[redacted]

Title: **Genetic Control of Inflammatory Responses**
Sponsor Name: [redacted]

PI Name: [redacted] Protocol #: [redacted] Type: **Current View**
Species: **MICE** # Of Animals: **1053** Date Received: **March 26, 2008**

Study Staff

Name	Role	Degree	Organization
[redacted]	Principal Investigator	MD	[redacted] > [redacted] >
[redacted]	Co-Investigator		Partners
[redacted]	Research Technician		[redacted] > [redacted] >
[redacted]	Co-Investigator		[redacted] > [redacted] >

Linked Agreements

Record #	Fund	Project Period	PI Name	Sponsor	Record Type	Process	Link Date	Link Status
[redacted]		04/01/10-03/31/16	[redacted]	[redacted] ECOR Interim Support Fund	RM – Funded Agreement	IR	06/11/08	Approved
[redacted]		09/01/16-08/31/18	[redacted]	[redacted]	RM – Funded Agreement	AME19	05/02/16	Approved
[redacted]		11/01/08-02/28/11	[redacted]	[redacted]	RM – Funded Agreement	IR	06/11/08	Approved
[redacted]		06/21/10-05/31/12	[redacted]	[redacted]	RM – Funded Agreement	IR	06/11/08	Approved
[redacted]		04/01/11-03/31/12	[redacted]	[redacted]	RM – Funded Agreement	IR	06/11/08	Approved
[redacted]		06/15/11-06/20/16	[redacted]	[redacted]	RM – Funded Agreement	TR2	01/28/14	Approved

[redacted]

Record #	Fund	Project Period	PI Name	Sponsor	Record Type	Process	Link Date	Link Status
[redacted]		[redacted]		[redacted]	RM – Funded Agreement	IR	06/11/08	Approved
[redacted]		06/21/16-05/31/21	[redacted]	[redacted]	RM – Funded Agreement	AME17	11/18/15	Approved

Linked Protocols

Protocol #	Relationship	Link Location	Overall Status	PI Name	Title	Process	Link Date	Link Status	Link Direction
[redacted]	Biosafety	Citrobacter rodentium, Salmonella enterica, Use of Animals	Active	Cherayil, [redacted] J	[redacted]	AME21/LR6	01/09/19	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 overall objective of the study is to find out how inflammation (an excessive, uncontrolled response by white blood cells of the immune system) in the gastrointestinal tract affects the handling of iron, an important micro-nutrient, and how iron status, including changes in the amount of iron in the diet, affects the immune response.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Detailed Research Plan, section A. Goals**. 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

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

The overall goals of the study are:

A. To clarify the mechanisms that regulate iron metabolism in different mouse models of colitis.

B. To clarify how alterations in dietary iron content affect immune responses to infection and vaccination.

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.]**

Many human diseases that are characterized by long-standing inflammation of various tissues lead to decreased production of red blood cells (anemia). The anemia is the result of abnormal handling of iron, a micro-nutrient that is an essential component of hemoglobin, the oxygen-carrying protein found in red blood cells. Our experiments will help to clarify how inflammation leads to abnormal iron handling. This information will be useful in developing new ways to treat the anemia associated with inflammation. Alterations in iron status that result from either inherited abnormalities of iron metabolism or from changes in dietary iron can have a significant impact on immune responses. For example, our earlier work has shown that mice with a genetic defect in sensing circulating iron levels do not respond normally to infection with the bacterial pathogen Salmonella Typhimurium. Our experiments will help us to understand the mechanisms underlying the effects of iron status on the immune response. This information will be useful in devising ways to correct the abnormalities.

