in body weight starting the night after infection and continued until three days after the challenge (Fig 4) that was less than 0.1% and not significantly different than control (Fig. 4). Overall, immunized mice and control did not gain weight, as observed in the primary immune response (Fig. 1), due to their stage of maturity. Thus, this immune challenge to adult mice did not affect body weight and indicates that the mice had developed protective immunity from their previous infection/immunization.

The next parameters examined were activity and sleep. We observed that starting at the time of infection through the second night after the challenge, mice immunized with the higher dose of *Listeria* had a decrease in their overall activity (Fig 5A). Initially, these mice had a ~66% reduction in activity, and on the second night after infection, they

had a ~33% reduction in activity (Fig. 5A). Conversely, mice infected with the higher dose of *Listeria* demonstrated increased time spend in quiet/sleep compared to control. (Fig. 5B). Interestingly, while we observed no difference between control and mice infected with the lower dose of *Listeria,* we observed an increase in quiet/sleep the first night after infection (Fig 5B).

To understand the systemic metabolic impact of a robust adaptive immune response, the energy expenditure and metabolic rate were monitored. We observed that the mice immunized with the high dose of *Listeria* had a decrease in energy expenditure the first night after infection (Fig. 6A). Additionally, these mice had a decrease in their metabolic rate the first night after infection (Fig. 6B). There was ~ 20% reduction in energy expenditure and metabolic rate compared to control. Additionally, the decrease in these parameters corresponds to the decrease in activity observed by the mice immunized with the high dose of bacteria (Fig. 5 &6). It is likely the decrease in activity could account for the decrease in metabolic rate, which in turn could decrease energy expenditure.

OVA-specific T cell Response

At the conclusion of the study to confirm immunization, we determined the IFN-γ production by OVA-specific T cells (Fig. 7). Splenocytes were cultured in the presence of OVA peptide to measure the OVA-specific T cell response in the form of IFN-γ production. Measurement of IFN-γ production was enabled by intracellular cytokine staining and flow cytometry compared to splenocytes from control (uninfected) mice. We observed the production of IFN-γ by OVA-specific T cells by both groups of infected

mice. Thus, confirming these mice had protective immunity to *Listeria* since they survived the immune challenge which is lethal to naïve mice (Fig. 7).

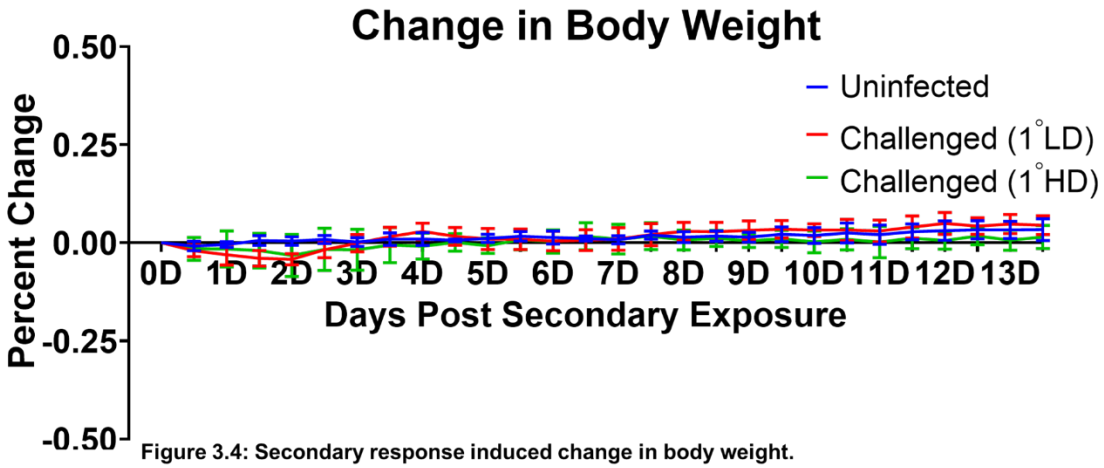

Figure 3.4: Secondary response induced change in body weight.
Analysis of change in body weight for a 12-hour light dark cycle over the 14-day experimental period. Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD.

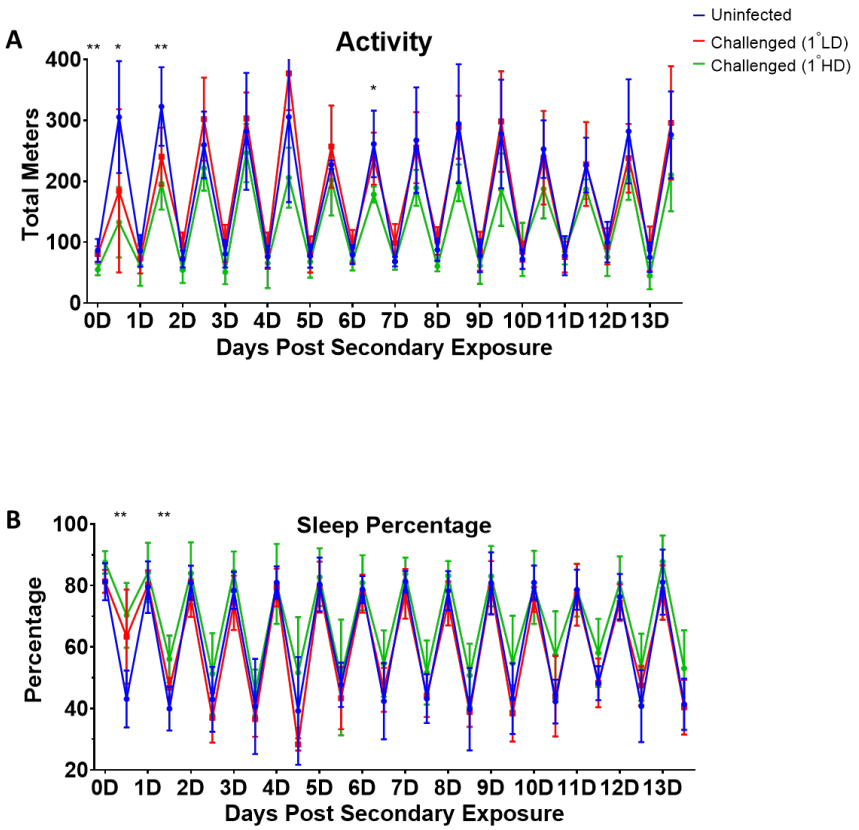

Figure 3.5: Secondary response induced lethargy.
Analysis of change in **(A)** Activity or **(B)** Sleep for a 12-hour light dark cycle over the 14-day experimental period. Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD. (* $p < 0.05$, ** $p < 0.01$, High dose compared to control, # $p < 0.05$, Low doe compared to control)

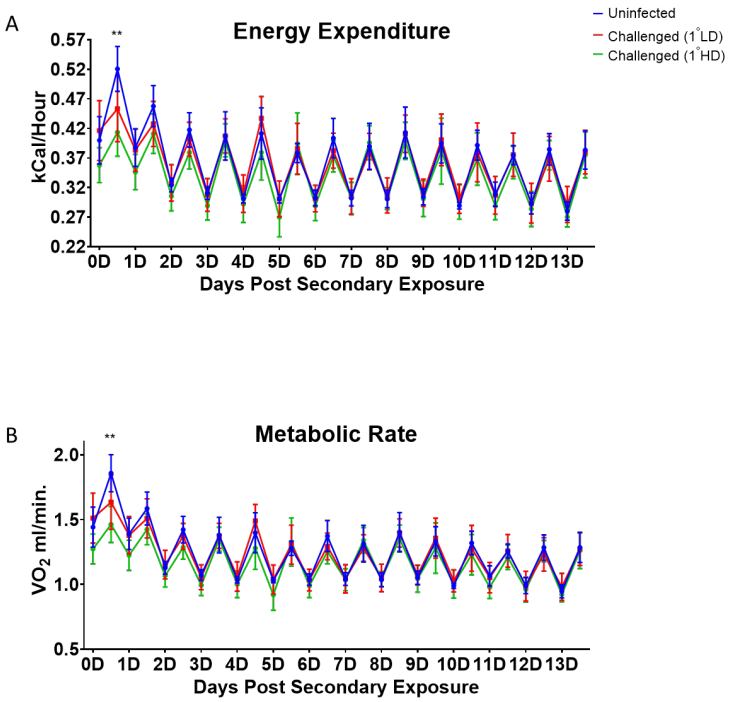

Figure 3.6: Secondary response induced changes in Energy Expenditure and Metabolic rate. Analysis of change in Energy Expenditure (A) or Metabolic rate (B) for a 12-hour light dark cycle over the 14-day experimental period. The Weir Equation was used to calculate EE (kcal/h = 60 × (0.003941 × VO_2 + 0.001106 × VCO_2)). Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD. (** $p < 0.01$, High dose compared to control)

