[redacted]

Title: **Commensal Antigen-Specific T cells**
Sponsor Name: [redacted] **ECOR Interim Support Fund, SUNDRY**
PI Name: [redacted] Protocol #: [redacted] Type: **Current View**
Species: **MICE** # Of Animals: **1740** Date Received: **January 09, 2013**

Study Staff

Name	Role	Degree	Organization
[redacted]	Principal Investigator	BS, Ph.D	[redacted] > [redacted] > [redacted]
[redacted]	Research Technician		[redacted] > [redacted] > [redacted]

Linked Agreements

Record #	Fund	Project Period	PI Name	Sponsor	Record Type	Process	Link Date	Link Status
[redacted]		[redacted]	[redacted]	SUNDRY	RM – Research Sundry	TR2	12/17/18	Approved
[redacted]		[redacted]	[redacted]	[redacted] ECOR Interim Support Fund	RM – Funded Agreement	AME10	03/13/19	Approved

Linked Protocols

Protocol #	Relationship	Link Location	Overall Status	PI Name	Title	Process	Link Date	Link Status	Link Direction
[redacted]	Biosafety	Citrobacter rodentium, Listeria monocytogenes, Use of Animals	Active	[redacted]	[redacted]	AME11/LA9	02/07/19	Approved	Two Way

Protocol Overview

Please answer the following questions using language a non-scientist will understand.

1. Study Goals

How would you explain the long term or overall scientific goals of the proposed work to a non-scientist? **[Please limit to 200 words.]**

The gastrointestinal tract is colonized by an enormous diversity of commensal microbes. Inflammatory Bowel Diseases (IBD) such as Crohn's Disease and

[redacted]

Ulcerative Colitis are thought to arise when the immune system erroneously attacks these microbes. A better understanding of how the immune system normally establishes tolerance to commensal microbes will be essential for the development of effective therapies. The overall goal of this protocol is to study T cells with specificity to commensal microbes in the gut and determine their role in inflammatory bowel diseases (IBD). We hypothesize that T cells normally become tolerized to gut commensal microbes and that a breakdown in this tolerance is an underlying cause of IBD. We will use the latest technologies to directly identify populations of T cells that recognize distinct strains of commensal bacteria in mice, and study their behavior during the different immune contexts brought upon by experimental models of colitis. By extensively characterizing the phenotype of these antigen-specific T cells during health and disease, we will unravel the mechanisms by which they mediate immune tolerance.

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The overall objective of this protocol is to characterize CD4+ T cells with specificity to commensal microbes in the gut and determine their role in inflammatory bowel diseases (IBD). We hypothesize that T cells normally become tolerized to gut commensal microbes and that a breakdown in this tolerance is an underlying cause of IBD. We will use the latest in peptide:MHC tetramer technology to directly study commensal antigen-specific T cells in mice and investigate the mechanisms mediating their tolerance.

2. Benefit to be Gained by Animal Research

How would you explain to a non-scientist that the potential benefits of the study, in terms of biomedical advancement, justify the proposed animal use? **[Please limit to 200 words.]**

Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder of the gastrointestinal system that affects over 1.4 million Americans and is quickly rising in prevalence in all developed parts of the world. There is no cure and the chronic debilitating nature of the disease contributes to substantial social and economic burdens on individuals, families, and society. The causes of IBD are unknown, but there is general agreement that the disease is the manifestation of dysregulated immune responses to normal gut microbiota. A better understanding of how the adaptive immune system normally establishes tolerance to these commensal microbes will be instrumental to the development of new therapies to treat IBD. Our study focuses on the biology of T cells, a major component of the adaptive immune system, specifically in the gut environment where they exert their influence on IBD. The extensive genetic, cellular, and molecular tools made available in mice over the past

several decades make this animal model by far and away the most powerful experimental system in which to study T cells in a physiologically relevant context, and accordingly, advances in the field have been dominated by studies using this model.

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Inflammatory Bowel Disease (IBD) is a chronic inflammatory disorder of the gastrointestinal system that affects some 1.4 million Americans. There is no cure and the chronic debilitating nature of the disease contributes to substantial social and economic burdens on individuals, families, and society. The causes of IBD are unknown, but there is general agreement that the disease is the manifestation of dysregulated immune responses to normal gut microbiota. These include both innate and adaptive immune functions, and recent studies of regulatory T cells (Treg) in particular have indicated a major role for adaptive immunity in both mediating and controlling gut inflammation.

Unfortunately, detailed studies of T cells involved in the development of IBD are severely lacking due to a dearth of knowledge about commensal microbiota and the relevant antigens from them that are recognized by T cells. Very few experimental tools are available to enable investigation of the important immunological questions surrounding T cell involvement in IBD. We have recently established a powerful new in vivo experimental system that will allow us to investigate in detail the immunobiology of CD4+ T cells with specificity to conserved antigens in commensal gut microbiota. We will use this system with established models of gut inflammation and infection in mice to determine how tolerance is established in these cells and what role they play in the development of IBD.

Research Objective: Research Objective 1

INSTRUCTIONS:
Complete a Research Objective form for each discrete aim of the protocol. To add an additional Research Objective, please click the **add New Research Objective** button at the end of this form.

Limit the discussion to activities involving animals. Do not describe *in vitro* procedures beyond collection of tissues, blood, or other biological products.

A. Rationale: [**Please limit to 200 words**]

Research Objective 1- T cell responses to commensal bacteria during epithelial barrier leakage

[redacted]

Numerous studies have linked IBD to genetic predispositions that result in impaired intestinal epithelial barrier integrity, leading to the hypothesis that excessive exposure of commensal bacteria from the lumen to the gut immune system may lead to a breakdown of T cell tolerance that results in chronic inflammation and disease. To investigate this possibility and delineate the underlying mechanisms, we will characterize the phenotype and behavior of commensal antigen-specific T cells in mice where the intestinal epithelial barrier is experimentally compromised by 1) the chemical irritant dextran sulfate sodium (DSS), and 2) the lack of secreted IgA through genetic ablation of the polymeric Ig receptor (pIgR). We have developed a panel of novel peptide:MHC tetramer reagents that enable us to directly identify populations of T cells that recognize specific antigens from distinct enteric commensal bacteria species and characterize their phenotype and function.

B. Experimental Design: For this research objective, outline the time-course indicating each activity. Describe each step and how it relates to an animal enrolled in this study. It should be clear what each animal will experience during the full course of this Research Objective.

- Include the length of time an animal is enrolled in an experiment
- Describe experimental endpoints
- Do not include descriptions of surgical and non-surgical procedures in the **Experimental Design**. Include this information in the specific **Procedure** forms.

Experiment 1- Acute colitis with single DSS treatment

To determine how T cell tolerance to commensal microbes of the gut is affected by inflammation caused by epithelial barrier disruption, we will study commensal antigen-specific T cell populations in naive mice as well as mice fed with the chemical irritant, dextran sulfate sodium (DSS), in their drinking water for 7 days. In this widely used model of colitis, the intestinal epithelial barrier is damaged, causing inflammation and leakage of lumenal bacterial contents into the underlying intestinal lamina propria, draining lymphatics, and bloodstream, where T cells within the immune system can recognize and respond to them. The extent of colitis will be monitored by measuring the weight of the mice daily, and when euthanized at set timepoints, measuring colon length and performing histological analysis of tissue sections.

To determine whether populations of commensal antigen-specific T cells increase or decrease, or exhibit functional changes, in response to their exposure to leaked commensal antigen, we will isolate these cells from the spleen, lymph nodes, or intestines of mice euthanized at defined timepoints throughout colitis development as monitored by animal weight loss. Weight loss peaks within the first 3 days after treatment is stopped and then recovers to baseline in the following week, so we will sample T cell populations throughout a 4 week timecourse. Isolated commensal antigen-specific T cells will be analyzed by tetramer staining and flow cytometry to calculate cell numbers and expression of surface markers associated with certain states of T cell development and function (i.e. naive, activated, memory, regulatory, etc.).

[redacted]

The use of Foxp3, IL-10, and IL-17 reporter genes in our transgenic mice will greatly aid in this analysis. In all experiments, populations of CD4+ T cells with specificity to foreign and self antigens will be simultaneously studied in each sample to provide a set of internal negative controls. For selected subsets of samples, antigen-specific T cell RNA will be harvested for high-powered RNA-seq analysis, and fecal pellets will be collected for 16S ribosomal sequence analysis of microbiome composition.

To directly test the immune function of these T cells, we will challenge DSS-treated mice at select timepoints with an intragastric dose of antigenic peptide and cholera toxin B subunit, and assay cytokine production by intracellular staining or reporter gene expression in these cells 7 days later. Effector T cells should produce IL-17, while anergic T cells would not produce cytokines at all, and regulatory T cells would produce IL-10 or TGFb.

Experiment 2- Chronic colitis with multiple rounds of DSS treatments

The establishment of T cell tolerance to a specific antigen is thought to be reinforced through multiple non-inflammatory rounds of exposure to the antigen. Therefore, to determine whether T cell tolerance to gut commensal antigens is developed gradually over chronic exposure to antigen, we will also study commensal antigen-specific T cells in mice receiving multiple rounds of DSS treatment over time in a chronic model of colitis. In these experiments, mice will receive three successive rounds of DSS treatment, with each treatment course consisting of one week of DSS treatment followed by two weeks of recovery, for a total experimental timecourse of 9 weeks. Mice will be euthanized at set timepoints and commensal antigen-specific or control T cell populations will be analyzed as described above.

Experiment 3- Chronic epithelial barrier leakage in pIgR KO mice

In another approach, we will study these T cells in pIgR knockout mice, which genetically lack the receptor that transports IgA antibody from the bloodstream into the intestinal lumen. As a result, these mice do not neutralize lumenal antigens, and the overall ability of the mucus-epithelium layer to provide barrier function is partially impaired. Consequently, there is a chronic low level leakage of lumenal contents, including commensal bacteria, into the surrounding tissue. However, the context in which this leakage occurs is perhaps less inflammatory than that during DSS treatment, thereby providing a greater chance that tolerance induction, rather than activation, may be the fate of commensal antigen-specific T cells in this system. We will simply examine these T cells in naive pIgR KO or wild type control mice at different ages ranging from 4 to 12 weeks using the same assays described above.

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review the information in this field as it may contain details useful in answering the **Experimental Design** question above. ***Use of this information is optional.***

The general window of experimentation for any mouse in this study is 3 months. Since most mice will be approximately 1-3 months of age at the start of experiments, all mice used for experiments will be euthanized by 6 months of age. The maximum age of mice used for breeding will be 1 year. Any mouse that shows signs of illness or suffering inconsistent with its experimental conditions will be immediately euthanized.

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C. Flow Chart: For this research objective, a schema or flow chart diagramming the overall picture of the study design and treatment groups must be included. The flow chart should include:

- all experimental groups
- the number of animals per group
- the procedures performed on the animal
- the length of time an animal is enrolled in the experiment

The IACUC must be able to understand the experience of each animal on the protocol. See TIPS for Creating Flow Charts in the FAQ pane for detailed information.

D. Health Status:

1. Describe the health status of the animals during this research objective. Include:

- Expected development and progression of clinical signs, including severity and time course
- Potential adverse events caused by the research model and/or experimental manipulations
- If a scoring system will be used to monitor animal health, please attach it to the protocol below.

All naive mice are expected to be healthy throughout the study. Mice experiencing DSS-induced colitis will experience weight loss and possibly some diarrhea that is reversible upon cessation of treatment. The dosage of DSS has been carefully titrated such that control mice will lose approximately 15-20% and not more than 25% of their original body weight peaking at day 9-10 post treatment initiation (day 2-3 post treatment cessation). Colitic mice will have inflamed intestines and may appear slightly lethargic, but should not exhibit other overt signs of illness. DSS treated mice will be monitored by weight daily throughout the course of treatment until the animal's original body weight is restored or the study endpoint is reached.

[redacted]

2. What action will be taken should clinical signs manifest?

The induction of colitis with DSS may cause some extended pain and distress, but analgesics cannot be used due to the detrimental side effects they will have on the physiology of the intestinal inflammation that we are studying (see Pain or Distress Study Justification). To help provide a nurturing environment for their recovery, all DSS-treated mice will receive an igloo and additional Enviro-Dry bedding material in their cages. However, any mouse that exhibits exceptional signs of pain or distress will be immediately euthanized. Any mouse exhibiting more than 25% weight loss will be immediately euthanized.

Migrated Data

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Following short-term isoflurane anesthesia, mice will be monitored until they resume normal behavior in their cages. Following immunization or infection, mice will be checked daily for any signs of illness or suffering for one week, then every two days for another week, and then once a week, until the study endpoint, maximum 3 months. DSS treated mice will be monitored by weight daily throughout the course of treatment until the animal's original body weight is restored or the study endpoint is reached. Immunized or infected mice will also be monitored for potential colitis by measuring their weight daily, every two days, or twice a week, depending on the experiment, until they recover their original body weight or the study endpoint is reached. Any signs of illness or suffering will result in immediate euthanasia. To help provide a nurturing environment for their recovery, all mice receiving any kind of experimental treatment (drug administration, cell transfer, immunization, or infection) will be placed in a cage containing an igloo and additional Enviro-Dry bedding material. Mice undergoing colitis or infection will also be given some Napa Nectar gel food supplements on the floors of their cages.

Attachments

Name	Mode
flowchart-1 (Flowchart)	Electronic

FLOW CHART

Research Objective 1- T cell responses to commensal bacteria during epithelial barrier leakage

Experimental strategy

Each euthanized timepoint analysis includes assessment of:

- Colon length
- Histological analysis of intestinal tissue
- Antigen-specific T cell frequency by flow cytometry
- Antigen-specific T cell phenotype by flow cytometry
- Antigen-specific T cell phenotype by single cell RNA-seq (some samples)
- Microbiome composition by 16S ribosomal sequencing (some samples)

Experiment 1- Acute colitis with single DSS treatment

Weigh mice daily
DSS treatment of **Triple reporter mice** for 7 days (t=0 to t=7)
No treatment for 21 days (t=7 to t=28)
A- Euthanize at timepoints t=0, 4, 8, 12, 16, 20, 28 days
4 mice x 7 timepoints = **28 mice** (4 Pain C, 24 Pain E)
B- Challenge additional mice at timepoints t=0, 20, 42 days
1- test peptide + cholera toxin i.g.
2- control peptide + cholera toxin i.g.
Euthanize 7 days later (t=7, t=27, t=49)
4 mice x 2 conditions x 3 timepoints = **24 mice** (8 Pain C, 16 Pain E)

Experiment 2- Chronic colitis with 3 repeated DSS treatments

Weigh mice every 1-3 days
DSS treatment of **Triple reporter mice** for 7 days (t=0 to t=7)
No treatment for 14 days (t=7 to t=21)
DSS treatment for 7 days (t=21 to t=28)
No treatment for 14 days (t=28 to t=42)
DSS treatment for 7 days (t=42 to t=49)
A- Euthanize at timepoints t=42, 46, 50, 54, 58, 62, 70, 77, 84 days
4 mice x 9 timepoints = **36 mice** (Pain E)
B- Challenge additional mice at timepoints t=62, 84 days
1- test peptide + cholera toxin i.g.
2- control peptide + cholera toxin i.g.
Euthanize 7 days later (t=69, t=91)
4 mice x 2 conditions x 2 timepoints = **16 mice** (Pain E)

Experiment 3- Chronic epithelial barrier leakage in pIgR KO mice

Triple reporter mice (control group)
pIgR KO mice (test group)
Weigh mice every 1-3 days
Euthanize mice at 4, 6, 8, 10, 12 weeks of age
4 mice x 5 timepoints = **20 mice (each strain)**(Pain C)
Challenge additional mice at 4, 12 weeks of age
1- test peptide + cholera toxin i.g.
2- control peptide + cholera toxin i.g.
Euthanize 7 days later (t=5, t=13 weeks)

4 mice x 2 conditions x 2 timepoints = **16 mice (each strain)**(Pain C)

Total experimental mice needed

28 + 24 + 36 + 16 + 20 + 16 = **140 Triple reporter mice** (48 Pain C, 92 Pain E)

20 + 16 = **36 pIgR KO mice** (36 Pain E)

[redacted]

Research Objective: Research Objective 4

INSTRUCTIONS:
Complete a Research Objective form for each discrete aim of the protocol. To add an additional Research Objective, please click the **add New Research Objective** button at the end of this form.