Migrated Data

This field may contain information that has been migrated from **Insight 3.6.4, Detailed Research Plan, section B. Background**. 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 **Benefit to be Gained** question above. ***Use of this information is optional.***

Many chronic inflammatory conditions such as inflammatory bowel disease (IBD) are associated with abnormalities of iron metabolism that lead to a type of anemia that is particularly resistant to treatment. An important factor that contributes to the development of this anemia is abnormal expression or function of various molecules involved in iron metabolism, including the hormone hepcidin. The mechanisms that lead to dysregulation of these molecules in the context of inflammation are not well understood. In our earlier work, we focused on the changes in expression of hepcidin induced by

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intestinal inflammation and characterized various factors tha hepcidin expression, e.g., the cytokines TNFalpha and IL-1beta, as well as commensal microbiota composition and erythropoietic activity. We would now like to extend these studies to identify additional factors that alter expression and function of hepcidin and other iron metabolism molecules during intestinal inflammation and further elucidate the mechanisms underlying the effects of TNFalpha, IL-1beta, erythropoiesis and the microbiota. We would also like to analyze the effects of changes in dietary iron content on the immune response to infection and vaccination.

Importance of the work: By clarifying the mechanisms that lead to abnormal iron metabolism during intestinal inflammation, it may be possible to devise strategies to correct the abnormalities and thus prevent or treat the anemia associated with diseases such as IBD. By clarifying the mechanisms that mediate the effects of altered iron status on immune function, we will have a better understanding of how iron deficiency and iron overload affect susceptibility to infectious disease and vaccine responses.

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**]

The overall goals of the study are:

A. To clarify the mechanisms that regulate iron metabolism in different mouse models of colitis.

B. To clarify how alterations in dietary iron content affect immune responses to infection and vaccination.

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. The goal of this experiment is to characterize the effects of DSS colitis on hepcidin expression and other molecules involved in iron metabolism

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in wild-type and IL-10-deficient C57BL/6 mice. Two groups of 6 week old wild-type C57BL/6 mice and two groups of the IL-10 knockout mice will be used, each group consisting of 30 mice, with roughly equal numbers of males and females. The mice will be bred at [redacted] and littermates (i.e., the progeny of the same IL-10 KO heterozygote x IL-10 KO heterozygote breeders) will be used if possible. Both homozygous wild-type and heterozygotes will be used in the wild-type groups. One group of each strain will be given regular water ad libitum while the other group of each strain will be given drinking water containing 3% w/v dextran sulfate sodium (DSS) ad libitum. The cages with DSS-containing drinking water will be marked with Special Husbandry cards indicating that study staff will be responsible for providing the water. The health status of the mice is expected to remain good, although the animals may experience mild, transient weight loss, softening of the stools and mild to moderate discomfort as a result of the colitis induced by the DSS treatment. The colitis is not severe enough to cause bloody diarrhea or dehydration. The mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. No analgesics will be provided since they will interfere with our analysis. However, if any animal appears to be in significant distress (as indicated by hunched posture, poor activity, ruffled, ungroomed fur) or experiences body weight loss that is more than 15% relative to the age- and sex-matched control mice, it will be euthanized immediately by controlled flow carbon dioxide asphyxia. In rare instances, the IL-10 KO mice may develop rectal prolapse in association with the colitis, in which case the affected animal will be euthanized immediately by controlled flow carbon dioxide asphyxia. The supply of DSS-containing water will also be monitored and will be replenished twice weekly or more frequently if required. The DSS treatment will be stopped on day 7, and 15 mice from each group will be euthanized by controlled flow carbon dioxide asphyxia at this time point in order to collect tissue samples for assessment of the effects of the intestinal inflammation on parameters of iron metabolism (serum and tissue iron, expression of hepcidin and other molecules, serum erythropoietin levels, splenic extra-medullary erythropoiesis, etc.). From day 7 to day 21 all the remaining mice will receive regular drinking water ad libitum to allow recovery from the colitis. Study staff will continue to monitor the health status of the mice as above. All mice will be euthanized by controlled flow carbon dioxide asphyxia and tissue samples collected to assess iron metabolism parameters as above. Total number of mice for this experiment: 120 (60 category C, 60 category E).