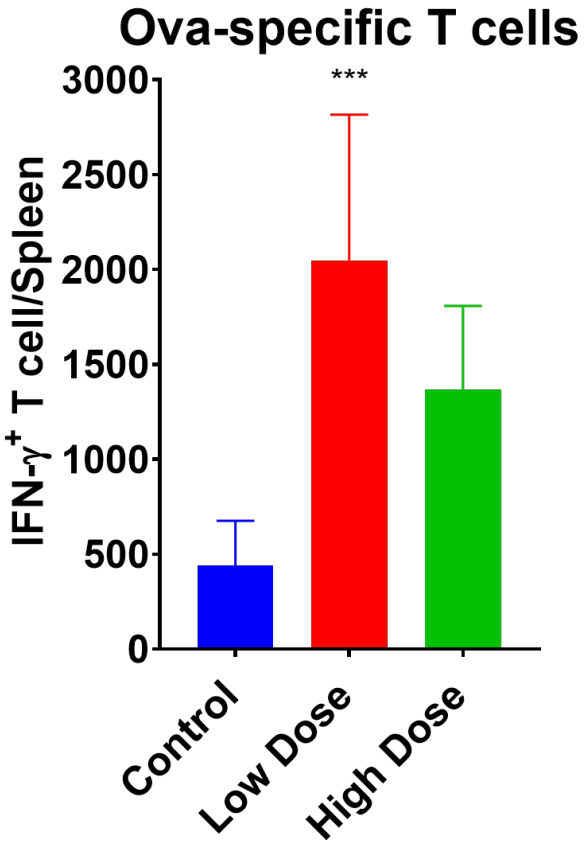

Figure 3.7: Enumeration of OVA-specific T cells in the Spleen.
OVA-specific IFN-γ producing T cells were enumerated at the conclusion of the secondary response in the spleen following a *Lm-OVA* infection. Significance was assessed using one-way ANOVA followed by a Dunnett Test for multiple comparison. Data are represented as mean ± SD. (** $p < 0.01$, compared to control)

Discussion

The goal of the current study was to compare and contrast the metabolic impact of a primary and secondary immune response in the context of a living pathogen. To achieve this goal, we longitudinally monitored the systemic metabolic phenotype throughout a primary and secondary immune response to *Listeria monocytogenes*. The utilization of this well-characterized system, murine listeriosis, allowed the study to focus on trade-offs without the need to characterize the innate and adaptive immune response. During the primary immune response, we observed a delay in growth only in the mice infected with the higher dose. However, the timing of this delayed growth corresponded to the timing of the innate immune response. When the immunized mice were challenged with *Listeria*, we observed a decrease in metabolism in the mice immunized with the higher dose. While the timing of the hypometabolic state corresponded to the timing of the innate immune response, innate immunity was likely not causing the shift in metabolism since it was only observed in one of the two groups of challenged mice.

The primary immune response to *Listeria* is characterized by an innate and adaptive immune response (34-37). Initially, the cells of the innate immune system control the infection until around day three post-infection (41, 42, 63-65). We observed a delay in growth during this time frame (Fig. 1). Cells of the innate immune system are responsible for the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-1β (38-40). These cytokines induce sickness behavior through their interaction on the brain (66, 67). Additionally, these cytokines have been implicated in interfering with growth through their action on insulin-like growth factors (68, 69). It is plausible that the

production of these cytokines from cells of the innate immune system during a *Listeria* infection led to the delayed growth of mice.

We did not observe any changes to systemic metabolism over the primary antigen-specific T cell response day 5-14 post-infection (28, 47, 48). Thus, during a primary immune response, it is likely the innate immune response induces trade-offs, not the adaptive immune response.

The data from this study does not fully align with the primary response observed in Chapter 2. This is likely due to the infectious doses. For Chapter 2, the doses used was $2x10^4$ CFU and $1x10^4$ CFU. The LD_{50} of wild type *Listeria* is $1x10^5$ CFU and the LD_{50} for *rLm*-OVA is $5x10^6$ CFU in C57Bl/6 mice (49, 70, 71). There was minimal impact to systemic metabolism in the $1x10^4$ dose; however, the $2x10^4$ dose had extensive impacts to metabolic phenotype. The does used in this study were all below 0.1 LD_{50}, and we failed to observe trade-offs to life-history traits. It is possible that the infectious dose of 0.1 LD_{50} is the threshold required for innate immunity induced life-history trait trade-offs. Both studies seem to not implicate adaptive immunity into trade-offs, so a secondary response, which primarily relies on adaptive immunity, would provide better evidence.

During the secondary response, we observed a decrease in activity, energy expenditure, and metabolic rate in mice immunized with the higher dose of *Listeria* until day two post-challenge (Fig. 5&6). Until day 3 post-infection, the cells of the innate immune system are controlling the infection. While the timing of systemic changes overlaps with the innate immune response, innate immunity is unlikely the cause in this case because innate immunity would be similar for both immunized groups, and this response is only observed in one of the two challenged groups. Upon reactivation,

memory T cells would have a shift in cellular metabolism, and the timing of this shift in cellular metabolism would overlap with the observed differences in metabolic phenotype (Fig. 5&6) (72, 73). It is likely that activation of memory T cells caused the observed systemic changes (more so in the high dose infection group). A possible explanation for it only been observed in the group of mice immunized with the higher dose of *Listeria* is these mice would have a higher frequency of memory T cells compared to the mice immunized with the lower dose of *Listeria* (28). Both groups of mice were challenged with a lethal dose of *Listeria* and survived the infection (49, 53). Thus, possibly only above a certain threshold of memory cell activation does adaptive immunity incur systemic trade-offs. From days 3-10 post-challenge, antigen-specific T cells go through clonal expansion, contraction, and re-establish memory (28, 47-49). The T cell expansion of subsequent immune challenges is greater in magnitude compared to a primary immune response (23, 24). We failed to observe any trade-offs during expansion or contraction, so it is unlikely that these processes are taxing to the host.

There is a significant difference in the number of T cells activated by non-specific activation, PHA, and antigen-specific activation, pathogen. *Perreau, et al.* stimulated 1 x 10^6 PBMC with PHA and observed about 50% of the cells became activated T cells (74). In contrast, there are only about 1000 naïve antigen-specific T cells for a pathogen (75-78). The previous observed trade-offs may be due to the number of cells activated. The immune challenge supports this idea since only the mice with a higher number of memory T cells showed systemic trade-offs.

There are discrepancies between the present study and previous studies on the effect adaptive immunity has on metabolism. These studies have determined that adaptive

immunity either increases metabolism or no effect on metabolism, and these studies examined the impact of adaptive immunity on resting metabolic rate (RMR). In contrast, our present study focuses on average daily metabolic rate (4, 5, 79, 80). RMR is an animal's metabolic rate while resting and fasting (5). Average daily metabolic rate is defined as the metabolic rate of a free-living animal and likely a more appropriate parameter to measure since it resembles an animal's natural environment by allowing for activity (81, 82). While we are unable to monitor RMR with our system, the multi-parameter monitoring of weight, food uptake, water uptake, metabolism, and energy expenditure over the entire immune response provides a more comprehensive understanding of systemic trade-offs induced by immunity.

Both the low dose (2×10^3 CFU/mouse) and the high dose (1×10^4 CFU/mouse) of *Listeria* were sub-lethal and provided protective immunity to an immune challenge. During their primary immune response, only the high dose of *Listeria* induced trade-offs; however, the timing of these trade-offs corresponds to the timing of innate immunity, not adaptive immunity. The immune challenge would provide the best evidence of adaptive immunity is driven trade-offs, and we observed trade-offs possibly associated with the activation of memory cells, but not the expansion, contraction, or re-established memory of these cells. Overall, it appears that trade-offs during a primary immune response are driven by innate immunity, and during an immune challenge, it is driven by activation of adaptive immunity.

References

1. Rauw, W. M. 2012. Immune response from a resource allocation perspective. *Frontiers in Genetics* 3.

2. Lochmiller Robert, L., and C. Deerenberg. 2003. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88: 87-98.

3. Zuk, M., and A. M. Stoehr. 2002. Immune defense and host life history. *The American naturalist* 160 Suppl 4: S9-s22.

4. Martin, L. B., A. Scheuerlein, and M. Wikelski. 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270: 153-158.

5. Nilsson, J.-Å., M. Granbom, and L. Råberg. 2007. Does the Strength of an Immune Response Reflect Its Energetic Cost? *Journal of Avian Biology* 38: 488-494.

6. Ots, I., A. B. Kerimov, E. V. Ivankina, T. A. Ilyina, and P. Hõrak. 2001. Immune challenge affects basal metabolic activity in wintering great tits. *Proceedings of the Royal Society of London B: Biological Sciences* 268: 1175-1181.

7. Terry, and S. Compton. 2003. Immune Response, Not Immune Maintenance, Is Energetically Costly in Wild White-Footed Mice (Peromyscus leucopus). *Physiological and Biochemical Zoology* 76: 744-752.

8. Demas, G. E., V. Chefer, M. I. Talan, and R. J. Nelson. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 273: R1631-R1637.

9. Crabtree, G. R. 1989. Contingent genetic regulatory events in T lymphocyte activation. *Science* 243: 355-361.

10. Movafagh, A., H. Heydary, S. A. Mortazavi-Tabatabaei, and E. Azargashb. 2011. The Significance Application of Indigenous Phytohemagglutinin (PHA) Mitogen on Metaphase and Cell Culture Procedure. *Iran J Pharm Res* 10: 895-903.