Limit the discussion to activities involving animals. Do not describe *in vitro* procedures beyond collection of tissues, blood, or other biological products.

A. Rationale: [**Please limit to 200 words**]

Research Objective 4- Elimination of regulatory T cell subsets to induce colitis

In this section, we will test the hypothesis that T cell responses against commensal bacteria are continually suppressed by regulatory T cells in the steady state. We will examine the role of two major regulatory T cell subsets, Foxp3+ regulatory T cells (Treg) and T regulatory type 1 cells (Tr1), by specifically eliminating them in vivo, and observing the impact on commensal antigen-specific T cells and intestinal health.

To study the role of Tregs, we will use Foxp3-DEREG mice, which express a BAC transgene that results in expression of the diphtheria receptor exclusively on Tregs. This makes them selectively sensitive to death by diphtheria toxin (DT) treatment while all other cells in the mouse are resistant. Thus, Tregs from these mice can be specifically and acutely ablated in vivo by systemic DT administration with minimal toxicity to other cells and organs. Because a small percentage of Tregs (~2-5%) do not express the transgene, they survive ablation and protect the mouse from autoimmunity induced death. Although these cell eventually expand to reconstitute a resistant population of Tregs, the treatment creates a period of a few weeks of severely impaired Treg function during which we hypothesize inflammatory T cell responses against commensal bacteria can initiate a progression towards IBD.

To study the role of IL-10+ cells, which include Tr1 cells and some Tregs, we will use 10BiT reporter mice, in which a transgenic IL-10 promoter drives the expression of the Thy1.1 surface protein. Systemic administration of a Thy1.1-specific antibody (clone 19E12) results in acute in vivo depletion of these cells. Finally, we will also use IL-10 KO mice to study how commensal antigen-specific T cells develop in the steady state absence of IL-10+ regulatory T cell subsets. These mice have global defects in immune tolerance and spontaneously develop colitis over 2-3 months of life. We will track commensal antigen-specific T cell development during this course of spontaneous disease development.

B. Experimental Design: For this research objective, outline the time-course indicating each activity. Describe each step and how it relates to an animal enrolled in this study. It should be clear what each animal will experience during the full course of this Research Objective.

[redacted]

- Include the length of time an animal is enrolled in an experiment
- Describe experimental endpoints
- Do not include descriptions of surgical and non-surgical procedures in the **Experimental Design**. Include this information in the specific **Procedure** forms.

Experiment 1- Treg ablation

To study the role of Tregs, we will inject Foxp3-DEREG mice intraperitoneally with diphtheria toxin (DT) to inducibly ablate all Foxp3+ Tregs, and then examine commensal antigen-specific T cell development and intestinal health. Control mice will be injected with PBS. We will perform 3 injections spaced apart by 2 days (t=0, 2, 4 days), after which remaining Tregs will no longer be sensitive to ablation. Commensal antigen-specific T cells will be isolated from the spleen, lymph nodes, and GALT of mice euthanized at set timepoints throughout a 12 week timecourse, and as before, we will conduct flow cytometric analysis of these cell populations to calculate their frequencies and phenotype based on surface marker expression. For selected subsets of samples, antigen-specific T cell RNA will be harvested for high-powered RNA-seq analysis, and fecal pellets will be collected for 16S ribosomal sequence analysis of microbiome composition. At the same time, we will be carefully monitoring these immunized mice for signs of intestinal inflammation and colitis. Mice will be weighed at every few days following immunization and inspected for signs of diarrhea, lethargy, or any other overt signs of illness. In addition, in each of the mice euthanized at defined timepoints, intestines will be dissected, measured, and sectioned for immunohistochemistry to determine if inflammation and/or T cell infiltration is occurring.

Experiment 2- IL-10 expressing cell depletion

To study the role of IL-10+ T cells which include Tr1 cells and a subset of Foxp3+ Tregs, we will inject 10BiT expressing triple reporter mice with a Thy1.1 antibody to induce depletion of any IL-10 expressing cell. Additional mice will be injected with an isotype-matched control antibody of irrelevant antigen specificity. As in Experiment 1, one set of mice will be euthanized early at day 2 to assess the efficiency of cell depletion, while the rest of the mice will be studied over a 12 week timecourse with antibody injections repeated every 2 weeks. Commensal antigen-specific T cells and intestinal health will be analyzed as in Experiment 1.

Experiment 3- Steady state IL-10 deficiency

To study the role of IL-10+ T cells in the steady state, we will simply track commensal antigen-specific T cells and intestinal health throughout the first 18 weeks of life in IL-10 KO mice using the same assays described in Experiments 1 and 2.

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Endpoints and Euthanasia, section 1. Study Duration. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** question above. ***Use of this information is optional.***

Migrated Data

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C. Flow Chart: For this research objective, a schema or flow chart diagramming the overall picture of the study design and treatment groups must be included. The flow chart should include:

- all experimental groups
- the number of animals per group
- the procedures performed on the animal
- the length of time an animal is enrolled in the experiment

The IACUC must be able to understand the experience of each animal on the protocol. See TIPS for Creating Flow Charts in the FAQ pane for detailed information.

D. Health Status:

1. Describe the health status of the animals during this research objective. Include:

- Expected development and progression of clinical signs, including severity and time course
- Potential adverse events caused by the research model and/or experimental manipulations
- If a scoring system will be used to monitor animal health, please attach it to the protocol below.

Foxp3-DEREG mice treated with DT develop systemic autoimmunity, but because the Treg ablation is only about 95-98% effective, the few surviving Tregs eventually expand to restore immune homeostasis. The mice do not display any overt signs of disease and rarely die. IL-10 KO mice develop spontaneous colitis over a 2-3 month period of life marked by progressive weight loss and eventually death. Acute IL-10+ cell depletion with antibody should cause less health issues but may phenocopy IL-10 KO mice over extended periods of time. In each case, mice will be monitored by weight every 1-3 days throughout the course of the experiment. Mice experiencing colitis, which we anticipate to occur in at least some of these experiments, experience weight loss and some diarrhea, and upon euthanasia will be evidenced by shortened colons. Depending on the severity of disease, colitic mice may appear slightly lethargic, but should not exhibit other overt signs of illness.

2. What action will be taken should clinical signs manifest?

[redacted]

Colitis may cause prolonged distress and pain, but analgesics cannot be used due to the detrimental side effects they will have on the physiology of the intestinal inflammation that we are studying (see Pain or Distress Study Justification). To help provide a nurturing environment during potential colitis development, all treated mice will receive an igloo and additional Enviro-Dry bedding material in their cages. We have set a 25% weight loss limit at which mice will be immediately euthanized. However, any mouse that exhibits exceptional signs of extended pain or distress regardless of amount of weight loss will also be immediately euthanized.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 2, "Describe the investigator's responsibilities during the post-surgical and/or post-experimental period..."**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Experimental Design** field above. ***Use of this information is optional.***

Attachments

Name	Mode
flowchart-4 (Flowchart)	Electronic

FLOW CHART

Research Objective 4- Elimination of regulatory T cell subsets to induce colitis

Experimental Strategy

Each euthanized timepoint analysis includes assessment of:

- Colon length
- Histological analysis of intestinal tissue
- Antigen-specific T cell frequency by flow cytometry
- Antigen-specific T cell phenotype by flow cytometry
- Antigen-specific T cell phenotype by single cell RNA-seq (some samples)
- Microbiome composition by 16S ribosomal sequencing (some samples)

Experiment 1- Treg ablation

Foxp3-DEREG mice

A- Inject diphtheria toxin i.p. at t=0, 2, 4 days to ablate Tregs

B- No ablation (PBS control)

Weigh mice every 1-3 days

Euthanize mice at t=1, 2, 4, 6, 8, 10, 12 weeks

4 mice x 2 conditions x 7 timepoints = **56 Foxp3-DEREG mice** (Pain E)

Experiment 2- IL-10 expressing cell depletion

Triple reporter mice

A- Inject Thy1.1 antibody i.p. every 2 weeks to deplete IL-10 expressing cells

B- No depletion (PBS control)

Weigh mice every 1-3 days

Euthanize mice at t=1, 2, 4, 6, 8, 10, 12 weeks

4 mice x 2 conditions x 7 timepoints = **56 Triple reporter mice** (Pain E)

Experiment 3- Steady state IL-10 deficiency

IL-10 KO mice

Weigh mice every 1-3 days

Euthanize mice at t=4, 6, 8, 10, 12, 14, 16, 18 weeks for analysis of colitis

4 mice x 9 timepoints = **36 IL-10 KO mice** (Pain C)

Total experimental mice needed

56 Foxp3-DEREG mice (56 Pain E)
56 Triple reporter mice (56 Pain E)
36 IL-10 KO mice (36 Pain C)

[redacted]

Research Objective: Research Objective 4

INSTRUCTIONS:
Complete a Research Objective form for each discrete aim of the protocol. To add an additional Research Objective, please click the **add New Research Objective** button at the end of this form.

Limit the discussion to activities involving animals. Do not describe *in vitro* procedures beyond collection of tissues, blood, or other biological products.

A. Rationale: [**Please limit to 200 words**]

Research Objective 3- Activation of commensal antigen-specific T cells to induce colitis

An underlying cause of IBD is thought to be a misdirected adaptive immune attack against commensal bacteria of the gut. The experiments in this section will formally test the concept that the generation of adaptive T cell responses to gut commensal bacteria can directly cause intestinal inflammation and colitis, ultimately contributing to the etiology of disease. We will use various methods to immunize mice with specific antigens from enteric commensal bacteria and study the resulting T cell immune response and intestinal health of the animals.

B. Experimental Design: For this research objective, outline the time-course indicating each activity. Describe each step and how it relates to an animal enrolled in this study. It should be clear what each animal will experience during the full course of this Research Objective.

- Include the length of time an animal is enrolled in an experiment
- Describe experimental endpoints
- Do not include descriptions of surgical and non-surgical procedures in the **Experimental Design**. Include this information in the specific **Procedure** forms.

Experiment 1- Immunize mice from HPPF room with commensal bacterial antigenic peptide

We will immunize mice with peptide corresponding to defined antigenic epitopes of commensal bacteria. To maximize our chances of achieving the proper conditions for successful commensal-specific T cell activation, three different routes of administration with three different adjuvants will be tried. Intravenous injection with lipopolysaccharide (LPS) establishes immunization at a systemic level, where all T cells in all lymphoid organs are affected. Subcutaneous injection with complete Freund's adjuvant (CFA) establishes a very strong Th17 response that is chronic due to the persistence of the oil emulsified antigen at the injection site. Finally, intragastric gavage with cholera toxin B subunit (CTB) provides an enteric route of immunization that results in a strong Th17 response in the same tissue environment as CBir1-expressing commensal bacteria.

[redacted]

We will activate commensal antigen-specific T cells with each of these immunization models and assess their activity throughout a 12 week timecourse. Commensal antigen-specific T cells will be isolated from the spleen, lymph nodes, and GALT of mice euthanized at set timepoints. As described before, we will conduct flow cytometric analysis of these cell populations to calculate their frequencies and phenotype based on surface marker expression. For selected subsets of samples, antigen-specific T cell RNA will be harvested for high-powered RNA-seq analysis, and fecal pellets will be collected for 16S ribosomal sequence analysis of microbiome composition.

At the same time, we will be carefully monitoring these immunized mice for signs of intestinal inflammation and colitis. Mice will be weighed at every few days following immunization and inspected for signs of diarrhea, lethargy, or any other overt signs of illness. In addition, in each of the mice euthanized at defined timepoints, intestines will be dissected, measured, and sectioned for immunohistochemistry to determine if inflammation and/or T cell infiltration is occurring.

These experiments will be performed under HPPF (Helicobacter Pasteurella Pneumoniae Free) colony conditions in this section and under standard SPF colony conditions in Experiment 2, to ascertain the impact of the presence of the pathobiont species Helicobacter hepaticus in tipping the balance of commensal bacteria-specific immunity towards inflammation over tolerance. Because 10BiT reporter mice are unavailable in HPPF rooms, we will use double reporter mice (IL-10-GFP x Foxp3-RFP) to maximize our analysis of Tr1 and Treg cell biology here.

Experiment 2- Immunize mice with commensal bacterial antigenic peptide

These experiments will be the same as those in Experiment 1, but with the use of triple reporter mice under SPF conditions in which Helicobacter hepaticus is endemic.

Experiment 3- Infect mice with Listeria expressing commensal bacterial peptide

To provide a more complete inflammatory immune context which adjuvants alone may not be able to constitute, we will also infect mice systemically with a transgenic strain of Listeria expressing a peptide epitope from a commensal bacterial antigen. Commensal and Listeria-specific T cells will be simulataneously analyzed as described in Research Objective 2, and the mice will monitored for the development of intestinal inflammation and colitis.

Experiment 4- Infect mice with Citrobacter expressing commensal bacterial peptide

We will recapitulate the experiments in Experiment 3, but using an enteric infection model with a transgenic strain of Citrobacter.

[redacted]

Migrated Data

This field may contain information that has been migrated from **Insight 4.3.6, Duration, Clinical Signs, Endpoints and Euthanasia, section 1. Study Duration**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** question above. ***Use of this information is optional.***

Migrated Data

This field may contain information that has been migrated from **Insight 4.3.6, Tumor Production, question 6. "What are the experimental endpoints used for this tumor study?"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** question above. ***Use of this information is optional.***

C. Flow Chart: For this research objective, a schema or flow chart diagramming the overall picture of the study design and treatment groups must be included. The flow chart should include:

- all experimental groups
- the number of animals per group
- the procedures performed on the animal
- the length of time an animal is enrolled in the experiment

The IACUC must be able to understand the experience of each animal on the protocol. See TIPS for Creating Flow Charts in the FAQ pane for detailed information.

D. Health Status:

1. Describe the health status of the animals during this research objective. Include:

- Expected development and progression of clinical signs, including severity and time course
- Potential adverse events caused by the research model and/or experimental manipulations
- If a scoring system will be used to monitor animal health, please attach it to the protocol below.

Mice immunized with intravenously with peptide plus LPS, or intragastrically with peptide plus CTB, may experience temporary discomfort, but should tolerate the treatment very well with no overt signs of distress. Subcutaneous immunization of mice with CFA can sometimes cause ulceration or scarring of the skin at the injection site, possibly accompanied with prolonged discomfort or pain.