Experiment 2. The goal of this experiment is to determine the requirement for macrophages in the alterations of iron metabolism associated with intestinal inflammation. Three groups of 6 week old wild-type C57BL/6 mice (obtained from Jackson) will be used, with 15 mice in each group and roughly equal

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numbers of males and females per group. One group will serve as the control and will not be subjected to any intervention or treatment. The other 2 groups will be injected intraperitoneally on day 1 with 0.2 ml of a liposomal formulation of either the drug clodronate or the vehicle PBS. Clodronate is taken up by macrophages and inactivates these cells. On day 3, the 2 groups that received the liposomes will be given 3% w/v DSS in their drinking water ad libitum, while the control group will remain on regular drinking water. The cages with DSS-containing drinking water will be marked with Special Husbandry cards indicating that study staff will be responsible for providing the water. The health status of the mice is expected to remain good, although the animals may experience mild, transient weight loss, softening of the stools and mild to moderate discomfort as a result of the colitis induced by the DSS treatment. The colitis is not severe enough to cause bloody diarrhea or dehydration. The mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. No analgesics will be provided since they will interfere with our analysis. However, if any animal appears to be in significant distress (as indicated by hunched posture, poor activity, ruffled, ungroomed fur) or experiences body weight loss that is more than 15% relative to the control mice, it will be euthanized immediately by controlled flow carbon dioxide asphyxia. The supply of DSS-containing water will also be monitored and will be replenished twice weekly or more frequently if required. All the mice will be euthanized by controlled flow carbon dioxide asphyxia on day 10 and tissues collected to analyze various parameters of iron metabolism as described in Experiment 1. Total number of mice for this experiment: 45 (15 category C, 30 category E).

Experiment 3. The goal of this experiment is to characterize the effects of trinitrobenzene sulfonic acid (TNBS)-induced colitis on the expression of hepcidin and other molecules involved in iron metabolism in wild-type and IL-10 KO C57BL/6 mice. Two groups of 6 week old wild-type C57BL/6 mice and two groups of the IL-10 knockout mice will be used, each group consisting of 30 mice, with roughly equal numbers of males and females. The mice will be bred at [redacted] and littermates (i.e., the progeny of the same IL-10 KO heterozygote x IL-10 KO heterozygote breeders) will be used if possible. Both homozygous wild-type and heterozygotes will be used in the wild-type groups. Using brief manual restrain, each animal will be anesthetized by intra-peritoneal injection of Avertin (tribromoethanol), 250 mg/kg body weight. Tribromoethanol provides rapid, reliable anesthesia with prompt, uncomplicated recovery and has no known effects on the TNBS-induced intestinal inflammation. It will be used only once in any given animal so it is unlikely to cause peritoneal adhesions or ileus. Although pharmaceutical grade tribromoethanol is no longer available, it will be prepared using the highest purity reagents available, and will be stored and used according to IACUC

[redacted]

guidelines. We cannot use isoflurane or ketamine/xylazine anesthesia for this experiment because isoflurane has been reported to reduce gut motility (e.g., [redacted] et al., [redacted]. 2005; 294: 65, [redacted] et al., [redacted]. 2014; 26: 1477) and both isoflurane and ketamine have been shown to have significant attenuating effects on inflammation in various tissues, including the intestine ([redacted] et al., [redacted]. 2004; 10: 1028, [redacted] et al., [redacted]. 2010; 111: 1051, [redacted] et al., [redacted]; 178: e17, [redacted] et al., [redacted] 2013; 119: 901, [redacted] et al., [redacted]. Res. 2015; 194: 599). These potential side effects of isoflurane and ketamine/xylazine anesthesia could adversely affect the outcome of our experiment. The level of anesthesia will be assessed by lack of limb withdrawal on toe pinch (usually within a few minutes), and also by visual monitoring of mucous membrane color, capillary reflex, and respiratory rate, rhythm and tidal volume. Once a stable and adequate level of anesthesia has been achieved, the mouse will be placed on its back and an epidural catheter (FlexTip Plus, Arrow International) will be inserted into the rectum and gently advanced into the colon for a distance of 4 cm. One hundred microliters of 2.5% w/v TNBS in 50% ethanol or just 50% ethanol without TNBS will be instilled through the catheter. After instillation, the catheter will be withdrawn and the mice will be held in a head-down position for 1 minute to minimize loss of the instilled material. They will then be returned to their cages and monitored until they recover the righting reflex (usually 30-40 minutes). The health status of the mice is expected to remain good, although the animals may experience mild, transient weight loss, softening of the stools and mild to moderate discomfort as a result of the colitis induced by the TNBS. The colitis is not severe enough to cause bloody diarrhea or dehydration. The mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. No analgesics will be provided since they will interfere with our analysis. However, if any animal appears to be in significant distress (as indicated by hunched posture, poor activity, ruffled, ungroomed fur) or experiences body weight loss that is more than 15% relative to the age- and sex-matched control mice, it will be euthanized immediately by controlled flow carbon dioxide asphyxia. In rare instances, the IL-10 KO mice may develop rectal prolapse in association with the colitis, in which case the affected animal will be euthanized immediately by controlled flow carbon dioxide asphyxia. Fifteen mice from each group will be euthanized by controlled flow carbon dioxide asphyxia on days 7 and 14 after TNBS administration in order to collect tissue samples for assessment of the effects of the intestinal inflammation on parameters of iron metabolism as described in Experiment 1. Total number of mice for this experiment: 120 (60 category C, 60 category E).