11. O'Neill, L. A. J., R. J. Kishton, and J. Rathmell. 2016. A guide to immunometabolism for immunologists. *Nature Reviews Immunology* 16: 553-565.

12. Kominsky, D. J., E. L. Campbell, and S. P. Colgan. 2010. Metabolic Shifts in Immunity and Inflammation. *The Journal of Immunology* 184: 4062-4068.

13. Gaber, T., C. Strehl, and F. Buttgereit. 2017. Metabolic regulation of inflammation. *Nat Rev Rheumatol* 13: 267-279.

14. Ganeshan, K., and A. Chawla. 2014. Metabolic Regulation of Immune Responses. *Annual Review of Immunology* 32: 609-634.

15. Pearce, E. L., and E. J. Pearce. 2013. Metabolic Pathways in Immune Cell Activation and Quiescence. *Immunity* 38: 633-643.

16. Everts, B., E. Amiel, G. J. W. van der Windt, T. C. Freitas, R. Chott, K. E. Yarasheski, E. L. Pearce, and E. J. Pearce. 2012. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. *Blood* 120: 1422.

17. Krawczyk, C. M., T. Holowka, J. Sun, J. Blagih, E. Amiel, R. J. DeBerardinis, J. R. Cross, E. Jung, C. B. Thompson, R. G. Jones, and E. J. Pearce. 2010. Toll-like receptor–induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 115: 4742.

18. Greiner, E. F., M. Guppy, and K. Brand. 1994. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. *J Biol Chem* 269: 31484-31490.

19. Frauwirth, K. A., J. L. Riley, M. H. Harris, R. V. Parry, J. C. Rathmell, D. R. Plas, R. L. Elstrom, C. H. June, and C. B. Thompson. 2002. The CD28 Signaling Pathway Regulates Glucose Metabolism. *Immunity* 16: 769-777.

20. Jacobs, S. R., C. E. Herman, N. J. MacIver, J. A. Wofford, H. L. Wieman, J. J. Hammen, and J. C. Rathmell. 2008. Glucose Uptake Is Limiting in T Cell Activation and Requires CD28-Mediated Akt-Dependent and Independent Pathways. *The Journal of Immunology* 180: 4476.

21. Brzoza, K. L., A. B. Rockel, and E. M. Hiltbold. 2004. Cytoplasmic Entry of Listeria monocytogenes Enhances Dendritic Cell Maturation and T Cell Differentiation and Function. *The Journal of Immunology* 173: 2641-2651.

22. Mitchell, L. M., K. L. Brzoza-Lewis, C. J. Henry, J. M. Grayson, M. M. Westcott, and E. M. Hiltbold. 2011. Distinct responses of splenic dendritic cell subsets to infection with Listeria monocytogenes: maturation phenotype, level of infection, and T cell priming capacity ex vivo. *Cell Immunol* 268: 79-86.

23. Haring, J. S., V. P. Badovinac, and J. T. Harty. 2006. Inflaming the CD8+ T Cell Response. *Immunity* 25: 19-29.

24. Busch, D. H., and E. G. Pamer. 1999. T lymphocyte dynamics during Listeria monocytogenes infection. *Immunology letters* 65: 93-98.

25. Condotta, S. A., M. J. Richer, V. P. Badovinac, and J. T. Harty. 2012. Probing CD8 T cell responses with Listeria monocytogenes infection. *Adv Immunol* 113: 51-80.

26. Sprent, J., and C. D. Surh. 2002. T CELLMEMORY. *Annual Review of Immunology* 20: 551-579.

27. Kaech, S. M., E. J. Wherry, and R. Ahmed. 2002. Effector and memory T-cell differentiation: implications for vaccine development. *Nature Reviews Immunology* 2: 251-262.

28. Badovinac, V. P., B. B. Porter, and J. T. Harty. 2002. Programmed contraction of CD8+ T cells after infection. *Nat. Immunol.* 3: 619-626.

29. Masopust, D., and R. Ahmed. 2004. Reflections on CD8 T-Cell Activation and Memory. *Immunologic Research* 29: 151-160.

30. Pennock, N. D., J. T. White, E. W. Cross, E. E. Cheney, B. A. Tamburini, and R. M. Kedl. 2013. T cell responses: naive to memory and everything in between. *Adv Physiol Educ* 37: 273-283.

31. Opferman, J. T. 1999. Linear Differentiation of Cytotoxic Effectors into Memory T Lymphocytes. *Science* 283: 1745-1748.

32. Lakkis, F. G. 2003. Memory T Cells: A Hurdle to Immunologic Tolerance. *Journal of the American Society of Nephrology* 14: 2402-2410.

33. Kursar, M., K. Bonhagen, A. Köhler, T. Kamradt, S. H. E. Kaufmann, and H.-W. Mittrücker. 2002. Organ-Specific CD4+ T Cell Response During Listeria monocytogenes Infection. *The Journal of Immunology* 168: 6382-6387.

34. Pamer, E. G. 2004. Immune responses to Listeria monocytogenes. *Nature Reviews Immunology* 4: 812-823.

35. Zenewicz, L. A., and H. Shen. 2007. Innate and adaptive immune responses to Listeria monocytogenes: a short overview. *Microbes and Infection* 9: 1208-1215.

36. Radoshevich, L., and P. Cossart. 2017. Listeria monocytogenes: towards a complete picture of its physiology and pathogenesis. *Nature Reviews Microbiology* 16: 32.

37. D'Orazio, S. E. F. 2019. Innate and Adaptive Immune Responses during Listeria monocytogenes Infection. *Microbiol Spectr* 7.

38. Ohga, S., K. Ueda, Y. Yoshikai, Y. Takeda, K. Hiromatsu, and K. Nomoto. 1991. Kinetics of fever and its related cytokines in mice after intraperitoneal infection with listeria monocytogenes. *Journal of Thermal Biology* 16: 103-107.

39. Wang, A., S. C. Huen, H. H. Luan, S. Yu, C. Zhang, J.-D. Gallezot, C. J. Booth, and R. Medzhitov. Opposing Effects of Fasting Metabolism on Tissue Tolerance in Bacterial and Viral Inflammation. *Cell* 166: 1512-1525.e1512.

40. Ganeshan, K., J. Nikkanen, K. Man, Y. A. Leong, Y. Sogawa, J. A. Maschek, T. Van Ry, D. N. Chagwedera, J. E. Cox, and A. Chawla. 2019. Energetic Trade-Offs and Hypometabolic States Promote Disease Tolerance. *Cell* 177: 399-413.e312.

41. Jia, T., N. V. Serbina, K. Brandl, M. X. Zhong, I. M. Leiner, I. F. Charo, and E. G. Pamer. 2008. Additive Roles for MCP-1 and MCP-3 in CCR2-mediated Recruitment of Inflammatory Monocytes During Listeria monocytogenes Infection. *Journal of immunology (Baltimore, Md. : 1950)* 180: 6846-6853.

42. Serbina, N. V., and E. G. Pamer. 2006. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol* 7: 311-317.

43. Seki, E., H. Tsutsui, N. M. Tsuji, N. Hayashi, K. Adachi, H. Nakano, S. Futatsugi-Yumikura, O. Takeuchi, K. Hoshino, S. Akira, J. Fujimoto, and K. Nakanishi. 2002. Critical Roles of Myeloid Differentiation Factor 88-Dependent Proinflammatory Cytokine Release in Early Phase Clearance of Listeria monocytogenes in Mice. *The Journal of Immunology* 169: 3863.

44. Dyatlov, V. A., and D. A. Lawrence. 2002. Neonatal Lead Exposure Potentiates Sickness Behavior Induced by Listeria monocytogenes Infection of Mice. *Brain, Behavior, and Immunity* 16: 477-492.

45. Jelley-Gibbs, D. M., N. M. Lepak, M. Yen, and S. L. Swain. 2000. Two Distinct Stages in the Transition from Naive CD4 T Cells to Effectors, Early Antigen-Dependent and Late Cytokine-Driven Expansion and Differentiation. *The Journal of Immunology* 165: 5017-5026.

46. Veiga-Fernandes, H., U. Walter, C. Bourgeois, A. McLean, and B. Rocha. 2000. Response of naïve and memory CD8+ T cells to antigen stimulation in vivo. *Nat. Immunol.* 1: 47-53.

47. Corbin, G. A., and J. T. Harty. 2004. Duration of Infection and Antigen Display Have Minimal Influence on the Kinetics of the CD4+ T Cell Response to Listeria monocytogenes Infection. *The Journal of Immunology* 173: 5679-5687.

48. Williams, M. A., and M. J. Bevan. 2004. Shortening the Infectious Period Does Not Alter Expansion of CD8 T Cells but Diminishes Their Capacity to Differentiate into Memory Cells. *The Journal of Immunology* 173: 6694-6702.

49. Wirth, T. C., J. T. Harty, and V. P. Badovinac. 2010. Modulating numbers and phenotype of CD8+ T cells in secondary immune responses. 40: 1916-1926.

50. Harty, J. T., and M. J. Bevant. 1995. Specific immunity to listeria monocytogenes in the absence of IFNγ. *Immunity* 3: 109-117.