Mice infected intravenously with Listeria develop an acute systemic infection that is completely cleared within one week or even earlier with ActA deficient strains. The mice do not exhibit any overt signs of illness, but may lose some weight and will have enlarged lymphoid organs and livers seen after euthanasia. Mice infected with Citrobacter develop an enteric infection that is cleared within 3 weeks. The mice do not exhibit overt signs of illness with the exception of some diarrhea. They may lose some weight and will have enlarged lymphoid organs and inflamed intestines during this time.

[redacted]

All immunized or infected mice will be monitored by weight every 1-3 days throughout the course of the experiment. Mice experiencing colitis, which we anticipate to occur in at least some of these experiments, experience weight loss and some diarrhea, and upon euthanasia will be evidenced by shortened colons. Depending on the severity of disease, colitic mice may appear slightly lethargic, but should not exhibit other overt signs of illness.

2. What action will be taken should clinical signs manifest?

CFA injected mice and mice experiencing colitis may experience some extended pain and distress, but analgesics cannot be used due to the detrimental side effects they will have on the physiology of the intestinal inflammation that we are studying (see Pain or Distress Study Justification). Igloos and Enviro-Dry bedding will be given to immunized or infected mice to help nurture them through their illness and/or potential development of colitis. We have set a 25% weight loss limit at which mice will be immediately euthanized. However, any mouse that exhibits exceptional signs of extended pain or distress regardless of amount of weight loss will also be immediately euthanized.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 2, "Describe the investigator's responsibilities during the post-surgical and/or post-experimental period..."**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Experimental Design** field above. ***Use of this information is optional.***

Attachments

Name	Mode
flowchart-3 (Flowchart)	Electronic

FLOW CHART

Research Objective 3- Activation of commensal antigen-specific T cells to induce colitis

Experimental Strategy

Each euthanized timepoint analysis includes assessment of:
- Colon length
- Histological analysis of intestinal tissue
- Antigen-specific T cell frequency by flow cytometry
- Antigen-specific T cell phenotype by flow cytometry
- Antigen-specific T cell phenotype by single cell RNA-seq (some samples)
- Microbiome composition by 16S ribosomal sequencing (some samples)

Experiment 1- Immunize mice from HPPF room with commensal bacterial antigenic peptide
Immunize **Double reporter mice** with:
A- commensal bacterial peptide + LPS i.v.
B- commensal bacterial peptide + CFA s.c.
C- commensal bacterial peptide + cholera toxin i.g.
Weigh mice and collect fecal pellets every 1-3 days
Euthanize mice at t=0, 1, 2, 3, 4, 6, 8, 12 weeks
4 mice x 3 conditions x 8 timepoints = **96 mice** (12 Pain C, 56 Pain D, 28 Pain E)

Experiment 2- Immunize mice with commensal bacterial antigenic peptide
Immunize **Triple reporter mice** with:
A- commensal bacterial peptide + LPS i.v.
B- commensal bacterial peptide + CFA s.c.
C- commensal bacterial peptide + cholera toxin i.g.
Weigh mice and collect fecal pellets every 1-3 days
Euthanize mice at t=0, 1, 2, 3, 4, 6, 8, 12 weeks
4 mice x 3 conditions x 8 timepoints = **96 mice** (12 Pain C, 56 Pain D, 28 Pain E)

Experiment 3- Infect mice with Listeria expressing commensal bacterial peptide
Infect **Triple reporter mice** with Listeria-peptide i.v.
Weigh mice and collect fecal pellets every 1-3 days
Euthanize mice at t=0, 1, 2, 3, 4, 6, 8, 12 weeks
4 mice x 8 timepoints = **32 mice** (4 Pain C, 28 Pain D)

Experiment 4- Infect mice with Citrobacter expressing commensal bacterial peptide
Infect **Triple reporter mice** with Citrobacter-peptide i.g.
Weigh mice and collect fecal pellets every 1-3 days
Euthanize mice at t=0, 1, 2, 3, 4, 6, 8, 12 weeks
4 mice x 8 timepoints = **32 mice** (4 Pain C, 28 Pain D)

Total experimental mice needed
96 Double reporter mice (12 Pain C, 56 Pain D, 28 Pain E)
96 + 32 + 32 = **160 Triple reporter mice** (20 Pain C, 112 Pain D, 28 Pain E)

[redacted]

Research Objective: Research Objective 5

INSTRUCTIONS:
Complete a Research Objective form for each discrete aim of the protocol. To add an additional Research Objective, please click the **add New Research Objective** button at the end of this form.

Limit the discussion to activities involving animals. Do not describe *in vitro* procedures beyond collection of tissues, blood, or other biological products.

A. Rationale: [**Please limit to 200 words**]

Research Objective 5- Transfer of commensal antigen-specific T cells to induce colitis

In this section, we will assess the colitic potential of commensal antigen-specific T cells using an established T cell transfer model of colitis. In this model, donor T cells devoid of regulatory T cells are adoptively transferred into T cell deficient RAG knockout host mice, and colitis develops as the transferred conventional T cells become activated, proliferate, and attack intestinal microbial flora in the absence of immune suppression by regulatory T cells. Our experiments will be a refinement of this model in that we will first enrich donor T cell populations for T cells specific for commensal bacterial antigens before adoptive transfer.

Our studies will address whether commensal antigen-specific T cells can cause colitis, and to what extent they are suppressed by Treg and Tr1 cells. We will use Foxp3-DEREG and 10BiT mice as sources of donor T cells in which Treg and Tr1 cell subsets, respectively, can be easily depleted as described in Research Objective 4. We will use the CBir TCR transgenic mouse as a control source of donor T cells which should cause disease as reported in the literature, but we will also build upon this finding by testing the role of Treg and Tr1 cells in suppressing their colitic activity.

B. Experimental Design: For this research objective, outline the time-course indicating each activity. Describe each step and how it relates to an animal enrolled in this study. It should be clear what each animal will experience during the full course of this Research Objective.

- Include the length of time an animal is enrolled in an experiment
- Describe experimental endpoints
- Do not include descriptions of surgical and non-surgical procedures in the **Experimental Design**. Include this information in the specific **Procedure** forms.

Experiment 1- T cell transfer with Treg ablation

We will isolate commensal antigen-specific T cells from Foxp3-DEREG mice using tetramer-based cell enrichment and adoptively transfer them into RAG1 KO mice by intravenous injection. Host mice receiving these cells will be

[redacted]

injected intraperitoneally with DT at t=0, 2, and 4 days to ablate the Foxp3-expressing Treg subset of the transferred T cells. Injections with PBS will be performed for control transferred mice in which Tregs are not ablated. As before, commensal antigen-specific T cells will be isolated from the spleen, lymph nodes, and GALT of mice euthanized at set timepoints throughout a 12 week timecourse, and we will conduct flow cytometric analysis of these cell populations to calculate their frequencies and phenotype based on surface marker expression. For selected subsets of samples, antigen-specific T cell RNA will be harvested for high-powered RNA-seq analysis, and fecal pellets will be collected for 16S ribosomal sequence analysis of microbiome composition. To assess the development of intestinal inflammation and colitis, mice will be weighed every few days and inspected for signs of diarrhea, lethargy, or any other overt signs of illness. Intestines of mice euthanized at each timepoint will be dissected, measured, and sectioned for immunohistochemistry to determine the extent of inflammation and/or T cell infiltration.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 1. Study Duration**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** question above. ***Use of this information is optional.***

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Tumor Production, question 4. "What are the experimental endpoints used for this tumor study?"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** question above. ***Use of this information is optional.***

C. Flow Chart: For this research objective, a schema or flow chart diagramming the overall picture of the study design and treatment groups must be included. The flow chart should include:

- all experimental groups
- the number of animals per group
- the procedures performed on the animal
- the length of time an animal is enrolled in the experiment

The IACUC must be able to understand the experience of each animal on the protocol. See TIPS for Creating Flow Charts in the FAQ pane for detailed information.

D. Health Status:

1. Describe the health status of the animals during this research objective. Include:

- Expected development and progression of clinical signs, including severity and time course
- Potential adverse events caused by the research model and/or experimental manipulations

- If a scoring system will be used to monitor animal health, please attach it to the protocol below.

Ablation of Tregs in RAG1 KO mice transferred with commensal antigen-specific T cells should develop colitis over a 1-2 month period, as should RAG1 KO mice receiving a transfer of CBir TCR transgenic T cells. It is yet unclear whether depletion of IL-10+ cells in RAG1 KO mice receiving either of these transfers will develop colitis. In each case, mice will be monitored by weight every 1-3 days throughout the course of the experiment. For any mouse developing colitis, we anticipate weight loss, some diarrhea, and shortened colons upon dissection. Depending on the severity of disease, colitic mice may appear slightly lethargic, but should not exhibit other overt signs of illness.

2. What action will be taken should clinical signs manifest?

Colitis may cause prolonged distress and pain, but analgesics cannot be used due to the detrimental side effects they will have on the physiology of the intestinal inflammation that we are studying (see Pain or Distress Study Justification). To help provide a nurturing environment for potential colitis development, all treated mice will receive an igloo and additional Enviro-Dry bedding material in their cages. We have set a 25% weight loss limit at which mice will be immediately euthanized. However, any mouse that exhibits exceptional signs of extended pain or distress regardless of amount of weight loss will also be immediately euthanized.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 2, "Describe the investigator's responsibilities during the post-surgical and/or post-experimental period..."**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Experimental Design** field above. ***Use of this information is optional.***

Attachments

Name	Mode
flowchart-5 (Flowchart)	Electronic

FLOW CHART

Research Objective 5- Transfer of commensal antigen-specific T cells to induce colitis

Experimental Strategy

Each euthanized timepoint analysis includes assessment of:

- Colon length
- Histological analysis of intestinal tissue
- Antigen-specific T cell frequency by flow cytometry
- Antigen-specific T cell phenotype by flow cytometry
- Antigen-specific T cell phenotype by single cell RNA-seq (some samples)
- Microbiome composition by 16S ribosomal sequencing (some samples)

Experiment 1- T cell transfer with Treg ablation

Tetramer-enrichment/sorting of commensal antigen-specific T cells from **Foxp3-DEREG mice**
Adoptive transfer into **RAG1 KO mice** (1:1 donor:recipient ratio)
A- Inject diphtheria toxin i.p. to ablate Tregs
B- No ablation (PBS control)
Weigh mice every 1-3 days
Euthanize mice at t=1, 2, 4, 6, 8, 10, 12 weeks
4 donor and 4 recipient mice x 2 conditions x 7 timepoints = **56 Foxp3-DEREG mice** (Pain C)
56 RAG1 KO mice (Pain E)

Experiment 2- T cell transfer with IL-10+ cell depletion

Tetramer-enrichment/sorting of commensal antigen-specific T cells from **Triple reporter mice**
Adoptive transfer into **RAG1 KO mice** (1:1 donor:recipient ratio)
A- Inject Thy1.1 antibody i.p. to deplete IL-10 expressing cells
B- No depletion (PBS control)
Weigh mice every 1-3 days
Euthanize mice at t=1, 2, 4, 6, 8, 10, 12 weeks
4 donor and 4 recipient mice x 2 conditions x 7 timepoints = **56 Triple reporter mice** (Pain C)
56 RAG1 KO mice (Pain E)

Experiment 3- TCR transgenic T cell transfer with IL-10+ cell depletion

T cells from **CBir mice**
Adoptive transfer into **RAG1 KO mice** (1:8 donor:recipient ratio)
A- Inject Thy1.1 antibody i.p. to deplete IL-10 expressing cells
B- No depletion (PBS control)
Weigh mice every 1-3 days
Euthanize mice at t=1, 2, 4, 6, 8, 10, 12 weeks
0.5 donor and 4 recipient mice x 2 conditions x 7 timepoints = **7 CBir mice** (Pain C)
56 RAG1 KO mice (Pain E)

Total experimental mice needed

56 Foxp3 DEREG mice (56 Pain C)
56 Triple reporter mice (56 Pain C)
7 CBir mice (7 Pain C)
56 + 56 + 56 = **168 RAG1 KO mice** (168 Pain E)

[redacted]

Research Objective: Research Objective 4

INSTRUCTIONS:
Complete a Research Objective form for each discrete aim of the protocol. To add an additional Research Objective, please click the **add New Research Objective** button at the end of this form.

Limit the discussion to activities involving animals. Do not describe *in vitro* procedures beyond collection of tissues, blood, or other biological products.

A. Rationale: [**Please limit to 422 words**]

Research Objective 2- T cell responses to commensal versus pathogenic enteric bacteria

A unresolved question in immunology is whether the adaptive immune system is able to sense and distinguish between antigens derived from commensal and pathogenic strains of bacteria in the intestinal tract. While strong immunity should be elicited against antigens from pathogenic strains, tolerance might be induced against specific antigens from commensal strains. We will investigate this possibility by simultaneously tracking populations of T cells with specificity to defined antigens derived from commensal and pathogenic bacteria during infection.

To this end, we will employ two established models of bacterial infection in mice: one that is systemic and another that is localized to the intestine. Listeria monocytogenes is a pathogenic enteric bacterium with a broad host range, but because mice are not very susceptible to enteric infection, it is usually administered intravenously to produce an acute systemic infection. Citrobacter rodentium is a non-invasive pathogenic bacterium that colocalizes with commensal bacteria in the intestine, but causes acute attaching effacing infection of the epithelium.

0. BEperimental x esiDn: For this research objective, outline the time-course indicating each activity. Describe each step and how it relates to an animal enrolled in this study. It should be clear what each animal will experience during the full course of this Research Objective.

- Include the length of time an animal is enrolled in an experiment
- Describe experimental endpoints
- Do not include descriptions of surgical and non-surgical procedures in the **BEperimental x esiDn**. Include this information in the specific **Procedgre** forms.

Experiment 1- Systemic infection with Listeria

To determine whether immune responses to a systemic pathogenic bacterial infection will also cause activation of T cell populations specific for commensal bacteria, we will infect mice intravenously with Listeria to generate a systemic infection, during which we will simultaneously track T cells specific for

[redacted]

commensal as well as Listeria derived antigens. The course of the disease and overall health of the animals will be monitored by measuring their weight daily. Antigen-specific T cell populations will be isolated from the spleen, gut-associated lymphoid tissues (GALT), and livers of mice euthanized at defined timepoints throughout the one week infection and five week followup period to assess their development into effector or tolerant phenotypes. As before, flow cytometry will be used to calculate the frequencies of these cells as well as assess the expression of phenotypic surface markers. For selected subsets of samples, antigen-specific T cell RNA will be harvested for high-powered RNA-seq analysis, and fecal pellets will be collected for 16S ribosomal sequence analysis of microbiome composition. Mice at select timepoints will also be challenged intragastrically with CBir1 peptide (or LLO as a control) plus cholera toxin and stained for intracellular expression of cytokines 7 days later to determine whether these cells are responsive to antigen and whether they produce pro-inflammatory or tolerogenic signals. Also, as before, T cells specific for a model foreign antigen will be analyzed as a control.