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Experiment 4. The goal of this experiment is to characterize the effects of T cell transfer-induced colitis on the expression of hepcidin and other molecules involved in iron metabolism. Two groups of 6 week old, T cell-deficient Rag2 knockout mice (bred at [redacted] 30 mice per group with roughly equal numbers of males and females, will be used. One group will be injected intravenously with 0.5 million CD4+CD45RBhi T cells suspended in 0.2 ml of sterile PBS. The other group (control) will be injected intravenously with 0.2 ml of just PBS, without cells. The CD4+CD45RBhi T cells will be purified from the spleens of female wild-type C57BL/6 mice (obtained from Jackson). CD4+CD45RBhi T cells from female mice have to be used because male cells express the sex-specific H-Y antigen. If H-Y-expressing male cells are transferred into female recipients, an immune response to the male antigen could result in rejection of the transferred cells. Since female cells do not express the H-Y antigen, they will not be rejected by either male or female recipients. Based on a yield of 1 million of CD4+CD45RBhi T cells per mouse, 15 female mice will be required to provide enough of these cells to carry out the experiment. The health status of the mice is expected to remain good, although the animals may experience mild, transient weight loss, softening of the stools and mild to moderate discomfort as a result of the colitis induced by the T cell transfer. The colitis is not severe enough to cause bloody diarrhea or dehydration. The mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. No analgesics will be provided since they will interfere with our analysis. However, if any animal appears to be in significant distress (as indicated by hunched posture, poor activity, ruffled, ungroomed fur) or experiences body weight loss that is more than 15% relative to the age- and sex-matched control mice, it will be euthanized immediately by controlled flow carbon dioxide asphyxia. Fifteen mice from each group will be euthanized by controlled flow carbon dioxide asphyxia at each of 2 time points, i.e., 6 and 8 weeks after the T cell transfer. At necropsy, tissue samples will be collected to assess parameters of iron metabolism as described in Experiment 1. Total number of mice for this experiment: 75 (45 category C, 30 category E).

Experiment 5. The goal of this experiment is to determine the effects of dietary iron on the expression of hepcidin and other molecules involved in iron metabolism. Accordingly, 3 groups of 6 week old wild-type male C57BL/6 mice (Jackson, 15 mice per group, equal numbers of males and female per group) will be placed on ad libitum amounts of standard chow, iron-deficient chow (Harlan-Teklad, 2-6 ppm of elemental iron) or iron-supplemented chow (Harlan-Teklad, 225 mg of elemental iron per kg chow) on day 1. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special chows. No adverse effects of the diets on health are expected. Nevertheless, the mice will be monitored closely

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by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. On day 14, all the mice will be euthanized by controlled flow carbon dioxide asphyxia and tissue samples collected for analysis of iron metabolism parameters as described in Experiment 1. Total number of mice for this experiment: 45 (all category C).