51. Kägi, D., B. Ledermann, K. Bürki, H. Hengartner, and R. M. Zinkernagel. 1994. CD8+ T cell-mediated protection against an intracellular bacterium by perforin-dependent cytotoxicity. *European Journal of Immunology* 24: 3068-3072.

52. Tripp, C. S., O. Kanagawa, and E. R. Unanue. 1995. Secondary response to Listeria infection requires IFN-gamma but is partially independent of IL-12. *The Journal of Immunology* 155: 3427.

53. Schiemann, M., V. Busch, K. Linkemann, K. M. Huster, and D. H. Busch. 2003. Differences in maintenance of CD8+ and CD4+ bacteria-specific effector-memory T cell populations. 33: 2875-2885.

54. Geginat, G., S. Schenk, M. Skoberne, W. Goebel, and H. Hof. 2001. A Novel Approach of Direct Ex Vivo Epitope Mapping Identifies Dominant and Subdominant CD4 and CD8 T Cell Epitopes from Listeria monocytogenes. *The Journal of Immunology* 166: 1877-1884.

55. Pope, C., S.-K. Kim, A. Marzo, K. Williams, J. Jiang, H. Shen, and L. Lefrançois. 2001. Organ-Specific Regulation of the CD8 T Cell Response to Listeria monocytogenes Infection. *The Journal of Immunology* 166: 3402-3409.

56. Shen, H., J. F. Miller, X. Fan, D. Kolwyck, R. Ahmed, and J. T. Harty. 1998. Compartmentalization of Bacterial Antigens: Differential Effects on Priming of CD8 T Cells and Protective Immunity. *Cell* 92: 535-545.

57. Shen, H., M. K. Slifka, M. Matloubian, E. R. Jensen, R. Ahmed, and J. F. Miller. 1995. Recombinant Listeria monocytogenes as a live vaccine vehicle for the induction of protective anti-viral cell-mediated immunity. *Proceedings of the National Academy of Sciences* 92: 3987-3991.

58. Woodie, L. N., Y. Luo, M. J. Wayne, E. C. Graff, B. Ahmed, A. M. O'Neill, and M. W. Greene. 2018. Restricted feeding for 9h in the active period partially abrogates the detrimental metabolic effects of a Western diet with liquid sugar consumption in mice. *Metabolism* 82: 1-13.

59. Luo, Y., C. M. Burrington, E. C. Graff, J. Zhang, R. L. Judd, P. Suksaranjit, Q. Kaewpoowat, S. K. Davenport, A. M. O'Neill, and M. W. Greene. 2016. Metabolic phenotype and adipose and liver features in a high-fat Western diet-induced mouse model of obesity-linked NAFLD. *American Journal of Physiology - Endocrinology and Metabolism* 310: E418-E439.

60. Weir, J. B. d. V. 1949. New methods for calculating metabolic rate with special reference to protein metabolism. *The Journal of Physiology* 109: 1-9.

61. Stemberger, C., K. M. Huster, M. Koffler, F. Anderl, M. Schiemann, H. Wagner, and D. H. Busch. 2007. A Single Naive CD8+ T Cell Precursor Can Develop into Diverse Effector and Memory Subsets. 27: 985-997.

62. Bahjat, K. S., W. Liu, E. E. Lemmens, S. P. Schoenberger, D. A. Portnoy, T. W. Dubensky, and D. G. Brockstedt. 2006. Cytosolic Entry Controls CD8+-T-Cell Potency during Bacterial Infection. *Infection and Immunity* 74: 6387-6397.

63. Humann, J., and L. L. Lenz. 2010. Activation of naive NK cells in response to Listeria monocytogenes requires IL-18 and contact with infected dendritic cells. *J Immunol* 184: 5172-5178.

64. Edelson, Brian T., Tara R. Bradstreet, K. Hildner, Javier A. Carrero, Katherine E. Frederick, W. Kc, R. Belizaire, T. Aoshi, Robert D. Schreiber, Mark J. Miller, Theresa L. Murphy, Emil R. Unanue, and Kenneth M. Murphy. 2011. CD8α+ Dendritic Cells Are an Obligate Cellular Entry Point for Productive Infection by Listeria monocytogenes. *Immunity* 35: 236-248.

65. Westcott, M. M., C. J. Henry, J. E. Amis, and E. M. Hiltbold. 2010. Dendritic Cells Inhibit the Progression of Listeria monocytogenes Intracellular Infection by Retaining Bacteria in Major Histocompatibility Complex Class II-Rich Phagosomes and by Limiting Cytosolic Growth. *Infection and Immunity* 78: 2956-2965.

66. McCusker, R. H., and K. W. Kelley. 2013. Immune–neural connections: how the immune system's response to infectious agents influences behavior. *The Journal of Experimental Biology* 216: 84.

67. Dantzer, R. 2001. Cytokine-Induced Sickness Behavior: Where Do We Stand? *Brain, Behavior, and Immunity* 15: 7-24.

68. DeBoer, M. D., V. Vijayakumar, M. Gong, J. L. Fowlkes, R. M. Smith, F. Ruiz-Perez, and J. P. Nataro. 2017. Mice with infectious colitis exhibit linear growth failure and subsequent catch-up growth related to systemic inflammation and IGF-1. *Nutr Res* 39: 34-42.

69. O'Connell, R. M., S. K. Saha, S. A. Vaidya, K. W. Bruhn, G. A. Miranda, B. Zarnegar, A. K. Perry, B. O. Nguyen, T. F. Lane, T. Taniguchi, J. F. Miller, and G. Cheng. 2004. Type I interferon production enhances susceptibility to Listeria monocytogenes infection. *J Exp Med* 200: 437-445.

70. Czuprynski, C. J., and J. F. Brown. 1986. The relative difference in anti-Listeria resistance of C57BL/6 and A/J mice is not eliminated by active immunization or by transfer of Listeria-immune T cells. *Immunology* 58: 437-443.

71. Foulds, K. E., L. A. Zenewicz, D. J. Shedlock, J. Jiang, A. E. Troy, and H. Shen. 2002. Cutting Edge: CD4 and CD8 T Cells Are Intrinsically Different in Their Proliferative Responses. *The Journal of Immunology* 168: 1528.

72. Gubser, P. M., G. R. Bantug, L. Razik, M. Fischer, S. Dimeloe, G. Hoenger, B. Durovic, A. Jauch, and C. Hess. 2013. Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat. Immunol.* 14: 1064-1072.

73. Pearce, E. L., M. C. Walsh, P. J. Cejas, G. M. Harms, H. Shen, L.-S. Wang, R. G. Jones, and Y. Choi. 2009. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* 460: 103.

74. Perreau, M., and E. J. Kremer. 2005. Frequency, Proliferation, and Activation of Human Memory T Cells Induced by a Nonhuman Adenovirus. 79: 14595-14605.

75. Moon, J. J., H. H. Chu, M. Pepper, S. J. McSorley, S. C. Jameson, Ross, and M. K. Jenkins. 2007. Naive CD4+ T Cell Frequency Varies for Different Epitopes and Predicts Repertoire Diversity and Response Magnitude. *Immunity* 27: 203-213.

76. Obar, J. J., K. M. Khanna, and L. Lefrançois. 2008. Endogenous Naive CD8+ T Cell Precursor Frequency Regulates Primary and Memory Responses to Infection. *Immunity* 28: 859-869.

77. Blattman, J. N., R. Antia, D. J. D. Sourdive, X. Wang, S. M. Kaech, K. Murali-Krishna, J. D. Altman, and R. Ahmed. 2002. Estimating the Precursor Frequency of Naive Antigen-specific CD8 T Cells. *Journal of Experimental Medicine* 195: 657-664.

78. Qiu, Z., C. Khairallah, and B. Sheridan. 2018. Listeria Monocytogenes: A Model Pathogen Continues to Refine Our Knowledge of the CD8 T Cell Response. *Pathogens* 7: 55.

79. Otálora-Ardila, A., L. G. Herrera M, J. J. Flores-Martínez, and K. C. Welch. 2016. Metabolic Cost of the Activation of Immune Response in the Fish-Eating

Myotis (Myotis vivesi): The Effects of Inflammation and the Acute Phase Response. *PLOS ONE* 11: e0164938.

80. Cox, C. L., R. T. Peaden, and R. M. Cox. 2015. The metabolic cost of mounting an immune response in male brown anoles (Anolis sagrei). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* 323: 689-695.

81. Nagy, K. A. 1987. Field Metabolic Rate and Food Requirement Scaling in Mammals and Birds. *Ecological Monographs* 57: 112-128.

82. Lifson, N., and R. McClintock. 1966. Theory of use of the turnover rates of body water for measuring energy and material balance. *Journal of Theoretical Biology* 12: 46-74.

Chapter 5

Discussion and Conclusion

Summary of Work

This dissertation described the trade-offs in life history traits caused by a live pathogenic infection over a primary and secondary immune response. During a primary immune response, a dose dependent trade-off in life-history traits and sickness behavior was observed as early as one day after infection. Furthermore, these behaviors persisted until 5 days after infection, which corresponds to the timing of innate immunity.