Experiment 2- Enteric infection with Citrobacter

To determine whether the host immune system can distinguish between pathogenic and commensal bacteria when they are localized in the same gut tissue environment, we will also infect mice intragastrically with Citrobacter and then simultaneously track T cells specific for commensal as well as Citrobacter derived antigens. We will use the same assays as those outlined for Listeria experiments above.

u iDrated x ata

This field may contain information that has been migrated from **InsiDht M3.6, x gration, Clinical SiDns, Bndpoints and Bgthanasia, section 1. Stgdy x gration**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **BEperimental x esiDn** question above. ***Use of this information is optional.***

u iDrated x ata

This field may contain information that has been migrated from **InsiDht M3.6, Tgmor Prodgction, qgestion 6. "What are the eEperimental endpoints gsed for this tgmor stgdy?"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **BEperimental x esiDn** question above. ***Use of this information is optional.***

C. Flow Chart: For this research objective, a schema or flow chart diagramming the overall picture of the study design and treatment groups must be included. The flow chart should include:

- all experimental groups
- the number of animals per group
- the procedures performed on the animal
- the length of time an animal is enrolled in the experiment

[redacted]

The IACUC must be able to understand the experience of each animal on the protocol. See TIPS for Creating Flow Charts in the FAQ pane for detailed information.

x. Health Statgs:

1. Describe the health status of the animals during this research objective. Include:

- Expected development and progression of clinical signs, including severity and time course
- Potential adverse events caused by the research model and/or experimental manipulations
- If a scoring system will be used to monitor animal health, please attach it to the protocol below.

Listeria is tolerated well by C57BL/6 mice and usually cleared within a week of infection. Listeria infected mice experience enlarged lymphoid organs and liver, but do not show any overt signs of illness. Citrobacter is also tolerated well by C57BL/6 mice and is cleared by 3 weeks post-infection. Infected mice experience intestinal inflammation with some potential diarrhea and weight loss, but do not exhibit other overt signs of suffering. For each type of infection, mice will be monitored by weight every 1-3 days throughout the course of the experiment.

2. What action will be taken should clinical signs manifest?

Both Listeria and Citrobacter are well tolerated by C57BL/6 mice and are cleared quickly. However, any mouse that exhibits exceptional signs of pain or distress will be immediately euthanized. Any mouse exhibiting more than 25% weight loss will be immediately euthanized. Igloos and Enviro-Dry bedding will be given to infected mice to help nurture them during their illness.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 2, "Describe the investigator's responsibilities during the post-surgical and/or post-experimental period..."**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Experimental Design** field above. ***Use of this information is optional.***

Attachments

Name	u ode
flowchart-2 (Flowchart)	Electronic

FLOW CHART

Research Objective 2- T cell responses to commensal versus pathogenic enteric bacteria

Experimental Strategy

Each euthanized timepoint analysis includes assessment of:

- Colon length
- Histological analysis of intestinal tissue
- Antigen-specific T cell frequency by flow cytometry
- Antigen-specific T cell phenotype by flow cytometry
- Antigen-specific T cell phenotype by single cell RNA-seq (some samples)
- Microbiome composition by 16S ribosomal sequencing (some samples)

Experiment 1- Systemic infection with Listeria

Infect **Triple reporter mice** i.v. with Listeria
Weigh mice and collect fecal pellets every 1-3 days
A- Euthanize at timepoints t=0, 4, 8, 12, 16, 20, 28, 35, 42 days
4 mice x 9 timepoints = **36 mice** (4 Pain C, 32 Pain D)
B- Challenge additional mice at timepoints t=0, 20, 42 days
1- test peptide + cholera toxin i.g.
2- control peptide + cholera toxin i.g.
Euthanize 7 days later (t=7, t=27, t=49)
4 mice x 2 conditions x 3 timepoints = **24 mice** (Pain D)

Experiment 2- Enteric infection with Citrobacter

Infect **Triple reporter mice** i.g. with Citrobacter
Weigh mice and collect fecal pellets every 1-3 days
A- Euthanize at timepoints t=0, 4, 8, 12, 16, 20, 28, 35, 42 days
4 mice x 9 timepoints = **36 mice** (4 Pain C, 32 Pain D)
B- Challenge additional mice at timepoints t=0, 20, 42 days
1- test peptide + cholera toxin i.g.
2- control peptide + cholera toxin i.g.
Euthanize 7 days later (t=7, t=27, t=49)
4 mice x 2 conditions x 3 timepoints = **24 mice** (Pain D)

Total experimental mice needed

36 + 24 + 36 + 24 = **120 Triple reporter mice** (8 Pain C, 112 Pain D)

[redacted]

Animals

The IACUC restricts protocols to a single species only. If the protocol will require xenografts, identify the donor species, and the applicable protocol number, in the appropriate **Research Objective** section of the protocol.

1. Select a species from the drop down list:
Mice (Mus)

1.a Select breed(s), or strain(s), or specific type(s).

Other

Please list other breed(s), strain(s), or specific type(s). See FAQ for institution-specific examples.

Foxp3-RFP, IL-17-GFP, IL-10-GFP, 10BiT, Foxp3-DEREG, IL-10 KO, pIgR KO, RAG1 KO, CBir, and various crosses of these strains

---

2. Do any of the animals have a genetic alteration and/or phenotype that is expected to have any impact on animal health and/or requirements for animal care?

◉ Yes O No

If Yes, please describe the phenotype for each affected strain.

IL-10 KO mice develop spontaneous colitis at 2-4 months of age, usually resulting in death. All of these mice will be used in experiments or euthanized before this happens.

---

3. Animal Source
Select all that apply:

☑ Animals will be acquired from an approved vendor - no quarantine is required. See FAQ for institution-specific approved vendors.
☑ Animals will be acquired through import. See FAQ for institution-specific procedures.
☑ Animals will be bred as part of this protocol

Please complete a **Breeding** form.

☐ Animals will be transferred from another protocol at this institution
☐ Animals will be acquired from an outside institution

[redacted]

4. Sex

O Male O Female ◉ Both

---

5. Indicate the method(s) of identification that will be used to track these animals *(select all that apply)*:

- ☐ Implant/microchip (See FAQ for SOP)
- ☑ Ear tag or notch (See FAQ for SOP)
- ☐ Tattoos (See FAQ for SOP)
- ☐ Collar
- ☑ Cage card
- ☐ Other

---

6. The species chosen is appropriate because *(select all that apply)*:

- ☑ The process resembles that in humans
- ☑ Prior research has been conducted in this species
- ☑ Tissues and/or other substances needed are best/uniquely provided be this species
- ☑ Species lower on the phylogenetic scale cannot be used
- ☐ The size or anatomy of this species is best/uniquely suited to the procedure(s)
- ☐ Tissues and/or other substances to be harvested require an animal of this size
- ☐ Other

Potential Pain and Distress

1. Total number of animals requested for this three-year approval

Enter the number of animals in each pain and distress category. Each animal must be assigned to a category based on the most invasive procedure or the procedure that has the greatest potential to cause pain or distress. See FAQ for definitions and examples.

- If an animal will be used in more than one **Research Objective**, count it only once, in the highest pain category that it will experience.
- If animals are bred in-house, include the progeny that may be culled. Progeny used for experiments should be counted in the specific **Research Objectives**. All other animals should be counted in **Other** as follows:

	Category
Breeders	B
Progeny culled without genotyping	B
Progeny culled after genotyping (<21 days old)	C
Progeny culled after genotyping (>21 days old)	D

[redacted]

TOTAL NUMBER OF ANIMALS REQUESTED USDA Pain & Distress Category (See FAQ for information)					
Animals	**B**	**C**	**D**	**E**	**Total**
Research Objective 1	0	48		128	
Research Objective 2		8	112		
Research Objective 3		32	168	56	
Research Objective 4		36		112	
Research Objective 5		119		168	
Other (e.g. breeding, training):	0	753			
Total requested	0	996	280	464	1740
Animals currently in house					
Total approved for purchase	0	996	280	464	1740

2. Justification for the number of animals requested (select all that apply):

☑ Power analyses indicated that the proposed sample size, number of groups and/or number of experiments is the lowest required for statistically valid tests of the hypothesis (i.e., 80% power with 0.05 type I error).
☑ Differences from controls are expected to be small, and large sample sizes are necessary to distinguish differences reliably.
☑ Based on previous and/or published data, the numbers of animals requested are the minimum needed to achieve sufficient statistical power.
☐ These animals will be used to produce antibodies or tissues, and numbers are based on yield.
☐ The numbers of animals or group sizes have been established by federal guidelines/requirements.
☐ This is a pilot/feasibility study that uses the minimum number of animals required to provide meaningful, but not statistically significant data (i.e., model development).
☑ This model involves breeding of genetically modified rodents. Based on Mendelian genetics, it is expected that ¼ of all pups will be homozygous and ¼ will be wild type, with the remaining ½ heterozygous. The homozygous and wild type mice will be used to generate data for the experiment, and the heterozygotes will be used to replace the breeding stock or will be euthanized.
☐ Other

The following tools can be used to determine minimum sample size:

- Sample Size Calculations in Animal Research (W. W. LaMorte, BUMC)
- ClinCalc Sample Size Calculator
- Jackson Laboratories Breeding Colony Size Planning Worksheet

3. Does the number of animals requested include extra animals to cover anticipated failures or to train or familiarize the staff with the procedures described?

[redacted]

O Yes ◉ No

4. The protocol includes animals in USDA Pain & Distress Category E, animals subjected to potentially painful or stressful procedures that are not relieved with anesthetics, analgesics, and/or other drugs; therefore, strong scientific justification must be provided.

a. Explain the procedure(s) that will produce pain and/or distress.

DSS treatment (Research Objective 1) results in colitis, which likely produces pain and distress that is marked by weight loss, some diarrhea, and some lethargy. In this model, DSS causes chemical injury to the intestinal epithelium, leading to inflammation and disruption of the epithelial barrier. Upon cessation of treatment, the tissue heals and the mice recover.

We anticipate that the activation of commensal antigen-specific T cells by immunization (Research Objective 3), elimination of regulatory T cells (Research Objective 4), or transfer into lymphopenic RAG1 KO mice (Research Objective 5) will also cause colitis in at least some of our experiments. In this model, T cells directly attack commensal bacteria and associated cells within the gut, producing cytokines and other immune mediators that cause inflammation and tissue damage. The onset of colitis will likely vary considerably in timing, ranging from 1 to 12 weeks. It is unclear whether colitis in any of these mice would resolve naturally.

Subcutaneous immunization of mice with CFA (Research Objective 3) can sometimes cause ulceration or scarring of the skin at the injection site, and may possibly be accompanied with prolonged discomfort or pain.

b. Provide scientific justification why pain and/or distress cannot be relieved with anesthetics, analgesics, and/or tranquilizers. State the reasons why relief of pain and/or distress would interfere with test results.

Unfortunately, analgesics cannot be used in any of our experimental mice due to the detrimental side effects they will have on the physiology of the intestinal inflammation that we are studying. This is true for both non-steroidal anti-inflammatory drugs (NSAIDs) which can exacerbate colitis, and opioids which can ameliorate it (see attached refs 1, 2). The mechanisms of action invariably involve immune functions, and therefore the use of these drugs may confound interpretations of our experiments.

c. Describe any measures that will be used to minimize pain and distress (e.g., special bedding, supplemental food, heat packs, etc.)

Igloos and Enviro-Dry bedding will be added to cages to help nurture infected or colitic mice. Napa nectar, however, will not be provided as it may possibly alter their gut microbiomes, thereby complicating results of our experiments, which focus on gut mucosal immunity. All of our experiments involve set

[redacted]

timepoints for euthanasia, so any pain or distress will be ultimately limited by duration of time.

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Replacement, Reduction, and Refinement

The 3 Rs – replacement, reduction, and refinement – represent a practical strategy for researchers to apply when considering the use of animals in research and in designing humane animal research studies. Government policy and regulatory agencies require the IACUC to assure that researchers consider the 3 Rs when preparing research protocols.

- The Guide for the Care and Use of Laboratory Animals
- U.S. Government Principles for the Utilization and Care of Vertebrate Animals Used in Testing, Research, and Training
- USDA Policies 11 and 12

1. Alternatives to Animal Models

☑ Mathematical models are not a suitable alternative to live animals
☑ Computer simulations (in silico models) are not a suitable alternative to live animals
☑ In vitro biological systems are not a suitable alternative to live animals
☐ Other

---

2. Duplication of Research

Unnecessarily duplicative research should be avoided for scientific and ethical reasons. Have the results fulfilling the experimental goals of this study been published in medical, scientific, or veterinary journals?

O Yes ◉ No

---

3. Search for Alternatives to Painful and/or Distressful Procedures

A literature search for alternative procedures must be performed for each procedure that has the potential to cause pain or distress, including prolonged use of restraint devices. Along with the literature search, consultation with experts in the field and attendance at scientific or professional meetings can be used to identify alternatives to painful and/or distressful procedures.

a. Indicate resources used to search for alternatives to painful and/or distressful procedures. In addition to the selections below, other useful resources can be found at IACUC Central , the NIH Office of Laboratory Animal Welfare, and the USDA Animal Welfare Information Center.

☐ Medline (http://library.massgeneral.org/)
☑ Pubmed (http://library.massgeneral.org/)

[redacted]

- ☐ Agricola (https://agricola.nal.usda.gov/)
- ☐ ALTBIB (https://toxnet.nlm.nih.gov/altbib.html)
- ☐ ALTWEB (http://altweb.jhsph.edu/)
- ☐ Animal Welfare Institute (https://awionline.org/)
- ☐ Google Scholar (http://scholar.google.com/)
- ☐ Other databases (please list):
- ☐ Consultation with experts with knowledge of alternatives within this specific field. Provide name(s) and qualifications/credentials, date, and content of the consultation.
- ☐ Scientific/professional meetings attended to remain current with pertinent information regarding alternatives in this specific field. Provide meeting name, date, and relevant topic.

b. Indicate the date the literature search was completed. The search must be conducted within the last 6 months.

Click here to enter a date.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Literature Search, Refine, Question Bii, Date of Literature Search**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Literature Search Date** field above. Literature search dates for field above for all migrated protocols were defaulted to the date of migration. ***Use of this information is optional.***

12/9/15

c. Indicate the time period surveyed in the literature search:

1947-2018

d. Indicate the procedure(s) and keyword(s) searched for each potentially painful or distressful procedure or condition described in this protocol.

Procedure	Keywords
Procedure: DSS treatment	Keywords: experimental colitis, acute, chronic, chemical, mouse, analgesic, pain, NSAID, opioid, alternative
Procedure: Intraperitoneal injection	Keywords: intraperitoneal injection, mouse, alternative
Procedure: Subcutaneous injection	Keywords: subcutaneous injection, immunization, mouse, T cells, CFA, complete Freund's adjuvant, alternative
Procedure: Citrobacter infection	Keywords: enteric infection, zoonotic, Citrobacter, survival, symptoms, mouse, alternative
Procedure: Oral gavage	Keywords: oral gavage, intragastric, mouse, inoculation, alternative
Procedure: Listeria infection	Keywords: systemic infection, zoonotic, Listeria, survival, symptoms, mouse, alternative
Procedure: Euthanasia for tissue harvest	Keywords: euthanasia, tissue harvest, mouse, dissection, lymphoid organs, alternative

[redacted]

Procedure	Keywords
Procedure: Tail vein injection	Keywords: tail vein injection, immunization, mouse, T cells, adoptive transfer, LPS, lipopolysaccharide, alternative
Procedure: Tail snip	Keywords: Tail snip, mouse, genotyping, DNA isolation, tail DNA, alternative

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Literature Search, Refine, Question Biv: "Indicate the procedure and keyword(s) used"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the infromation in this field as it may contaiin details useful in answering the **Literature Search** Date field above. ***Use of this information is optional.***

Procedure: Oral gavage

Keywords: oral gavage, intragastric, mouse, inoculation, alternative

Procedure: Intraperitoneal injection

Keywords: intraperitoneal injection, mouse, alternative

Procedure: Subcutaneous injection

Keywords: subcutaneous injection, immunization, mouse, T cells, CFA, complete Freund's adjuvant, alternative

Procedure: Tail vein injection

Keywords: tail vein injection, immunization, mouse, T cells, adoptive transfer, LPS, lipopolysaccharide, alternative

Procedure: Euthanasia for tissue harvest

Keywords: euthanasia, tissue harvest, mouse, dissection, lymphoid organs, alternative

Procedure: Tail snip

Keywords: Tail snip, mouse, genotyping, DNA isolation, tail DNA, alternative

Procedure: DSS treatment

Keywords: experimental colitis, acute, chronic, chemical, mouse, analgesic, pain, NSAID, opioid, alternative

Procedure: Citrobacter infection

Keywords: enteric infection, zoonotic, Citrobacter, survival, symptoms, mouse, alternative

[redacted]

Procedure: Listeria infection

Keywords: systemic infection, zoonotic, Listeria, survival, symptoms, mouse, alternative

e. Results of the Search for Alternatives to Painful and/or Distressful Procedures

☑ The literature search conducted indicates that there are no alternative procedures that would involve less pain or distress.
☐ There are alternative procedures, however, they cannot be used for these experiments.

If there are any relevant citations or other documents that are needed to support this search for alternatives, please attach them to this form.

Humane Endpoint Disposition and Euthanasia

A. Humane Endpoints

Mammals:

- Persistent recumbence; inability to rise; loss of righting reflex
- Pain or distress that cannot be alleviated by analgesics
- Difficulty with ambulation (paralysis, fractures, trauma, etc.)
- Severe central nervous system signs (e.g., circling, rolling, persistent seizures or convulsions)
- Abnormal breathing (dyspnea) and cyanosis
- Body condition score of 2 (out of 5) or less (see FAQ for links to species-specific body condition scoring charts)
- Excessive weight loss (see institution-specific guidelines)
- Vomiting/diarrhea resulting in severe dehydration
- Tumor production specific endpoints (see FAQ for links to institution-specific guidelines)
- Other model-specific endpoints (please describe)

Please choose:

O Animals will be removed from the study and euthanized if any of the above clinical signs/conditions are found
◉ Some or all of the criteria listed above cannot apply to this study. Animals will be euthanized if the following criteria are met.

Indicate which criteria do not apply, and explain

Some mice in this study will experience pain or distress that cannot be alleviated by analgesics due to their detrimental side effects. This has been

[redacted]

justified in the Potential Pain and Distress Form. This mainly applies to mice injected subcutaneously with CFA. However, if persistent open wounds are noticed in these mice, they will be immediately euthanized.

Some mice will experience diarrhea and weight loss as a consequence of experimental colitis or enteric infection. We have set a quantifiable 25% loss of body weight as an endpoint (instead of the usual 15%) because a standard 7 day DSS treatment regimen for colitis typically results in a weight loss of about 20% from which mice almost always recover (see attached reference 3, fig 1). A 25% cutoff provides enough of a margin to enable the creation of a robust colitic state without the excessive loss of animals from forced euthanasia. This is in accordance with the 25-30% threshold that is generally recommended by other IACUCs for this popular experimental model (see attached reference 4, page 2). I have discussed this and received approval from IACUC in the past protocol period.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 5. Endpoints**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Humane Endpoints** question above. ***Use of this information is optional.***

All studies end with euthanasia at defined timepoints, and with the exception of diarrhea and weight loss, all of the experimental mice are not expected to display any overt signs of illness or suffering at any time. However, any mouse that does exhibit pre-defined clinical signs of suffering will be removed from the study immediately and euthanized. For any experiment, this will include a loss of greater than 25% of a mouse's body weight, or any overt signs of illness or suffering except diarrhea. These include rectal prolapse, lethargy, ruffled fur, tremors, paresis, paralysis, head tilt, or abnormal locomotion. If persistent open wounds are noticed in mice receiving subcutaneous injections, they will be immediately euthanized.

Migration Data

This field may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoints and Euthanasia, section 5. "Please describe other endpoints"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Experimental Design** field above. ***Use of this information is optional.***

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Tumor Form, Question 5. "Indicate other humane endpoints used"**. The information in this section could not be mapped from your approved application to a

[redacted]

new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain details useful in answering the **Humane Endpoints** question above. ***Use of this information is optional.***

B. Moribundity and Mortality

The IACUC acknowledges that some studies may require moribundity (a clinically irreversible condition leading inevitably to death) or mortality (a fatal outcome) as an endpoint. The committee recommends that consideration be given to surrogate markers that can be utilized for a more humane endpoint, such as serial imaging or biomarkers that may permit the detection of experimental endpoints that precede the development of significant clinical signs, rather than allowing the animal to proceed to moribundity or mortality.

The use of death as an endpoint is strongly discouraged and requires scientific justification Rationale

1. Will this protocol include models with severe clinical signs expected?

O Yes ◉ No

2. Will this protocol use death as an endpoint?

O Yes ◉ No

C. Animal Transfer and Disposition

Select all that apply:

☑ Euthanasia or Terminal Procedure
☐ Transfer to another protocol at this institution (see FAQ for institution-specific guidelines)
☐ Transfer to another institution
☐ Release (field studies only)
☐ Animals may be considered for adoption.
☐ Animals may be considered for retirement.

D. Euthanasia Method

Euthanasia methods must be consistent with the AVMA Guidelines for the Euthanasia of Animals, 2013 edition. See FAQ for institution-specific guidelines/SOPs.

• A method must be indicated even if the protocol procedures are not terminal, for use in the event of an emergency.
• [redacted] protocols only: A secondary physical method to confirm euthanasia by carbon dioxide overdose or Isoflurane anesthesia overdose is recommended, but not required.

Species:

Mice (Mus)

[redacted]

☐ Pentobarbital euthanasia solution (Euthasol, Fatal Plus, etc.); 100 mg/kg IP (0.22 mL/kg IP)
☐ Pentobarbital anesthetic overdose; 150-200 mg/kg pentobarbital IP
☐ Ketamine/xylaine anesthetic overdose: 240-300 mg/kg ketamine + 15-30 mg/kg xylazine IP
☐ General anesthesia, followed by non-survival surgery or exsanguination. *Please complete a procedure form to cover this method of euthanasia.
☐ Isoflurane anesthetic overdose (5% isoflurane with secondary physical method).
☐ Euthanex Multi-Chamber Units (CO2 overdose with no secondary physical method)
☑ Carbon dioxide overdose (with secondary physical method)

Death by Carbon Dioxide Overdose will be confirmed by:

- ☐ Decapitation
- ☐ Bilateral thoracotomy
- ☐ Removal of vital organs
- ☐ Exsanguination
- ☐ Other
- ☑ Cervical dislocation

☑ Cervical dislocation (without anesthesia) - animals
☐ Decapitation (without anesthesia) by rodent guillotine. *Proficiency must be observed and documented before this method of euthanasia may be performed independently by users.
☐ Hypothermia/cryoanesthesia, followed by a secondary physical method - neonates
☐ Other

Will a sedative, tranquilizer, or anesthetic be administered prior to euthanasia?

○ Yes ◉ No

Migrated Data for [redacted] Protocols Only

This filed may contain information that has been migrated from **Insight 3.6.4, Duration, Clinical Signs, Endpoint, Question 6.c. "If you plan to deviate from the approved [redacted] CCM Euthanasia SOPs, describe the euthanasia method"**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it may contain useful in answering the **Humane Endpoints** question above. ***Use of this information is optional.***

Housing [redacted]

[redacted]

I. HOUSING LOCATIONS

A. CCM Centralized Facilities
Select all applicable housing areas.

[redacted]

B. Investigator-Managed Facilities or Satellite/Laboratory Housing Areas

- Please note that permission to house animals in investigator-managed centralized facilities must be obtained from the appropriate satellite facility manager. See FAQ for contact information.
- All new satellite/laboratory housing areas must be inspected and approved by the IACUC and the Center for Comparative Medicine. Research cannot be conducted until the area has been inspected and notification of approval has been received.

Select applicable housing areas.

☐ [redacted]
☐ [redacted]

☐ Other IACUC approved satellite/laboratory housing area

☐ New satellite/laboratory housing area

C. Offsite Housing
All offsite housing locations must be inspected and approved by the IACUC and the Director, Center for Comparative Medicine. Animals may not be housed in a new location until it has been inspected and notification of approval has been received.

☐ [redacted]
☐ Other

II. SPECIAL HANDLING, HUSBANDRY, OR HOUSING REQUIREMENTS

Will the animals on this protocol require any special handling, husbandry, or housing requirements? This includes anything outside of normal routine husbandry/handling services utilized by CCM, as defined in the species specific SOPs (e.g., alterations in bedding types, cage change frequencies, housing densities, special diets/fluids, deviations from currently approved IACUC policies, etc). See Species Specific Social Housing SOPs for more information.

Please discuss all special handling, husbandry, or housing requirements with CCM facility managers and/or veterinarians.

[redacted]

☑ No special housing or husbandry is required

☐ Breeding (i.e., delayed weaning requirements, harem breeding strategies, etc.)

☐ Immunocompromised

☐ Genetically modified animals (includes knock-outs, knock-ins, and transgenics)

☐ Specialized diet or fluid

☐ Alteration of cage / pen change frequency

☐ Alteration of light cycle

☐ Alteration of temperature and/or humidity

☐ Non-standard caging (e.g., metabolic cages, raised floor)

☐ Other

Exemptions from the Environmental Enhancement Program that are defined and approved by the IACUC Policy on Environmental Enrichment, Social housing and Exercise of Laboratory Animals do not need to be described in the protocol. A flow chart detailing the social housing policy is available to assist in the determination if planned single housing is covered by the policy.

☐ Non-social housing of social animals

☐ Withholding all cage, pen, or tank environmental enrichment

☐ Exemption from canine exercise program

If there are any relevant citations or other documents that are needed to support these special

[redacted]

housing, husbandry, or handling requirements, please attach them to this form.

Anesthesia Regimen: Isoflurane via precision vaporizer

Please assign a label for this anesthesia regimen (e.g. Isoflurane Option, Surgical – Minor Procedure, Imaging Sedation, etc.). This label will be used in dropdown lists for other forms in this protocol.

Isoflurane via precision vaporizer

1. Enter the agents that will be used for this anesthesia regimen. Include sedatives, paralytic agents, and anesthetic reversal agents. Do not include local anesthetics or other drugs used for analgesia. **See FAQ pane for institution-specific formularies.**

Agent	Dose	Route	Frequency
Isoflurane	up to 5%	Inhalation in chamber with precision vaporizer (with scavenging system)	once

2. Are any of the agents listed paralytics?

O Yes ◉ No

3. The IACUC requires that all anesthetics administered to any animal species be of pharmaceutical grade (USP grade), if that agent is available in pharmaceutical grade. Are all agents in this anesthetic regimen of pharmaceutical grade (USP grade)? **See FAQ for definition of pharmaceutical grade.**

◉ Yes O No

Species:
Mice (Mus)

4. The adequacy or depth of anesthesia will be monitored by (select all that apply):

- ☑ Respiratory rate
- ☐ Toe pinch
- ☐ Corneal or palpebral (blink) reflex
- ☐ Other (please describe)

5. How frequently will the depth of anesthesia be assessed?

See the Policy on Anesthesia and Analgesia for documentation guidelines for USDA-regulated and non-regulated species.

[redacted]

Animal will be monitored continuously with depth assessed at least every 15 minutes

Procedures: Subcutaneous injection

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Subcutaneous injection

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Injection SC

[redacted]

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

◉ Yes ○ No

Select the anesthesia regimen that will be used for this procedure, including induction and maintenance regimens. Please select a regimen that is appropriate for the duration of the procedure.

Isoflurane via precision vaporizer

---

2. Will pre-operative/pre-emptive analgesics be used?

○ Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Mice will be anesthetized with isoflurane (optional). A 0.2 ml syringe + 28 gauge needle will be inserted at a shallow angle subcutaneously (just below the skin) into the back of the mouse between the shoulder blades, and a 50 ul dose of immunogen pre-warmed to room temperature will be injected. The needle will then be removed and inserted subcutaneously at the base of the tail, avoiding all blood vessels, where another 50 ul dose will be injected. If the mouse is not anesthetized, it will be placed in a tailveiner device. The mouse

[redacted]

returned to its cage where it will be monitored for 5 minutes to ensure its recovery from anesthesia and resumption of normal behavior. If the animal displays signs of illness or suffering, it will be euthanized.

In this study, subcutaneous injection will be used to administer water-oil emulsions of peptide and complete Freund's adjuvant (CFA). Each dose will contain 50 ug peptide dissolved in 25 ul of PBS and 25 ul of CFA in the emulsion. Each mouse will receive two doses, one in the back and one in the tail, for a total of 100 ug peptide, 100 ul PBS, and 100 ul CFA. No further dosings will take place.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

---

5. Immediate post-procedural care and monitoring plan.

a. Supportive therapy

- ☐ Warming pad/blanket
- ☐ Incubator or ICU chamber/cage
- ☐ Intravenous fluids
- ☐ Subcutaneous fluids
- ☑ Other

Describe other supportive therapy

Mice will be monitored for 5 minutes to ensure recovery from anesthesia and resumption of normal behavior.

---

b. What criteria will be used to determine the animals are stable and have recovered from anesthesia before being returned to their housing/holding room? Please note that animals must be monitored continuously until they have recovered from anesthesia.

- ☐ Animal maintains sternal recumbency
- ☐ Animal can sit upright (NHPs)
- ☑ Animal is ambulatory
- ☐ Other

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with

[redacted]

CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

Continuously for 5 min until ambulatory. Additional observations are in accordance with the experimental group for the animal as described in the Duration, Clinical Signs, Endpoints and Euthanasia Form.

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

O Not painful/not required

◉ Painful, but analgesia cannot be used

Provide scientific justification for not using analgesia for this painful procedure

All suitable analgesics (e.g. Cox inhibitors, opioids) will affect the immune environment of the gut mucosa and thereby undermine experimental results.

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

O Yes ◉ No O Not applicable

Please include all non-pharmaceutical grade agents on the **Controlled** and **Non-Pharmaceutical Grade Substances** form.

Procedures: Euthanasia for tissue harvest

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Euthanasia for tissue harvest

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

[redacted]

○ Survival ◉ Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Tissue Harvest

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☐ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

○ Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required ○ Painful, but anesthesia cannot be used

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

[redacted]

Mice will be euthanized in the appropriate animal procedure room ([redacted]) or in the lab ([redacted] transport to room 6102, the body will be pinned to a dissection board in a supine position, sprayed with 70% ethanol, and an incision will be made with sharp surgical scissors through the skin along the entire mid-ventral line of the body. The skin will be retracted and pinned down, and the inguinal, axillary, brachial, cervical, and mandibular lymph nodes will be removed using angled surgical forceps. The abdominal cavity will then be cut open with sharp surgical scissors, and the spleen, mesenteric, periaortic, and pancreatic lymph nodes as well as the Peyer's patches and intestines will be removed with angled surgical forceps and scissors. All of the organs will be placed in T cell media and kept on ice until processing. Remaining carcasses will be bagged and disposed in CCM designated freezers.

---

a. Is this a tumor production procedure?