During the time of maximal demand by the adaptive immune system, T cell expansion, there were no observed differences between control and infected mice. However, when a primary and secondary response were compared, trade-offs were observed that corresponded to the timing of reactivation by cells of the adaptive immune system. Thus, the innate immune response is likely metabolically taxing to the host as compared to the adaptive immune response. Additionally, the immunometabolism induced by a live-pathogenic infection was characterized. There was an observed shift toward aerobic glycolysis in cells of the innate immune system that persisted up to 5 days after infection. However, there was no observed shift to aerobic glycolysis in T cells even though a strong *Listeria*-specific T cell response was detected.

The impact of a primary and secondary immune response on Life-history traits and its implications for trade-offs with growth and maintenance.

Life history theory offers postulates aimed at explaining how animals with access to scarce resources allocate them between different life history traits. Growth, reproduction, and maintenance are the major life history traits that compete for these resources. Allocation toward one or more of these affects an animal's overall fitness. When the demand for resources by one trait is greater than others, a trade-off is inevitable. Throughout an organism's lifespan, it must survive environmental insults. A good example of this is a pathogenic invasion. The host immune system is responsible for defense to pathogenic invasions, which relies on maintenance programs. For this reason, the immune system is part of maintenance (1-5). Numerous studies have examined the trade-offs induced by immunocompetence. Immunocompetence is the ability to mount an effective immune response to a pathogen, which relies on an innate and adaptive immune system. However, these studies have primarily relied on non-specific activation of innate cells or T cells to examine the trade-offs (6-10). Thus, these studies do not actually investigate the full range of responses encompassed by immunocompetence because these studies examine the cost of either the innate or adaptive immune system not both. Our study, utilizing a live pathogen, examined the trade-offs to Life-history traits (growth and maintenance) induced by immunocompetence. Our present studies have some similarities with the current literature that has investigated the trade-offs induced by immunity.

We found that during a primary immune response we observed a decrease in body weight, activity, energy expenditure, metabolic rate, and an increase in sleep coinciding with the timing of innate immune response. Multiple studies have presented similar results as our study, in different animals such as bats, birds, and rodents. When bats were

injected with LPS, Otalora-Ardila et al. observed a 140-185% increase in resting metabolic rate (RMR) (6), and Stockmaier et al. observed a decrease in activity and body weight (7). Similar treatment in house sparrows resulted in a described a decrease in bodyweight, activity, and reproduction as described by Bonneaud et al. (8). Studies in rodents described reduction in weight and activity as well as metabolic rate and food consumption following LPS treatment (9, 10).

Whereas many commonalities are found within the literature and our study, there are also a few discrepancies with the change in metabolism. Otalora-Ardila et al., observed an increase in metabolic rate while Ganeshan et al., and our study observed a decrease (6, 10). The discrepancy is due to the way metabolic rate is defined in the studies. Otalora-Ardila et al., examined RMR while is an animal's metabolic rate while Ganeshan et al., and our study. Collectively, these studies suggest that innate immunity is the driver of trade-offs and sickness behavior which our conclusions support. This could relate to the potential demand caused by the innate immune response during cellular activation, effector molecule production, recruitment and myelopoiesis.

As previously mentioned, cells of the innate immune system demonstrate a shift toward aerobic glycolysis upon activation (11-14). However, these studies have all used LPS as a stimulus to generate these results (15-20). Collectively, these studies have demonstrated that the change in cellular metabolism is essential for effector function, and inhibition of aerobic glycolysis impairs effector function as demonstrated by Everts et al. (15, 20). Our present study supports these findings because we observed a shift towards aerobic glycolysis in cells of the innate immune system as determined by increased expression of Glut1 and glucose uptake which was sustained throughout the timing of

innate immunity. A *Listeria* infection is highly complex and better reflects the numerous challenges by infection by having multiple PAMPs (flagellin, CpG, and Lipoteichoic acid) and the toxin LLO (21-24). However, as previously mentioned, the shift towards aerobic glycolysis in cells of the innate immune system has only been demonstrated with the use of LPS. Thus, we extended the current understanding of PRRs activation that can lead towards aerobic glycolysis (21-24). Aerobic glycolysis requires more nutrients for energy since only 2 ATP are generated vs 36 ATP from OXPHOS. Therefore, the shift towards aerobic glycolysis has the potential to induce the trade-offs observed to provide the immune system with nutrients and energy.

Activated cells have an increased demand for biosynthetic precursors and energy to produce immune-derived products. While we did not directly measure the demand for these cellular processes, we indirectly measured them through the T cell expansion phase since these cells would need the above mentioned nutrients and energy for immune-derived products and proliferation (25-30). These studies demonstrated the importance of glycolysis and glutaminolysis for effector function of T cells but not for energy (28-30) while Chang et al., demonstrated that T cells rely on OXPHOS for generation of ATP (31). Because we failed to observe differences during the T cell expansion phase of a primary immune response, cell proliferation and cellular processes needed for effector function are not likely very taxing on the host. Since the shift towards aerobic glycolysis by these cells was not observed as in cells of the innate immune system, it provided evidence that the shift towards aerobic glycolysis is the inducer of trade-offs. The nutritional and energy requirement for cellular recruitment and myelopoiesis remain poorly characterized.

To our knowledge, there have been no studies to directly investigate the metabolic demand of myelopoiesis or cellular recruitment. To thoroughly determine their demand, these processes would need to be inhibited. However, we can possibly gain insight into their cellular demand from T cells. As previously mentioned, activated T cells rely on OXPHOS, glycolysis and glutaminolysis, and multipotent progenitor cells have the same cellular metabolism (32-34) Because we did not observe trade-offs during T cell activation, it is unlikely cellular differentiation is taxing to the host. Because the innate immune system appears to be more metabolically demanding to the host and induces sickness behavior, this could be a way to reallocate resources of nutrients and energy towards the immune system.

Sickness behavior is characterized by sleepiness, inactivity, reclusiveness, fever, lack of appetite which is driven by the cytokines TNF-α, IL-6 and IL-1β (35-38). Prostaglandin E_2 (PGE_2) interacting with its receptor, EP3, in the medial preoptic nucleus within the hypothalamus induced fever (39, 40), which is a well characterized innate immune response that is metabolically taxing to the host (39-42). Furthermore, Kluger et al., established that for each 1 °C rise in core body temperature requires a 10-12% increase in metabolic rate (43). Because sickness behavior is observed across vertebrates, it is likely an evolutionary mechanism used to shift resources towards the immune system. Sickness behavior increases drowsiness and decreases activity, shifting energy away from locomotion towards immunity. We observed the decrease in activity coinciding with the timing of the innate immune response supporting this idea. Previous studies have generally relied on mitogens or non-pathogenic antigen, which is why the demand of adaptive immunity remains poorly characterized.

Studies have previously used PHA, SRBCs, or KLH to determine the impact of the adaptive immune system. PHA induced non-specific activation of T cells while SRBCs and KLH are non-pathogenic antigens. Generally, these studies have failed to observe any trade-offs induced by an activated adaptive immune system (6, 44-47). However, a couple of studies have observed a cost. Martin et al., observed that house sparrows injected with PHA had an increase in RMR (48), and Demas, et al., observed an increase in metabolic rate in mice injected with KLH (49). Thus, generalization about cost of the adaptive immune response is difficult. To better determine if the adaptive immune response induces trade-offs, we monitored the primary and secondary response to a live pathogen.

The results during the innate immune response of Chapter 3 did not resemble what was observed in Chapter 2. As previously discussed, this is likely due to differences in LD_{50} of the two strains of *Listeri*a. However, the results during the adaptive immune response were similar for both studies, in which there were no observed trade-offs. Because a secondary response has a higher magnitude of clonal expansion, this response has a greater potential to induce adaptive immune response trade-offs (50, 51). We failed to observe trade-offs during the time of detectable clonal expansion in the primary response, indicating primary clonal expansion may not be metabolically taxing. However, we did observe trade-offs within the first 24 hours during the time of reactivation. Gubser et al., demonstrated that reactivation of memory T cells initially requires a rapid shift towards aerobic glycolysis for cytokine production (52). Thus, this shift towards aerobic upon reactivation potentially caused these trade-offs.

Collectively, these studies suggest the shifts in cellular metabolism towards aerobic glycolysis in cells of the immune system as the possible inducer of trade-offs. A possible mechanism is the pro-inflammatory cytokines (TNF-α, IL-1β and IL-6) interacting on the brain to initiate sickness behavior which allows for reallocation of resources which are all produced during a *Listeria* infection (10, 53-58). During this timing of reallocation, these mice become insulin resistant as observed by increased HOMA-IR. This type of response has previously been observed to be induced by TNF-α or IFN-γ as determined by Hotamisligil et al and Sestan et al respectively (59, 60). Given that these animals are systemically insulin resistant and utilize lipids as an energy source as observed by a decrease in RER, these two findings are likely linked. One explanation for this link might be that insulin resistance allows for reallocation of available glucose towards cells of the immune system. These cells likely need excess glucose for energy given that aerobic glycolysis generates only 2 ATP per glucose molecule vs. OXPHOS which generates 36 ATP.

Implications of These Findings

These studies explore the cost of immunocompetence on life-history traits and immunometabolism to a live pathogenic infection. These studies have implications for ecological immunology and immunomodulation research. The first implication is to provide current approaches to determine the cost of immunocompetence. The second implication is advancing the field of immunometabolism by examining the immunometabolism induced by a pathogen.