O Yes ◉ No

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Ear Notch or Punch for Mice

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody

[redacted]

production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Ear Notch or Punch for Mice

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Ear Punch

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):
[redacted]

---

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

[redacted]

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required

O Painful, but anesthesia cannot be used

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

a. Animals ≥ 2 weeks of age are manually restrained by scruffing

b. The ear(s) is notched by cutting the minimum amount of tissue needed (approx. 2-3 mm) using a clean sharp scissors or punched using a commercial rodent ear punch device

---

a. Is this a tumor production procedure?

O Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

[redacted]

1. Indicate the frequency of post-procedural observations

Animals will be monitored immediately after the procedure to ensure no adverse effects.

---

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

---

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required O Painful, but analgesia cannot be used

---

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

---

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

---

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

[redacted]

Procedures: Tail snip

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Tail snip

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Tail Snip

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

2. Indicate other preoperative preparation:

- ☐ Eye lubricant
- ☐ Withdrawal of food
- ☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required

O Painful, but anesthesia cannot be used

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Mice that need to be genotyped will be tail snipped and marked by ear punch/slice by 21 days of age. Animals will be restrained manually or using a plastic restraining device with the tail exposed. The distal part of the tail will be wiped with 70% ethanol and the minimal amount of tissue (approx. 2 mm) removed using sterile surgical scissors or blade. The scissors/blade will be cleaned and disinfected between animals. After collection, gentle pressure will be applied to the cut portion of the tail using gauze. Animals will be returned to the cage only after bleeding has been confirmed to have stopped.

a. Is this a tumor production procedure?

[redacted]

O Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

Animals will be observed immediately post procedure to ensure bleeding stops. Additional observations are in accordance with the experimental group for the animal as described in the Duration, Clinical Signs, Endpoints and Euthanasia Form.

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

[redacted]

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

◉ Yes O No O Not applicable

Procedures: Tail vein injection

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

Tail vein injection

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

[redacted]

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Tail vein injections

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☐ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required O Painful, but anesthesia cannot be used

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

[redacted]

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Mice will be briefly warmed under a heat lamp for 1-2 minutes and then restrained with a plexiglass tailveiner device from Braintree Scientific. The base of the tail will be swabbed with an alcohol pad and let dry. A 1 ml syringe + 25 gauge needle or 0.2 ml syringe + 28 gauge needle will be inserted at a shallow angle directly into either the left or right tail vein, and a 200-300 ul dose of immunogen, inoculum, or cells in room temperature PBS will be injected. The needle will then be removed and direct pressure with sterile gauze will immediately be applied to the injection site to help stop bleeding. After the bleeding is controlled, the mouse will be returned to its cage, where it will be continuously observed for 5 minutes and daily thereafter for signs of infection or distress.

In this study, tail vein injection will be used to administer peptide + lipopolysaccharide (LPS) for immunization, Listeria for infection, or T cells for adoptive transfer purposes. Each dose will contain 50-100 ug peptide and 5 ug of LPS in a total volume of 300 ul PBS, 10^3-10^4 cfu Listeria in a total volume of 200 ul PBS, or 1-20 million cells in a total volume of 300 ul PBS. All of these injections will be single doses.

Agents to be injected: Peptide + LPS, Listeria, T cells

Site: Right or left lateral tail vein

Volume: ≤ 0.2 ml mouse using ≤ 25 gauge needle

Dose for each agent: 100 ug peptide + 5 ug LPS, 10^3-10^8 cfu Listeria, 1-20 x 10^6 T cells

Dosing schedule for each agent: once

Method:

1. The tail can be vasodilated by working under a heat lamp or placing the tail in warm water (37oC).

2. Animal will be restrained with plastic rodent restrainer.

3. About half-way up the tail, the needle will be inserted into the vein bevel side up, and the substance injected, ensuring the fluid flows easily into the vein with temporary blanching at the site of injection.

4. Gentle compression using gauze will be applied to stop any bleeding.

[redacted]

a. Is this a tumor production procedure?

○ Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

Continuously for 5 min until bleeding stops. Additional observations are in accordance with the experimental group for the animal as described in the Duration, Clinical Signs, Endpoints and Euthanasia Form.

---

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

---

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required ○ Painful, but analgesia cannot be used

---

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

---

3. Will post-operative/post-procedural antibiotics be administered?

○ Yes ◉ No

---

4. Will other miscellaneous post-operative/post-procedural medications be administered?

○ Yes ◉ No

[redacted]

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

O Yes ◉ No O Not applicable

Please include all non-pharmaceutical grade agents on the **Controlled** and **Non-Pharmaceutical Grade Substances** form.

Procedures: Oral gavage

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Oral gavage

A. Procedure Type

1. What is the type of procedure?

[redacted]

O Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Oral Gavage

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☐ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required O Painful, but anesthesia cannot be used

---

[redacted]

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

---

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Mice will be fasted (given only water) for approximately 6-12 hours before inoculation. A 22 gauge feeding needle attached to a 1 ml syringe will be carefully threaded down the esophagus of the mouse as it is firmly held by the scruff of the neck and back. A 100-500 uL dose of drug or inoculum pre-warmed to room temperature will be dispensed into the stomach. The mouse will be returned to its cage where it will be monitored for 5 minutes for any adverse signs. If the animal displays signs of illness or suffering, it will be euthanized. For peptide immunizations, a single dose of 100 ug peptide + 10 ug cholera toxin in a total volume of 500 ul PBS will be used. For Citrobacter inoculation, a single dose of 10^8 to 10^9 cfu in a total volume of 100 ul PBS will be used.

---

a. Is this a tumor production procedure?

O Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

---

1. Indicate the frequency of post-procedural observations

Continuously for 5 min until resumption of normal behavior. Additional observations are in accordance with the experimental group for the animal as described in the Duration, Clinical Signs, Endpoints and Euthanasia Form.

---

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

[redacted]

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

---

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

---

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

O Yes ◉ No O Not applicable

Please include all non-pharmaceutical grade agents on the **Controlled** and **Non-Pharmaceutical Grade Substances** form.

Procedures: Oral administration

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination

[redacted]

purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:

Oral administration

A. Procedure Type

1. What is the type of procedure?

○ Surgical Procedure ◉ Non-Surgical Procedure

a. This procedure is:

◉ Survival ○ Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)

Drug Delivery, Oral

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant

☐ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required

O Painful, but anesthesia cannot be used

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

The cage water bottle will be replaced with a water bottle containing 150 ml drinking water containing the drug (in this case, 2-3% (w/v) dextran sulfate sodium). After 2 and 4 days, the drug-containing water will be replaced with fresh preparations. At 7 days, the bottle will be replaced with a normal water bottle to resume normal water administration. Treated mice will be observed and weighed daily once treatment is started to assess the development of colitis. For chronic colitis experiments, this procedure will be repeated after the mice have recovered from the previous treatment, to a maximum of three total treatments over the course of the mouse's life.

a. Is this a tumor production procedure?

O Yes ◉ No

[redacted]

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

daily until mice recover from colitis as evidenced from normal weight

2. Will post-operative/post-procedural analgesics be administered?

○ Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

○ Not painful/not required ◉ Painful, but analgesia cannot be used

Provide scientific justification for not using analgesia for this painful procedure

All suitable analgesics (e.g. Cox inhibitors, opioids) will affect the immune environment of the gut mucosa and thereby undermine experimental results.

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

○ Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

○ Yes ◉ No

[redacted]

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

---

1. Will all analgesics, antibiotics, or other medications administered during the course of this procedure be of pharmaceutical grade?

O Yes ◉ No O Not applicable

Please include all non-pharmaceutical grade agents on the **Controlled** and **Non-Pharmaceutical Grade Substances** form.

Procedures: Intraperitoneal injection

Complete this form for each procedure/surgery to be performed.

A procedure is any manipulation of an animal for an experimental application, for examination purposes or for treatment of an induced or spontaneous disease or condition. For clarity of definition the IACUC uses the terms “surgical procedure”' or “non-surgical procedure” to describe all manipulations performed.

Non-surgical Procedure is used to describe injections, bandaging or casting, imaging, antibody production, collection of blood and other clinical samples, non-invasive physiological monitoring, breeding, behavior observations, euthanasia, etc.

Surgery usually involves an incision and exposure of a tissue for an operative method or the operative manipulation of physiologic or physical parameters to create a model of a clinical disease process or condition and/or treatment of a disease or condition.

Enter a title for this procedure:
Intraperitoneal injection

A. Procedure Type

1. What is the type of procedure?

O Surgical Procedure ◉ Non-Surgical Procedure

[redacted]

a. This procedure is:

◉ Survival O Non-Survival

2. Please select the procedure from the list.
(Select the item that best represents the procedure or approach used.)
Injection, IP

B. Location

Indicate the building where the surgery or procedure will be performed:

[redacted]

Indicate the room number(s):

[redacted]

---

2. Indicate other preoperative preparation:

☐ Eye lubricant
☐ Withdrawal of food
☐ Other

D. Procedure

1. Will anesthesia be used for this procedure?

O Yes ◉ No

---

a. Why will anesthesia not be used for this procedure?

◉ Not painful/not required O Painful, but anesthesia cannot be used

---

2. Will pre-operative/pre-emptive analgesics be used?

O Yes ◉ No

[redacted]

4. Description of procedure

Provide a complete description of the procedure. For surgical procedures, include the surgical approach used, the method(s) of wound closure, and intra-operative supportive care (e.g., IV fluids, mechanical ventilation)

Agents to be injected: diphtheria toxin, Thy1.1 antibody, control IgG2a antibody

Site: Lower right or left quadrant of the abdomen.

Volume: ≤ 3 ml per mouse using ≤ 21 gauge needle

Dose for each agent:

diptheria toxin: 0.8 - 1.0 ug per mouse (50 mg/kg) in PBS

antibodies: 250 ug per mouse (12.5 g/kg) in PBS

Dosing schedule for each agent:

diphtheria toxin: once every other day for 6 days (3 total injections)

antibodies: once every two weeks for 12 weeks (6 total injections)

1. Animal will be restrained by scruffing so that the abdomen is facing up.

2. The animal will be held with head tilted slightly lower than the rear, thus moving abdominal organs away from the injection site.

3. The needle will be inserted through the abdominal wall in the right or left caudal quadrant of the animal; the plunger will be pulled back to make sure no fluid is in the hub If there is fluid in the hub of the needle, the needle is removed, animal monitored and the procedure attempted again only if animal appears stable. If the hub is clean, material is injected.

4. The site of injection will be alternated between the lower quadrants of the animal for repeated injections.

---

a. Is this a tumor production procedure?

○ Yes ◉ No

E. Post-operative/Post-procedural Care

CCM provides routine veterinary oversight, but the investigators are responsible for all

[redacted]

monitoring and care of the research animals, unless a specific service has been pre-arranged with CCM by contract. See FAQ for links to Veterinary Care and Post Operative/Post Procedural Care policies.

1. Indicate the frequency of post-procedural observations

Continuously for 5 min until animal resumes normal behavior. Additional observations are in accordance with the experimental group for the animal as described in the Duration, Clinical Signs, Endpoints and Euthanasia Form.

2. Will post-operative/post-procedural analgesics be administered?

O Yes ◉ No

a. Why will post-operative/post-procedural analgesics not be used for this procedure?

◉ Not painful/not required

O Painful, but analgesia cannot be used

If signs of pain persist past administration of the last dose of the analgesic regimen, contact a CCM veterinarian.

3. Will post-operative/post-procedural antibiotics be administered?

O Yes ◉ No

4. Will other miscellaneous post-operative/post-procedural medications be administered?

O Yes ◉ No

F. Non-Pharmaceutical Grade Substances

The IACUC requires that all substances administered to any animal species be of pharmaceutical grade, if that substance is available in pharmaceutical grade.

[redacted]

1. Will all analgesics, antibiotics, or other medications administered durin procedure be of pharmaceutical grade?

O Yes ◉ No O Not applicable

Please include all non-pharmaceutical grade agents on the **Controlled** and **Non-Pharmaceutical Grade Substances** form.

Hazardous Agent Administration and Use: diphtheria toxin

All projects involving the use of any biological, chemical, or radiological hazard must be performed in accordance with [redacted] Safety Policies or [redacted] Safety and Biosafety Policies (see links below) for hazardous materials. Principal Investigators are responsible for informing employees of any potential risks associated with hazardous agents they will be expected to use.

Capture information related to all hazardous agents used in the protocol using this form. See FAQ for a description of exempt items that do not require a Hazardous Agent Administration and Use form.

For information or assistance, contact:

- [redacted] Environmental Health and Safety, [redacted]
- [redacted] Staff Health, Safety, Compliance, [redacted]

Please provide the emergency study contact(s).

Contact Name	Phone / Beeper number	Email (Partners email only)
[redacted]	[redacted]	[redacted]

A. Hazard Type

Indicate the type of hazard to be used

O Biological ◉ Chemical O Radioisotope/ Radionuclide

---

1. Please select the chemical hazard to be used.

diphtheria toxin

[redacted]

In order to ensure chemical safety in the workplace, information about the identities and hazards of the chemicals must be available and understandable to workers. All employers with hazardous chemicals in their workplaces must have labels and safety data sheets (MSDSs) for their exposed workers, and train them to handle the chemicals appropriately. MSDSs for many agents can be found through MSDS Source (log in with username: [redacted] password: [redacted]

2. Do you have a Chemical Hygiene Plan?

◉ Yes O No

B. Hazard Use Location

Please indicate the building where this agent will be administered to an animal

[redacted]

Indicate the room number(s):

[redacted]

- The rooms indicated on this form must be consistent with the rooms indicated on your Procedure form(s). Biological hazards and radiological hazards may be used only in the rooms approved on your relevant PIBC registration or radioisotope permit.
- If the hazard will be used within CCM Facilities, please contact the appropriate CCM facility manager **before** use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.
- Please consult with [redacted] Environmental Health and Safety ([redacted]) to discuss whether additional engineering controls are required for this agent.

C. Agent Administration

1. What is the expected dose (range) per animal?

1 microgram

2. What is the total number of doses an individual animal may receive?

3

3. How frequently will an individual animal be dosed with this agent?

once a day, up to 3 times over one week

4. Indicate the duration of time between administration of the hazardous agent and planned euthanasia of the animals.

2-12 weeks

[redacted]

5. Will animals be returned to CCM facilities after exposure to this agent?

- ◉ Yes
- O No
- O Not applicable - all work will be done within CCM facilities

Please note, if animals will be housed in CCM facilities after exposure to this agent or if the agent will be administered within CCM facilities:

- **Please contact the appropriate CCM facility manager** before **use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.**
- **Include all special requirements for animal housing, husbandry, and handling on the Housing form.**

Hazardous Agent Administration and Use: Cholera toxin B subunit

All projects involving the use of any biological, chemical, or radiological hazard must be performed in accordance with [redacted] Safety Policies or [redacted] Safety and Biosafety Policies (see links below) for hazardous materials. Principal Investigators are responsible for informing employees of any potential risks associated with hazardous agents they will be expected to use.

Capture information related to all hazardous agents used in the protocol using this form. See FAQ for a description of exempt items that do not require a Hazardous Agent Administration and Use form.

For information or assistance, contact:

- [redacted] Environmental Health and Safety, [redacted]
- [redacted] Lab Staff Health, Safety, Compliance, [redacted]

Please provide the emergency study contact(s).

Contact Name	Phone / Beeper number	Email (Partners email only)
[redacted]	[redacted]	[redacted]

A. Hazard Type

Indicate the type of hazard to be used

O Biological	◉ Chemical	O Radioisotope/ Radionuclide

[redacted]

1. Please select the chemical hazard to be used.

Cholera toxin B subunit

In order to ensure chemical safety in the workplace, information about the identities and hazards of the chemicals must be available and understandable to workers. All employers with hazardous chemicals in their workplaces must have labels and safety data sheets (MSDSs) for their exposed workers, and train them to handle the chemicals appropriately. MSDSs for many agents can be found through MSDS Source (log in with username: [redacted] password: [redacted]

2. Do you have a Chemical Hygiene Plan?

◉ Yes O No

B. Hazard Use Location

Please indicate the building where this agent will be administered to an animal

[redacted]

Indicate the room number(s):

[redacted]

- The rooms indicated on this form must be consistent with the rooms indicated on your Procedure form(s). Biological hazards and radiological hazards may be used only in the rooms approved on your relevant PIBC registration or radioisotope permit.
- If the hazard will be used within CCM Facilities, please contact the appropriate CCM facility manager **before** use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.
- Please consult with [redacted] Environmental Health and Safety ([redacted]) to discuss whether additional engineering controls are required for this agent.

C. Agent Administration

1. What is the expected dose (range) per animal?

10 micrograms

2. What is the total number of doses an individual animal may receive?

one

3. How frequently will an individual animal be dosed with this agent?

[redacted]

once

4. Indicate the duration of time between administration of the hazardous agent and planned euthanasia of the animals.

1-12 weeks

5. Will animals be returned to CCM facilities after exposure to this agent?

- ◉ Yes
- O No
- O Not applicable - all work will be done within CCM facilities

Please note, if animals will be housed in CCM facilities after exposure to this agent or if the agent will be administered within CCM facilities:

- **Please contact the appropriate CCM facility manager** before **use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.**
- **Include all special requirements for animal housing, husbandry, and handling on the Housing form.**

Hazardous Agent Administration and Use: Citrobacter rodentium

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All projects involving the use of any biological, chemical, or radiological hazard must be performed in accordance with [redacted] Safety Policies or [redacted] Safety and Biosafety Policies (see links below) for hazardous materials. Principal Investigators are responsible for informing employees of any potential risks associated with hazardous agents they will be expected to use.

Capture information related to all hazardous agents used in the protocol using this form. See FAQ for a description of exempt items that do not require a Hazardous Agent Administration and Use form.

For information or assistance, contact:

- [redacted] Environmental Health and Safety, [redacted]
- [redacted] Lab Staff Health, Safety, Compliance, [redacted]

Please provide the emergency study contact(s).

Contact Name	Phone / Beeper number	Email (Partners email only)
[redacted]	[redacted]	[redacted]

[redacted]

A. Hazard Type

Indicate the type of hazard to be used

◉ Biological | O Chemical | O Radioisotope/ Radionuclide

Any investigator wishing to work with biological hazards must register with the Partners Institutional Biosafety Committee (PIBC). PIBC forms are available in the Biosafety Module of Insight. The PIBC referenced must cover the agents, personnel, and locations indicated in the IACUC protocol. Please note that the IACUC protocol will not be approved until the PIBC registration is approved.

1. Please indicate the PIBC Registration Number

[redacted] - Infectious Agents to Stu...

2. Please select the biological agent that will be used

Citrobacter rodentium

3. Indicate the classification for the selected biological agent

☑ Biological Agent

- Biological Agents are viable infectious microorganisms and proteinaceous infectious particles (e.g. prions) regardless of their pathogenicity to humans.

a. Enter the common name of the agent to be used

Citrobacter

b. Enter the specific strain of the agent to be used

DBS100

☐ Recombinant and Synthetic Nucleic Acid Molecules

☐ Biological Toxin

☐ Nonhuman Primate Tissue/Sample

[redacted]

☐ Human Tissue/Sample

B. Hazard Use Location

Please indicate the building where this agent will be administered to an animal

[redacted]

Indicate the room number(s):

[redacted]

- The rooms indicated on this form must be consistent with the rooms indicated on your Procedure form(s). Biological hazards and radiological hazards may be used only in the rooms approved on your relevant PIBC registration or radioisotope permit.
- If the hazard will be used within CCM Facilities, please contact the appropriate CCM facility manager **before** use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.
- Please consult with [redacted] Environmental Health and Safety ([redacted]) to discuss whether additional engineering controls are required for this agent.

C. Agent Administration

1. What is the expected dose (range) per animal?

Approximately 10^8 to 10^9 cfu per mouse.

2. What is the total number of doses an individual animal may receive?

one

3. How frequently will an individual animal be dosed with this agent?

once

4. Indicate the duration of time between administration of the hazardous agent and planned euthanasia of the animals.

4-29 days

5. Will animals be returned to CCM facilities after exposure to this agent?

◉ Yes
O No
O Not applicable - all work will be done within CCM facilities

[redacted]

Please note, if animals will be housed in CCM facilities after exposure to this agent or if the agent will be administered within CCM facilities:

- **Please contact the appropriate CCM facility manager** before **use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.**
- **Include all special requirements for animal housing, husbandry, and handling on the Housing form.**

Hazardous Agent Administration and Use: Listeria monocytogenes

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All projects involving the use of any biological, chemical, or radiological hazard must be performed in accordance with [redacted] Safety Policies or [redacted] Safety and Biosafety Policies (see links below) for hazardous materials. Principal Investigators are responsible for informing employees of any potential risks associated with hazardous agents they will be expected to use.

Capture information related to all hazardous agents used in the protocol using this form. See FAQ for a description of exempt items that do not require a Hazardous Agent Administration and Use form.

For information or assistance, contact:

- [redacted] Environmental Health and Safety, [redacted]
- [redacted] Lab Staff Health, Safety, Compliance, [redacted]

Please provide the emergency study contact(s).

Contact Name	Phone / Beeper number	Email (Partners email only)
[redacted]	[redacted]	[redacted]

A. Hazard Type

Indicate the type of hazard to be used

◉ Biological O Chemical O Radioisotope/ Radionuclide

Any investigator wishing to work with biological hazards must register with the Partners Institutional Biosafety Committee (PIBC). PIBC forms are available in the Biosafety Module of Insight. The PIBC referenced must cover the agents, personnel, and locations indicated in the IACUC protocol. Please note that the IACUC protocol will not be approved until the PIBC registration is approved.

[redacted]

1. Please indicate the PIBC Registration Number

[redacted] - [redacted] - Infectious Agents to Stu...

2. Please select the biological agent that will be used

Listeria monocytogenes

3. Indicate the classification for the selected biological agent

☑ Biological Agent

- Biological Agents are viable infectious microorganisms and proteinaceous infectious particles (e.g. prions) regardless of their pathogenicity to humans.

a. Enter the common name of the agent to be used

Listeria

b. Enter the specific strain of the agent to be used

10403s, DPL1942

☐ Recombinant and Synthetic Nucleic Acid Molecules

☐ Biological Toxin

☐ Nonhuman Primate Tissue/Sample

☐ Human Tissue/Sample

B. Hazard Use Location

Please indicate the building where this agent will be administered to an animal

[redacted]

[redacted]

Indicate the room number(s):

[redacted]

- The rooms indicated on this form must be consistent with the rooms indicated on your Procedure form(s). Biological hazards and radiological hazards may be used only in the rooms approved on your relevant PIBC registration or radioisotope permit.
- If the hazard will be used within CCM Facilities, please contact the appropriate CCM facility manager **before** use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.
- Please consult with [redacted] Environmental Health and Safety ([redacted]) to discuss whether additional engineering controls are required for this agent.

C. Agent Administration

1. What is the expected dose (range) per animal?

10^3 cfu i.v. or 10^8 cfu i.g. for 10403s strain, 10^7 cfu i.v. or 10^10 cfu i.g. for DPL1942 strain

2. What is the total number of doses an individual animal may receive?

1-3

3. How frequently will an individual animal be dosed with this agent?

once

4. Indicate the duration of time between administration of the hazardous agent and planned euthanasia of the animals.

1-12 weeks

5. Will animals be returned to CCM facilities after exposure to this agent?

- ◉ Yes
- O No
- O Not applicable - all work will be done within CCM facilities

Please note, if animals will be housed in CCM facilities after exposure to this agent or if the agent will be administered within CCM facilities:

- **Please contact the appropriate CCM facility manager** before **use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.**
- **Include all special requirements for animal housing, husbandry, and handling on the Housing form.**

[redacted]

Hazardous Agent Administration and Use: Lipopolysaccharide

All projects involving the use of any biological, chemical, or radiological hazard must be performed in accordance with [redacted] Safety Policies or [redacted] Safety and Biosafety Policies (see links below) for hazardous materials. Principal Investigators are responsible for informing employees of any potential risks associated with hazardous agents they will be expected to use.

Capture information related to all hazardous agents used in the protocol using this form. See FAQ for a description of exempt items that do not require a Hazardous Agent Administration and Use form.

For information or assistance, contact:

- [redacted] Environmental Health and Safety, [redacted]
- [redacted] Lab Staff Health, Safety, Compliance, [redacted]

Please provide the emergency study contact(s).

Contact Name	Phone / Beeper number	Email (Partners email only)
[redacted]	[redacted]	[redacted]

A. Hazard Type

Indicate the type of hazard to be used

O Biological	◉ Chemical	O Radioisotope/ Radionuclide

1. Please select the chemical hazard to be used.

   Lipopolysaccharide

In order to ensure chemical safety in the workplace, information about the identities and hazards of the chemicals must be available and understandable to workers. All employers with hazardous chemicals in their workplaces must have labels and safety data sheets (MSDSs) for their exposed workers, and train them to handle the chemicals appropriately. MSDSs for many agents can be found through MSDS Source (log in with username: [redacted] password: [redacted]

2. Do you have a Chemical Hygiene Plan?

   ◉ Yes O No

[redacted]

B. Hazard Use Location

Please indicate the building where this agent will be administered to an animal

[redacted]

Indicate the room number(s):

[redacted]

- The rooms indicated on this form must be consistent with the rooms indicated on your Procedure form(s). Biological hazards and radiological hazards may be used only in the rooms approved on your relevant PIBC registration or radioisotope permit.
- If the hazard will be used within CCM Facilities, please contact the appropriate CCM facility manager **before** use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.
- Please consult with [redacted] Environmental Health and Safety ([redacted]) to discuss whether additional engineering controls are required for this agent.

C. Agent Administration

1. What is the expected dose (range) per animal?

5 micrograms

2. What is the total number of doses an individual animal may receive?

one

3. How frequently will an individual animal be dosed with this agent?

once

4. Indicate the duration of time between administration of the hazardous agent and planned euthanasia of the animals.

1-12 weeks

5. Will animals be returned to CCM facilities after exposure to this agent?

- ◉ Yes
- O No
- O Not applicable - all work will be done within CCM facilities

Please note, if animals will be housed in CCM facilities after exposure to this agent or if the agent will be administered within CCM facilities:

[redacted]

- **Please contact the appropriate CCM facility manager** before **use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.**
- **Include all special requirements for animal housing, husbandry, and handling on the Housing form.**

Hazardous Agent Administration and Use: Dextran Sulfate Sodium (DSS)

All projects involving the use of any biological, chemical, or radiological hazard must be performed in accordance with [redacted] Safety Policies or [redacted] Safety and Biosafety Policies (see links below) for hazardous materials. Principal Investigators are responsible for informing employees of any potential risks associated with hazardous agents they will be expected to use.

Capture information related to all hazardous agents used in the protocol using this form. See FAQ for a description of exempt items that do not require a Hazardous Agent Administration and Use form.

For information or assistance, contact:

- [redacted] Environmental Health and Safety, [redacted]
- [redacted] Lab Staff Health, Safety, Compliance, [redacted]

Please provide the emergency study contact(s).

Contact Name	Phone / Beeper number
[redacted]	[redacted]

A. Hazard Type

Indicate the type of hazard to be used

O Biological	◉ Chemical	O Radioisotope/ Radionuclide

---

1. Please select the chemical hazard to be used.

   Dextran Sulfate Sodium (DSS)

In order to ensure chemical safety in the workplace, information about the identities and hazards of the chemicals must be available and understandable to workers. All employers with hazardous chemicals in their workplaces must have labels and safety data sheets (MSDSs) for their exposed workers, and train them to handle the chemicals

[redacted]

appropriately. MSDSs for many agents can be found through MSDS Source (log in with username: [redacted] password: [redacted]

2. Do you have a Chemical Hygiene Plan?

◉ Yes O No

B. Hazard Use Location

Please indicate the building where this agent will be administered to an animal

[redacted]

Indicate the room number(s):

[redacted]

- The rooms indicated on this form must be consistent with the rooms indicated on your Procedure form(s). Biological hazards and radiological hazards may be used only in the rooms approved on your relevant PIBC registration or radioisotope permit.
- If the hazard will be used within CCM Facilities, please contact the appropriate CCM facility manager **before** use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.
- Please consult with [redacted] Environmental Health and Safety ([redacted]) to discuss whether additional engineering controls are required for this agent.

C. Agent Administration

1. What is the expected dose (range) per animal?

Mouse expected to drink 5 mL per day of 3% DSS solution or take in 0.15 g DSS per day maximum.

2. What is the total number of doses an individual animal may receive?

1-3

3. How frequently will an individual animal be dosed with this agent?

once or three times depending upon acute or chronic colitis model

4. Indicate the duration of time between administration of the hazardous agent and planned euthanasia of the animals.

4-91 days

5. Will animals be returned to CCM facilities after exposure to this agent?

[redacted]

◉ Yes
O No
O Not applicable - all work will be done within CCM facilities

Please note, if animals will be housed in CCM facilities after exposure to this agent or if the agent will be administered within CCM facilities:

- **Please contact the appropriate CCM facility manager** before **use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.**
- **Include all special requirements for animal housing, husbandry, and handling on the Housing form.**

Hazardous Agent Administration and Use: complete Freunds adjuvant

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All projects involving the use of any biological, chemical, or radiological hazard must be performed in accordance with [redacted] Safety Policies or [redacted] Safety and Biosafety Policies (see links below) for hazardous materials. Principal Investigators are responsible for informing employees of any potential risks associated with hazardous agents they will be expected to use.

Capture information related to all hazardous agents used in the protocol using this form. See FAQ for a description of exempt items that do not require a Hazardous Agent Administration and Use form.

For information or assistance, contact:

- [redacted] Environmental Health and Safety, [redacted]
- [redacted] Lab Staff Health, Safety, Compliance, [redacted]

Please provide the emergency study contact(s).

Contact Name	Phone / Beeper number	Email (Partners email only)
[redacted]	[redacted]	[redacted]

A. Hazard Type
Indicate the type of hazard to be used

O Biological ◉ Chemical O Radioisotope/ Radionuclide

[redacted]

1. Please select the chemical hazard to be used.
complete Freunds adjuvant

In order to ensure chemical safety in the workplace, information about the identities and hazards of the chemicals must be available and understandable to workers. All employers with hazardous chemicals in their workplaces must have labels and safety data sheets (MSDSs) for their exposed workers, and train them to handle the chemicals appropriately. MSDSs for many agents can be found through MSDS Source (log in with username: [redacted] password: [redacted]

2. Do you have a Chemical Hygiene Plan?

◉ Yes O No

B. Hazard Use Location

Please indicate the building where this agent will be administered to an animal

[redacted]

Indicate the room number(s):

[redacted]

- The rooms indicated on this form must be consistent with the rooms indicated on your Procedure form(s). Biological hazards and radiological hazards may be used only in the rooms approved on your relevant PIBC registration or radioisotope permit.
- If the hazard will be used within CCM Facilities, please contact the appropriate CCM facility manager **before** use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.
- Please consult with [redacted] Environmental Health and Safety ([redacted]) to discuss whether additional engineering controls are required for this agent.