There is a large collection of work investigating life-history trade-offs that occur during an immune response. However, these studies have relied on the use of PAMPs or mitogens to investigate the cost of immunocompetence which do not reflect an immune response to a pathogen. Additionally, these studies tend to utilize antiquated techniques to monitor an immune response. For example, a popular technique includes the measurement of swelling at injection site as indication of immune cell proliferation where some swelling is likely due to edema. Another technique used to measure the cellular composition is the ration of heterophil/lymphocyte which does not differentiate the cell types of the immune system. (6, 7, 47, 48, 61-63). Additionally, these studies tend to remove animals from home caging and place them into chambers to monitor systemic metabolism. These metabolic chambers restrict movement and likely stress the animal which could influences the readouts. Collectively, these methods do not properly reflect the conditions of an immune response. Our studies addressed these issues through well characterized approaches in immunology and the use of metabolic cages that resemble conventional home cages.

An immune response to a pathogen relies on the innate and adaptive immune system. Additionally, the adaptive immune response involves pathogen-specific cells. Previous studies have used the H:L ratio to determine the number of granulocytes and lymphocytes in a blood smear which provides quantitative but not qualitative information about immune cells. By using flow cytometry, we were able to provide both parameters. For example, we determined the number of innate immune cells, cell type, and activation statue of cells recruited to the spleen. This technique provided better resolution of an immune response compared to H:L ratio. The technique of measuring swelling at the site

of infection is an inappropriate way to monitor T cell proliferation/activation because it cannot determine the total number of T cells or proliferating T cells. Additionally, there is likely edema at the injection site skewing these interpretations. Since we used cell surface markers and IFN-γ production, we were able to determine the total number of T cells and number of antigen-specific T cell in the spleen. This is the typical adaptive immune response since it is antigen-specific not non-specific proliferation as observed with a mitogen. Applying these techniques used in our study provide quantitative and qualitative results which these antiquated techniques cannot achieve which provide better resolution of an immune response. If these techniques are applied to future studies involving Life-history theory, these studies will be robust studied compared to previous studies. These techniques allowed for the longitudinally analysis of immunometabolism induced by a pathogen.

Our current understanding of immunometabolism of cells of cells of the innate immune system relies on the use of LPS which has been shown to causes a shift in cellular metabolism towards aerobic glycolysis (15-18). Our study used *Listeria* (a Gram-positive pathogen) which lacks LPS, but we observed similar shift towards aerobic glycolysis by innate immune cells regardless of PRR detection. Additionally, the previous studies have used the Seahorse Bioanalyzer where they generally examine the cellular metabolism for 4 hours. Where the Seahorse Bioanalyzer may be more sensitive, we were able to examine cellular metabolism of immune cells over the course of an infection using flow cytometric analysis. In the spleen, we observed sustained aerobic glycolysis by cells of the innate immune system during the period of bacterial colonization. Our results align with previous studies but demonstrate sustained aerobic

glycolysis during the timing of pathogen colonization. Thus, this metabolism is likely sustained until pathogen clearance.

The threshold to induce protective immunity for an infection is below the threshold for trade-offs between growth, reproduction, and maintenance because we failed to observe overt cost by the adaptive immune system This phenomenon is used to great advantage in vaccination. Vaccines provide protective immunity through the development of strong adaptive immune responses without inducing disease symptoms including sickness behavior. Thus, dampening the innate immune response would alleviate the monetary cost associated with infections.

In the US alone, the cost for work absences due to illness is about $226 billion annually (41). With the use of immune modulation, we could dampen the immune response allowing for pathogen clearance without the cost of sickness behavior preventing absences due to illnesses. Several studies have examined the effect of immune modulation on cells of the innate immune system, however these studies use immune modulation prior to or at the time of immune activation (42, 43). Since people generally do not know the time of infection, these studies do not represent a real-world application. Future studies need to investigate the effects of immune modulation after immune activation.

Short-comings, Limitations, and Future works

This dissertation was the first to investigate the systemic and cellular cost of an immune response to a pathogen. To achieve the longitudinal analysis of the metabolic phenotype, immune response to *Listeria*, and cellular metabolism of immune system,

required the use of two cohorts of mice. An underlining assumption was that each cohort had similar responses to *Listeria*. To eliminate as many variables, the same infectious dose was used for all mice, similar age, same sex, and inbred strain of mice were used; however, there is no guarantee this was occurring. Future studies can get around this variability by increasing sample size.

While Chapter 2 was more robust of the two studies, it did have its short-comings and limitations. One short-coming is that only the immunometabolism of the high dose of infection was examined which would support the idea of the high dose being more metabolically demanding. Additionally, a limitation of this study was the systemic pro-inflammatory cytokines were not determined. Thus, future studies would include measuring immunometabolism of the low dose of infection and determine the systemic pro-inflammatory cytokines of both infectious doses. This study could support the idea that the high dose if more metabolically taxing than the low dose. Additionally, it might elucidate if the aerobic glycolysis by innate immune cells that is demanding, and the induced sickness behavior by these cells that leads to reallocation of resources towards the immune system.

Another limitation of this research is the differences in LD_{50} for the two strains of *Listeria* used in these studies. Recall that the use of *Lm*-OVA allows for the direct comparison of $CD4^+$ and $CD8^+$ T cells *ex vivo* during an infection (64-68). We determined that similar infectious doses of the different strains did not lead to similar bacterial colonization. Wirth et al., and other have determined the lethal dose of wild-type *Listeria* is 10-fold lower than that of Lm-OVA (69-71). For this reason, the direct comparison of the systemic metabolic profile of the two primary immune responses is not

appropriate. In the future, the more appropriate approach would be to use an infectious dose of *Listeria* for the two strains that would have similar bacterial colonization.

Immunomodulation is the therapeutic intervention aimed at modifying the immune response, and a few studies have applied this technique during infections. Varanasi et al. infected mice with herpes-simplex virus and administered 2-DG which prevents glycolysis (72). They observed pathogen clearance, but the off-target damage by IFN-γ was decreased because T cell cytokine production relies on glucose. Pearce et al. examined the manipulation of FAO on immunological memory (73). They determined that increased FAO lead to increased immunological memory. Both studies have manipulated to adaptive immune system with promising outcomes. A future study would be to administer 2-DG during the timing of innate immunity. This could dampen the innate immune response and decrease the cost of immunity on the host.

Conclusion

Overall, the shift towards aerobic glycolysis that drives trade-offs during an immune response is likely fueling the demand placed on the host by the immune system. During a primary response, we observed the mice infected with the highest dose of *Listeria* exhibited sickness behavior and a hypometabolic state. Furthermore, the timing of trade-offs corresponds to the timing of innate immunity. Thus, the threshold to induce protective immunity must be below the threshold for trade-offs between growth, reproduction, and maintenance. We observed sustained shifts towards aerobic glycolysis in cells of the innate immune system for up to five days after infection. While we failed to observe a shift towards aerobic glycolysis by T cells, we did observe their activation as

determined by IFN-γ production by *Listeria*-specific T cells. Thus, aerobic glycolysis is likely a good marker for innate immune cell activation but not for T cell activation. During a secondary response, we observed trade-offs to life-history traits that corresponded to the timing of activation of memory cells, but not the expansion, contraction, or re-established memory of these cells. Because we failed to observe clonal expansion of T cells during a primary and secondary response inducing trade-offs, it is unlikely that myelopoiesis, generation of effector molecules or cell recruitment induces trade-offs. Collectively, these studies, to our knowledge, provide the first comprehensive understanding of systemic and cellular metabolism to a live pathogen.

Reference

1. Norris, K. 2000. Ecological immunology: life history trade-offs and immune defense in birds. *Behavioral Ecology* 11: 19-26.

2. Lochmiller Robert, L., and C. Deerenberg. 2003. Trade-offs in evolutionary immunology: just what is the cost of immunity? *Oikos* 88: 87-98.

3. Zuk, M., and A. M. Stoehr. 2002. Immune defense and host life history. *The American naturalist* 160 Suppl 4: S9-s22.

4. Rauw, W. M. 2012. Immune response from a resource allocation perspective. *Frontiers in Genetics* 3.

5. Wang, A., H. H. Luan, and R. Medzhitov. 2019. An evolutionary perspective on immunometabolism. *Science* 363: eaar3932.

6. Otálora-Ardila, A., L. G. Herrera M, J. J. Flores-Martínez, and K. C. Welch. 2016. Metabolic Cost of the Activation of Immune Response in the Fish-Eating Myotis (Myotis vivesi): The Effects of Inflammation and the Acute Phase Response. *PLOS ONE* 11: e0164938.

7. Stockmaier, S., D. I. Bolnick, R. A. Page, and G. G. Carter. 2018. An immune challenge reduces social grooming in vampire bats. *Animal Behaviour* 140: 141-149.

8. Bonneaud, C., J. Mazuc, G. Gonzalez, C. Haussy, O. Chastel, B. Faivre, and G. Sorci. 2003. Assessing the Cost of Mounting an Immune Response. *The American naturalist* 161: 367-379.