C. Agent Administration

1. What is the expected dose (range) per animal?
50 ul total (50 ul of 1:1 PBS emulsion) x 2 injection sites

2. What is the total number of doses an individual animal may receive?
one

3. How frequently will an individual animal be dosed with this agent?
once

[redacted]

4. Indicate the duration of time between administration of the hazardous agent and planned euthanasia of the animals.

1-12 weeks

5. Will animals be returned to CCM facilities after exposure to this agent?

- ◉ Yes
- O No
- O Not applicable - all work will be done within CCM facilities

Please note, if animals will be housed in CCM facilities after exposure to this agent or if the agent will be administered within CCM facilities:

- **Please contact the appropriate CCM facility manager** before **use to ensure that any special requirements for animal housing, husbandry, and handling, and personnel safety are addressed.**
- **Include all special requirements for animal housing, husbandry, and handling on the Housing form.**

Controlled Substance and Non-Pharmaceutical Grade Substance

A. Controlled Substances

1. Are any of the agents (anesthetics, analgesics, test agents, etc.) used in this protocol DEA/Federally Controlled Substances?

O Yes ◉ No

B. Non-Pharmaceutical Grade Substances

Both OLAW and AAALAC provide guidance regarding the use of non-pharmaceutical grade compounds in laboratory animals.

Pharmaceutical-grade substances, when available, must be used to avoid toxicity or side effects that may threaten the health and welfare of vertebrate animals and/or interfere with the interpretation of research results. However, it is frequently necessary to use non-pharmaceutical-grade substances such as investigational substances, veterinarian- or pharmacy-compounded substances, and/or Schedule I controlled substances to meet scientific and research goals.

A listing of pharmaceutical-grade drugs and biologics is available through the FDA database.
· The Orange Book is the reference for FDA-approved human drugs.
· The Green Book is the reference for FDA-approved veterinary drugs.

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1. Are all substances to be administered to animals of pharmaceutical grade?

Examples of non-pharmaceutical grade substances include:

[redacted]

- Anesthetics and analgesics (e.g., Avertin)
- Euthanasia compounds (e.g., pentobarbital)
- Diluents and/or vehicles (e.g., DMSO, methyl cellulose)
- Test compounds

O Yes ◉ No

2. List the non-pharmaceutical grade substance(s) that will be used.

Please address the use of these non-pharmaceutical grade substance(s) in the appropriate section(s) of the protocol (Research Plan, Procedure forms, etc.).

Agent	Dose (range)	Route	Frequency	Duration
Complete Freund's adjuvant (CFA)	50-100 ul	s.c.	once	once
Thy1.1 antibody	250 ug	i.p.	once every 2 weeks	6 injections over 12 weeks
Peptide	50-100 ug	i.v., s.c., i.g.	once	once
Lipopolysaccharide (LPS)	5 ug	i.v.	once	once
Cholera toxin B subunit (CTB)	10 ug	i.g.	once	once
Dextran sulfate sodium (DSS)	2-3% in drinking water (	oral	7 days	1-3 times, separated by 2 week intervals
Diphtheria toxin (DT)	1 ug	i.p.	once per day, 3 times per week	one week

3. Non-pharmaceutical grade substances must be the highest grade available and must be formulated using biocompatible solutions appropriate for the route of administration, as described in the IACUC Policy on the Use of Non-Pharmaceutical Grade Substances in Laboratory Animals; departures from these guidelines must be described and justified below. In addition, non-pharmaceutical grade substances administered parenterally (e.g., IV, IP, IM, SC) will be sterilized according to the guidelines.

Describe and justify departures from the guidelines.

None of these substances are available in pharmaceutical grade.

4 Justification for use of non-pharmaceutical grade compounds (select all that apply):

☑ No equivalent veterinary or human drug is available for experimental use; this includes new investigational compounds.
☐ Pharmaceutical grade is not available in the appropriate concentration or formulation, or the appropriate vehicle control is unavailable.
☐ Non-pharmaceutical grade is required to generate data as part of an ongoing study or to generate data that are comparable to previous work
☐ Other

[redacted]

☑ The Principal Investigator attests the he/she has read the IACUC Policy on the Use of Non-Pharmaceutical Grade Substances in Laboratory Animals and will ensure that all protocol study staff will follow the policy.

Restraint

The Guide for the Care and Use of Laboratory Animals defines physical restraint as the use of manual or mechanical means to limit some or all of an animal's movement for the purpose of examination or experimental manipulation. Sedatives or anesthetics may be used to immobilize animals for the performance of non-painful procedures that might otherwise be painful or distressful to the animal.

Restraint

◉ Animals will undergo restraint as part of this research

1. Provide justification for the use and duration of restraint.

Mice will be held securely by the scruff of the neck/back as briefly as possible to obtain a tail snip or ear punch, or to enable intraperitoneal injection. The time of restraint will be as short as possible, typically ranging from 10 sec to 1 min per mouse.

2. Will the animals be conscious or sedated during the restraint?

◉ Conscious O Sedated

3. Indicate the type of restraint that will be used.

☑ Manual restraint

a. Indicate the duration of manual restraint (select all that apply):

☑ Routine - manual restraint for less than 15 minutes
☐ Restraint duration longer than 15 minutes, but less than 4 hours
☐ Restraint duration longer than 4 house

b. Indicate the frequency of manual restraint.

Mice will be restrained once per procedure. Depending on the experiment, mice will be restrained 0 to 3 times per lifetime.

[redacted]

c. Describe the methods used to train and acclimate the animal to manual restraint.

While holding the mouse by the tail in one hand, the investigator will very slowly push down on the animal's back and neck with the other hand until he/she can grab enough scruff to immobilize and lift up the animal. Moving slowly but deliberately will keep the animal calm and lessen the likelihood of it trying to escape.

d. Describe the plans for monitoring and care of the animals during the periods of manual restraint.

During manual restraint, the investigator will observe the animal's breathing and attempted movements. If the animal appears to be in pain, it will be immediately released and the manual restraint reapplied more carefully.

☑ Mechanical

Select all that apply.

- ☑ Rodent plexiglass, metal, or Bowman style restrainer
- ☐ Rabbit plexiglass or metal retrainer
- ☐ Full body sling
- ☐ NHP chair
- ☐ Stereotaxic device
- ☐ Squeeze cage
- ☐ Other

a. Indicate the duration of mechanical restraint (select all that apply).

- ☑ Routine mechanical restraint for less than 15 minutes
- ☐ Restraint duration longer than 15 minutes, but less than 4 hours
- ☐ Restraint duration longer than 4 hours

b. Indicate the frequency of mechanical restraint.

Mice will be restrained once per procedure. Depending on the experiment, mice will be restrained 0 to 2 times per lifetime.

c. Describe the methods used to train and acclimate animals to the mechanical restraint device.

Handling the mouse by the tail, the investigator will slowly pull it into the plexiglass restrainer and close the opening with the plastic plunger, reducing the volume so the mouse does not have room to try to jump free.

d. Describe the plans for monitoring and care of the animals during the periods of mechanical restraint.

[redacted]

During mechanical restraint, the investigator will observe the animal's breathing and attempted movements. If the animal appears to be in pain, it will be immediately released and the restraint reapplied more carefully.

The Principal Investigator is responsible for assuring that:

· Veterinary care will be provided if lesions or illnesses are observed

· The purpose and duration of restraint will be communicated to all personnel involved in the study.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Anesthesia Regimen, Label**. The information in this section could not be mapped from your approved application to a new form/field as part of the transition to Insight 4.0. Please review the information in this field as it contains details useful in answering the **Indicate the anesthesia regimen or sedative that will be used**, question above.

Device Acclimation

O This research includes devices to which animals must be acclimated, e.g., jackets/tethers

Breeding Studies

Please use this form to provide details related to the breeding aspect of your study. Users are required to keep a record of all animals born and weaned, as the IACUC may request this information for regulatory reporting requirements.

[redacted] **users:** records will be maintained by utilizing the census software, and rodents used for experiments prior to weaning should be reported to the facility supervisor.

For additional guidance, please refer to your institution's rodent breeding and cage density policy.

1. Breeding scheme
   a. Select the breeding scheme that will be used

[redacted]

- ☑ Monogamous pair - ONE (1) adult male and ONE (01
- ☐ Breeding trio - ONE (1) adult male and TWO (2) adult females
- ☑ Harem Breeding - ONE (1) adult male and up to FOUR (4) adult females
- ☐ Other

b. How will the breeding pairs or groups be selected?

Breeders will preferentially be set up as single pairs, and litters will be weaned at 21 days of age. In cases when parent mice are limiting, one male may be harem bred with 2-3 females for 1-2 weeks followed by separation of the pregnant females. Animals requiring genotyping will be tailed and marked by ear punch by 21 days of age. Breeding pairs will be replaced every 6 months. Mice at 6-8 weeks of age will selected for breeding based on their genotype, general appearance, and health.

2. Indicate the identification method(s) that will be used to track offspring.

- ☑ Ear notch
- ☐ Ear tag
- ☐ Tattoo
- ☑ Other

Please describe.

Ear punch/slice

3. At what age will the offspring be weaned?

- ☑ Standard (see FAQ for SOP)
- ☐ Not applicable (aquatics only)

- ☐ Non-standard

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Breeding Studies, question 3 -- "At what age will the offspring be weaned?"**. The information in this section could not be mapped from your approved application due to field format differences. Please review the information in this section and **ensure it is consistent with the defaulted checkbox - "Standard" - in question 3 above.**

4. Will any animals undergo genotyping to verify genetics?

◉ Yes O No

- Please note that animals to be genotyped must be assigned to an appropriate pain and distress category.

- Tail Snipping of animals over 21 days of age and toe clipping of animals over 7 days of age are considered category D procedures requiring anesthesia with/without analgesia and a procedure form must be completed.
- Procedure forms are not required for other genotyping procedures if the procedures are performed in accordance with institutional SOPs.

Select genotyping method(s) to be used.

☑ Ear punching/ear snipping
☐ Buccal (oral) swabs
☐ Fecal sampling
☐ Hair sampling
☐ Fin clipping

☑ Tail snipping, under 21 days of age

☐ Tail snipping, over 21 days of age

☐ Toe clipping under 7 days of age

Transportation

The transportation of animals must conform to IACUC policy and the Animal Welfare Act, as applicable.

1. Will live animals be transported to facilities outside the institution?

O Yes ◉ No

2. Will animals be moved within or between institution facilities? This includes moving animals between housing areas and laboratory or imaging areas.

◉ Yes O No

a. Will animals be moved between Biosafety Level 2 (BSL2) housing and laboratory or imaging areas?

O Yes ◉ No

b. Select the option that applies:

☑ Transfers will be performed by CCM

[redacted]

☐ Transfers will be performed by protocol study staff following institutional SOPs and/or guidelines (see FAQ for links to institution-specific SOPs/guidelines).
☐ Other (including any animal transport not covered by institutional SOPs or guidelines)

Initial Survey

INSTITUTIONAL ANIMAL CARE AND USE COMMITTEE

In accordance with federal regulations and hospital policies, all animal research conducted at or funded through the [redacted] must be reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) prior to initiation of the study. This policy applies to any vertebrate animal used for any type of research, teaching, or testing. The IACUC has the sole authority to approve, require modifications (in order to secure approval), or withhold approval of research protocols involving the use of animals at the selected Institution. Protocols can be approved for a maximum of three years, subject to satisfactory annual reviews where required. The IACUC also must review and approve **in advance** any changes or modifications to previously approved protocols.

Principal Investigator Eligibility: Please note that you must meet the eligibility requirements set by your institution's IACUC in order to serve as the principal investigator (PI) for an animal research protocol. See FAQ for links to institution-specific guidelines.

The questions below will help to identify if an IACUC protocol must be submitted to your institution's IACUC for your research project.

Please enter the full title of the study.
Commensal Antigen-Specific T cells

A. At which Institution will the research be conducted?

◉ [redacted] O [redacted] O [redacted] O Other Institution

B. The proposed research project will involve the following:

◉ The entire animal research protocol will be conducted at the selected institution.

[redacted]

O Only a portion of the animal research project will be conducted at the selected Institution. This includes, but is not limited to, housing, surgery procedures, behavior assessments, imaging sessions, etc.

If the IACUC grants approval, it will oversee only the research component that is performed at your institution. Any research component(s) conducted at an outside institution will be conducted under the auspices of that institution's IACUC.

For the protocol to remain active, the investigator must submit satisfactory IACUC annual progress reports (if required), as well as provide annual documentation of the relevant outside IACUC approvals.

For more information, please refer to the IACUC website for your institution:

- [redacted]

C. The proposed study involves the use of:

◉ Any live animal (ie. mouse, rat, rabbit, dog, cat, swine, sheep, nonhuman primate, etc)

O Animals tissues, products, or blood (including whole dead animal), not otherwise approved by the IACUC as part of the investigator's own animal research protocol.

Attachments

Name	Mode
[redacted] CHP 01-18-18 (Chemical Hygiene Plan)	Electronic
3-Floer et al (Citations or References)	Electronic
1-Morteau et al (Citations or References)	Electronic
2-Anselmi et al (Citations or References)	Electronic
4-Chassaing et al (Citations or References)	Electronic
flowchart-0 (Flowchart)	Electronic
DSS MSDS (MSDS Sheet)	Electronic
DT MSDS (MSDS Sheet)	Electronic
LPS MSDS (MSDS Sheet)	Electronic
CFA MSDS (MSDS Sheet)	Electronic
CT MSDS (MSDS Sheet)	Electronic
Citrobacter spp (Safety Info)	Electronic
Listeria monocytogenes (Safety Info)	Electronic
Study Staff certification (Staff certification)	Electronic
Study Staff certification (Staff certification)	Electronic

CBir (CBir TCR tg x IL-17-GFP x Foxp3-RFP x 10BiT)(Charles Elson, University of Alabama)
The mice express fixed T cell receptors with specificity to the CBir1 antigen of *Clostridia* bacteria and will be used as donor T cells in a T cell transfer model of colitis. They will be crossed with the Foxp3-RFP, IL-17-GFP, and 10BiT strains to facilitate the identification of T cell subsets.

Breeding Strategy

Foxp3-RFP, IL-17-GFP, IL-10-GFP, 10BiT
These reporter mice will be purchased or imported and used in initial crosses to generate our panel of experimental strains.

Double reporter, pIgR KO, RAG1 KO
Breed as homozygotes
100% offspring with useful genotype

Triple reporter, Foxp3-DEREG, CBir
Try to breed as homozygotes, but crosses likely to be mix of homo x homo, homo x het, het x het
Overall, ~92% offspring with useful genotype (average of 100%, 100%, and 75% outcomes)

IL-10 KO
Breed as heterozygotes
25% offspring with useful genotype

See table for summary of breeding strategy and mouse numbers for each strain

Total mice needed for experiments = **987**

Total mice needed for breeding = **246**

Total mice carried over from last period = 12

Total mice needed for breeding and experiments (excluding carryover) = **1221**

Total mice generated = **1740** (753 Pain C)