9. Bay-Richter, C., S. Janelidze, L. Hallberg, and L. Brundin. 2011. Changes in behaviour and cytokine expression upon a peripheral immune challenge. *Behav Brain Res* 222: 193-199.

10. Ganeshan, K., J. Nikkanen, K. Man, Y. A. Leong, Y. Sogawa, J. A. Maschek, T. Van Ry, D. N. Chagwedera, J. E. Cox, and A. Chawla. 2019. Energetic Trade-Offs and Hypometabolic States Promote Disease Tolerance. *Cell* 177: 399-413.e312.

11. O'Neill, L. A. J., R. J. Kishton, and J. Rathmell. 2016. A guide to immunometabolism for immunologists. *Nature Reviews Immunology* 16: 553-565.

12. Pearce, E. J., and B. Everts. 2015. Dendritic cell metabolism. *Nature reviews. Immunology* 15: 18-29.

13. Pearce, E. L., and E. J. Pearce. 2013. Metabolic Pathways in Immune Cell Activation and Quiescence. *Immunity* 38: 633-643.

14. Ganeshan, K., and A. Chawla. 2014. Metabolic Regulation of Immune Responses. *Annual Review of Immunology* 32: 609-634.

15. Everts, B., E. Amiel, G. J. W. van der Windt, T. C. Freitas, R. Chott, K. E. Yarasheski, E. L. Pearce, and E. J. Pearce. 2012. Commitment to glycolysis sustains survival of NO-producing inflammatory dendritic cells. *Blood* 120: 1422.

16. Krawczyk, C. M., T. Holowka, J. Sun, J. Blagih, E. Amiel, R. J. DeBerardinis, J. R. Cross, E. Jung, C. B. Thompson, R. G. Jones, and E. J. Pearce. 2010. Toll-like receptor–induced changes in glycolytic metabolism regulate dendritic cell activation. *Blood* 115: 4742.

17. Greiner, E. F., M. Guppy, and K. Brand. 1994. Glucose is essential for proliferation and the glycolytic enzyme induction that provokes a transition to glycolytic energy production. *J Biol Chem* 269: 31484-31490.

18. Kelly, B., and L. A. J. O'Neill. 2015. Metabolic reprogramming in macrophages and dendritic cells in innate immunity. *Cell Res* 25: 771-784.

19. Thwe, P. M., L. Pelgrom, R. Cooper, S. Beauchamp, J. A. Reisz, A. D'Alessandro, B. Everts, and E. Amiel. Cell-Intrinsic Glycogen Metabolism Supports Early Glycolytic Reprogramming Required for Dendritic Cell Immune Responses. *Cell Metabolism* 26: 558-567.e555.

20. Everts, B., E. Amiel, S. C.-C. Huang, A. M. Smith, C.-H. Chang, W. Y. Lam, V. Redmann, T. C. Freitas, J. Blagih, G. J. W. van der Windt, M. N. Artyomov, R. G. Jones, E. L. Pearce, and E. J. Pearce. 2014. TLR-driven early glycolytic reprogramming via the kinases TBK1-IKKε supports the anabolic demands of dendritic cell activation. *Nat. Immunol.* 15: 323-332.

21. D'Orazio, S. E. F. 2019. Innate and Adaptive Immune Responses during Listeria monocytogenes Infection. *Microbiol Spectr* 7.

22. Pamer, E. G. 2004. Immune responses to Listeria monocytogenes. *Nature Reviews Immunology* 4: 812-823.

23. Zenewicz, L. A., and H. Shen. 2007. Innate and adaptive immune responses to Listeria monocytogenes: a short overview. *Microbes and Infection* 9: 1208-1215.

24. Radoshevich, L., and P. Cossart. 2017. Listeria monocytogenes: towards a complete picture of its physiology and pathogenesis. *Nature Reviews Microbiology* 16: 32.

25. Vander Heiden, M. G., L. C. Cantley, and C. B. Thompson. 2009. Understanding the Warburg Effect: The Metabolic Requirements of Cell Proliferation. *Science (New York, N.Y.)* 324: 1029-1033.

26. Pollizzi, K. N., and J. D. Powell. 2014. Integrating canonical and metabolic signalling programmes in the regulation of T cell responses. *Nature Reviews Immunology* 14: 435-446.

27. DuPage, M., and J. A. Bluestone. 2016. Harnessing the plasticity of CD4+ T cells to treat immune-mediated disease. *Nature Reviews Immunology* 16: 149-163.

28. Carr, E. L., A. Kelman, G. S. Wu, R. Gopaul, E. Senkevitch, A. Aghvanyan, A. M. Turay, and K. A. Frauwirth. 2010. Glutamine Uptake and Metabolism Are Coordinately Regulated by ERK/MAPK during T Lymphocyte Activation. *The Journal of Immunology* 185: 1037.

29. Frauwirth, K. A., J. L. Riley, M. H. Harris, R. V. Parry, J. C. Rathmell, D. R. Plas, R. L. Elstrom, C. H. June, and C. B. Thompson. 2002. The CD28 Signaling Pathway Regulates Glucose Metabolism. *Immunity* 16: 769-777.

30. Jacobs, S. R., C. E. Herman, N. J. MacIver, J. A. Wofford, H. L. Wieman, J. J. Hammen, and J. C. Rathmell. 2008. Glucose Uptake Is Limiting in T Cell Activation and Requires CD28-Mediated Akt-Dependent and Independent Pathways. *The Journal of Immunology* 180: 4476.

31. Chang, C.-H., Jonathan, Leonard, B. Faubert, Alejandro, D. O'Sullivan, Stanley, Gerritje, J. Blagih, J. Qiu, Jason, Edward, Russell, and Erika. 2013. Posttranscriptional Control of T Cell Effector Function by Aerobic Glycolysis. *Cell* 153: 1239-1251.

32. Ito, K., and T. Suda. 2014. Metabolic requirements for the maintenance of self-renewing stem cells. *Nature Reviews Molecular Cell Biology* 15: 243-256.

33. Maciver, N. J., R. D. Michalek, and J. C. Rathmell. 2013. Metabolic Regulation of T Lymphocytes. *Annual Review of Immunology* 31: 259-283.

34. Inoue, S.-I., S. Noda, K. Kashima, K. Nakada, J.-I. Hayashi, and H. Miyoshi. 2010. Mitochondrial respiration defects modulate differentiation but not proliferation of hematopoietic stem and progenitor cells. 584: 3402-3409.

35. Hennessy, M. B., T. Deak, and P. A. Schiml. 2014. Sociality and sickness: Have cytokines evolved to serve social functions beyond times of pathogen exposure? *Brain, Behavior, and Immunity* 37: 15-20.

36. Dantzer, R., and K. W. Kelley. 2007. Twenty years of research on cytokine-induced sickness behavior. *Brain, Behavior, and Immunity* 21: 153-160.

37. Dantzer, R., C. J. Heijnen, A. Kavelaars, S. Laye, and L. Capuron. 2014. The neuroimmune basis of fatigue. *Trends in Neurosciences* 37: 39-46.

38. Adelman, J. S., and L. B. Martin. 2009. Vertebrate sickness behaviors: Adaptive and integrated neuroendocrine immune responses. *Integr. Comp. Biol.* 49: 202-214.

39. Ushikubi, F., E. Segi, Y. Sugimoto, T. Murata, T. Matsuoka, T. Kobayashi, H. Hizaki, K. Tuboi, M. Katsuyama, A. Ichikawa, T. Tanaka, N. Yoshida, and S. Narumiya. 1998. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 395: 281-284.

40. Lazarus, M., K. Yoshida, R. Coppari, C. E. Bass, T. Mochizuki, B. B. Lowell, and C. B. Saper. 2007. EP3 prostaglandin receptors in the median preoptic nucleus are critical for fever responses. 10: 1131-1133.

41. Evans, S. S., E. A. Repasky, and D. T. Fisher. 2015. Fever and the thermal regulation of immunity: the immune system feels the heat. *Nature reviews. Immunology* 15: 335-349.

42. Netea, M. G., B. J. Kullberg, and J. W. M. Van der Meer. 2000. Circulating Cytokines as Mediators of Fever. *Clinical Infectious Diseases* 31: S178-S184.

43. Kluger, M. J. 1979. Phylogeny of fever. *Fed Proc* 38: 30-34.

44. Amat, J. A., E. Aguilera, and G. H. Visser. 2006. Energetic and developmental costs of mounting an immune response in greenfinches (Carduelis chloris. *Ecol Res* 22: 282-287.

45. Merlo, J. L., A. P. Cutrera, F. Luna, and R. R. Zenuto. 2014. PHA-induced inflammation is not energetically costly in the subterranean rodent Ctenomys talarum (tuco-tucos). *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* 175: 90-95.

46. Terry, and S. Compton. 2003. Immune Response, Not Immune Maintenance, Is Energetically Costly in Wild White-Footed Mice (Peromyscus leucopus). *Physiological and Biochemical Zoology* 76: 744-752.

47. Cox, C. L., R. T. Peaden, and R. M. Cox. 2015. The metabolic cost of mounting an immune response in male brown anoles (Anolis sagrei). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology* 323: 689-695.

48. Martin, L. B., A. Scheuerlein, and M. Wikelski. 2003. Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society of London. Series B: Biological Sciences* 270: 153-158.

49. Demas, G. E., V. Chefer, M. I. Talan, and R. J. Nelson. 1997. Metabolic costs of mounting an antigen-stimulated immune response in adult and aged C57BL/6J mice. *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology* 273: R1631-R1637.

50. Opferman, J. T. 1999. Linear Differentiation of Cytotoxic Effectors into Memory T Lymphocytes. *Science* 283: 1745-1748.

51. Lakkis, F. G. 2003. Memory T Cells: A Hurdle to Immunologic Tolerance. *Journal of the American Society of Nephrology* 14: 2402-2410.

52. Gubser, P. M., G. R. Bantug, L. Razik, M. Fischer, S. Dimeloe, G. Hoenger, B. Durovic, A. Jauch, and C. Hess. 2013. Rapid effector function of memory CD8+ T cells requires an immediate-early glycolytic switch. *Nat. Immunol.* 14: 1064-1072.

53. Ohga, S., K. Ueda, Y. Yoshikai, Y. Takeda, K. Hiromatsu, and K. Nomoto. 1991. Kinetics of fever and its related cytokines in mice after intraperitoneal infection with listeria monocytogenes. *Journal of Thermal Biology* 16: 103-107.

54. Wang, A., S. C. Huen, H. H. Luan, S. Yu, C. Zhang, J.-D. Gallezot, C. J. Booth, and R. Medzhitov. Opposing Effects of Fasting Metabolism on Tissue Tolerance in Bacterial and Viral Inflammation. *Cell* 166: 1512-1525.e1512.

55. Jia, T., N. V. Serbina, K. Brandl, M. X. Zhong, I. M. Leiner, I. F. Charo, and E. G. Pamer. 2008. Additive Roles for MCP-1 and MCP-3 in CCR2-mediated Recruitment of Inflammatory Monocytes During Listeria monocytogenes Infection. *Journal of immunology (Baltimore, Md. : 1950)* 180: 6846-6853.

56. Serbina, N. V., and E. G. Pamer. 2006. Monocyte emigration from bone marrow during bacterial infection requires signals mediated by chemokine receptor CCR2. *Nat Immunol* 7: 311-317.

57. Seki, E., H. Tsutsui, N. M. Tsuji, N. Hayashi, K. Adachi, H. Nakano, S. Futatsugi-Yumikura, O. Takeuchi, K. Hoshino, S. Akira, J. Fujimoto, and K. Nakanishi. 2002. Critical Roles of Myeloid Differentiation Factor 88-Dependent Proinflammatory Cytokine Release in Early Phase Clearance of Listeria monocytogenes in Mice. *The Journal of Immunology* 169: 3863.

58. Dyatlov, V. A., and D. A. Lawrence. 2002. Neonatal Lead Exposure Potentiates Sickness Behavior Induced by Listeria monocytogenes Infection of Mice. *Brain, Behavior, and Immunity* 16: 477-492.

59. Hotamisligil, G. S., D. L. Murray, L. N. Choy, and B. M. Spiegelman. 1994. Tumor necrosis factor alpha inhibits signaling from the insulin receptor. *Proceedings of the National Academy of Sciences* 91: 4854-4858.

60. Sestan, M., S. Marinovic, I. Kavazovic, D. Cekinovic, S. Wueest, T. Turk Wensveen, I. Brizic, S. Jonjic, D. Konrad, F. M. Wensveen, and B. Polic. 2018. Virus-Induced Interferon-gamma Causes Insulin Resistance in Skeletal Muscle and Derails Glycemic Control in Obesity. *Immunity*.

61. Smits, J. E., G. R. Bortolotti, and J. L. Tella. 1999. Simplifying the phytohaemagglutinin skin-testing technique in studies of avian immunocompetence. *Functional Ecology* 13: 567-572.

62. Maxwell, M. H. 1993. Avian blood leucocyte responses to stress. *World's Poultry Science Journal* 49: 34-43.

63. Gross, W. B., and H. S. Siegel. 1983. Evaluation of the heterophil/lymphocyte ratio as a measure of stress in chickens. *Avian Dis* 27: 972-979.

64. Schiemann, M., V. Busch, K. Linkemann, K. M. Huster, and D. H. Busch. 2003. Differences in maintenance of CD8+ and CD4+ bacteria-specific effector-memory T cell populations. 33: 2875-2885.

65. Geginat, G., S. Schenk, M. Skoberne, W. Goebel, and H. Hof. 2001. A Novel Approach of Direct Ex Vivo Epitope Mapping Identifies Dominant and Subdominant CD4 and CD8 T Cell Epitopes from Listeria monocytogenes. *The Journal of Immunology* 166: 1877-1884.

66. Pope, C., S.-K. Kim, A. Marzo, K. Williams, J. Jiang, H. Shen, and L. Lefrançois. 2001. Organ-Specific Regulation of the CD8 T Cell Response to Listeria monocytogenes Infection. *The Journal of Immunology* 166: 3402-3409.

67. Shen, H., J. F. Miller, X. Fan, D. Kolwyck, R. Ahmed, and J. T. Harty. 1998. Compartmentalization of Bacterial Antigens: Differential Effects on Priming of CD8 T Cells and Protective Immunity. *Cell* 92: 535-545.

68. Shen, H., M. K. Slifka, M. Matloubian, E. R. Jensen, R. Ahmed, and J. F. Miller. 1995. Recombinant Listeria monocytogenes as a live vaccine vehicle for the induction of protective anti-viral cell-mediated immunity. *Proceedings of the National Academy of Sciences* 92: 3987-3991.

69. Wirth, T. C., J. T. Harty, and V. P. Badovinac. 2010. Modulating numbers and phenotype of CD8+ T cells in secondary immune responses. 40: 1916-1926.

70. Stemberger, C., K. M. Huster, M. Koffler, F. Anderl, M. Schiemann, H. Wagner, and D. H. Busch. 2007. A Single Naive CD8+ T Cell Precursor Can Develop into Diverse Effector and Memory Subsets. 27: 985-997.

71. Bahjat, K. S., W. Liu, E. E. Lemmens, S. P. Schoenberger, D. A. Portnoy, T. W. Dubensky, and D. G. Brockstedt. 2006. Cytosolic Entry Controls CD8+-T-Cell Potency during Bacterial Infection. *Infection and Immunity* 74: 6387-6397.

72. Varanasi, S. K., D. Donohoe, U. Jaggi, and B. T. Rouse. 2017. Manipulating Glucose Metabolism during Different Stages of Viral Pathogenesis Can Have either Detrimental or Beneficial Effects. *The Journal of Immunology*.

73. Pearce, E. L., M. C. Walsh, P. J. Cejas, G. M. Harms, H. Shen, L.-S. Wang, R. G. Jones, and Y. Choi. 2009. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. *Nature* 460: 103.

Appendix I

Contributions to Others Work

Auburn University strives for collaboration. While pursuing my Ph.D., I was able to collaborate with my fellow lab mates and other labs outside of the department. These collaborations honed my research skills and critical thinking, but unfortunately, did not directly connect with my dissertation work. I wish to describe these collaborations and my contributions below.

The first collaboration occurred with Dr. Michael Greene in the Department of Nutrition and my former labmate Dr. Keah Higgins. The goal of this study was to longitudinally monitor mice on a Western diet to determine when and how the intestinal microbiota shifts in composition, as well as the development of a metabolic phenotype resembling obesity. I aided in specimen collection and determined the intestinal inflammatory status through Lipocalin-2. Lipocalin-2 or neutrophil gelatinase-associated is a protein released by neutrophils to sequester iron; thus, limiting bacterial growth. Before this study, Chassaing, *et al.* had established Lipocalin-2 as a marker of intestinal inflammation in a mouse model of DSS-induced colitis (1). We wanted to determine if this marker could monitor the chronic "low-grade" inflammation observed in obesity. Unfortunately, the "low-grade" inflammation observed during obesity was not enough to elicit a detectable difference between lean and obese individuals. Thus, this marker is not a useful marker for chronic "low-grade" inflammation observed during obesity but is an excellent marker of acute inflammation as observed during DSS induced colitis.

The second collaboration involved Dr. Terry Brandebourg in the Department of Animal Sciences and my labmate, Haley Hallowell. This project had two goals. The first

was to define and characterize the Mangalica pig as a novel model of obesity. Secondly, after establishing this model, the goal was to determine whether overconsumption alone was enough to drive changes in the gut microbiota as piglets developed obesity. My contribution to this project was specimen collection, animal husbandry, and monitoring intestinal inflammation. Due to my bachelor's degree in Animal Sciences, I had the responsibility of animal husbandry. This study was occurring concurrently with my other collaborations, so we used the same fecal marker of intestinal inflammation Lipocalin-2. As observed with the mouse study, lipocalin-2 was not a useful marker of chronic "low-grade" inflammation.

Reference

1. Chassaing, B., G. Srinivasan, M. A. Delgado, A. N. Young, A. T. Gewirtz, and M. Vijay-Kumar. 2012. Fecal Lipocalin 2, a Sensitive and Broadly Dynamic Non-Invasive Biomarker for Intestinal Inflammation. *PLoS ONE* 7: e44328.