Experiment 6. The goal of this experiment is to determine the effect of a low-protein, low-fat diet on the expression of hepcidin and other molecules involved in iron metabolism. The experiment will begin with 2 groups of 3-4 week old, wild-type C57BL/6 mice (purchased from the Jackson Laboratory), 15 mice per group, with similar numbers of males and females in each group. On day 1, one group of mice will be placed on a low-protein, low-fat diet (6.5 gms% protein, 2.1 gms% fat, 81.9 gms% carbohydrate). As a control, the other group will be placed on a standard diet (19 gms% protein, 6.5 gms% fat, 63.1 gms% carbohydrate). Both diets provide the same amount of total calories (3.77 kcal/gm) and will be given to the mice ad libitum. The diets will be purchased from Research Diets, Inc., New Brunswick, NJ in the form of sterile irradiated pellets. These diets have been used previously in published experiments and data from those studies indicate that the mice on the low-protein, low-fat diet will grow at a reduced rate compared to the control mice (about 10% less weight gain over 1 month) but will otherwise remain healthy. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special diets. The amount of the food pellets in each cage will be monitored daily and will be replenished as needed. No adverse effects of the diets on health are expected. Nevertheless, the mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. On day 28, all the mice will be euthanized by controlled flow carbon dioxide asphyxia and tissue samples collected for analysis of iron metabolism parameters as described in Experiment 1. Total number of mice for this experiment: 30 (all category C).

Experiment 7. The goal of this experiment is to determine the effect of the gut commensal microbiota on the expression of hepcidin and other molecules involved in iron metabolism. The first group will not receive any treatment and will serve as the control. The second group will receive ad libitum medicated drinking water containing a mix of 4 different antibiotics (ampicillin 1 gram/liter, neomycin 1 gram/liter, metronidazole 1 gram/liter and vancomycin 0.5 gram/liter). This antibiotic mix has been shown by other investigators to

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reduce the total quantity of commensals by about 90% and to alter commensal composition. The cages with medicated drinking water will be marked with Special Husbandry cards indicating that study staff will be responsible for providing the water. No adverse effects of the antibiotics on health are expected. Nevertheless, the mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur,actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. The supply of medicated water will also be monitored and will be replenished twice weekly or more frequently if required. On day 14, all the mice will be euthanized by controlled flow carbon dioxide asphyxia and tissue samples collected for analysis of iron metabolism parameters as described in Experiment 1. Total number of mice for this experiment: 30 (all category C).

Experiment 8. The goal of this experiment is to determine the effect of dietary iron concentration on the immune response to oral infection by the rodent Gram-negative bacterial enteropathogen Citrobacter rodentium. Accordingly, 3 groups of wild-type C57BL/6 mice (Jackson, 15 mice per group, approximately equal numbers of males and females per group) will be placed on ad libitum amounts of standard chow, iron-deficient chow (Harlan-Teklad, 2-6 ppm of elemental iron) or iron-supplemented chow (Harlan-Teklad, 225 mg of elemental iron per kg chow) on day 1. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special chows. On day 14, all the mice will be transferred to the BL2 room (CNY114-[redacted] and infected by oral gavage with a single dose of 500 million colony forming units of Citrobacter rodentium strain DBS100 in 0.2 ml PBS per mouse. The mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. The health status of the mice is expected to remain good, although the animals may experience mild, transient weight loss, softening of the stools and mild to moderate discomfort as a result of the colitis induced by the Citrobacter infection. The colitis is not severe enough to cause bloody diarrhea or dehydration. No analgesics will be provided since they will interfere with our analysis. However, if any animal appears to be in significant distress (as indicated by hunched posture, poor activity, ruffled, ungroomed fur) or experiences body weight loss that is more than 15% relative to the weight of age- and sex-matched control mice on standard mouse growth charts, it will be euthanized immediately by controlled flow carbon dioxide asphyxia. Otherwise, all the mice will be euthanized on day 28 by controlled flow carbon dioxide asphyxia. At necropsy, the colon will be excised to evaluate the degree of intestinal inflammation

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based on histology and analysis of gene expression by ELISA and qRT-PCR. Mesenteric lymph node and spleen will be excised to prepare single cell suspensions for analysis by flow cytometry and to prepare RNA for gene expression analysis. Total number of mice for this experiment: 45 (all category E).

Experiment 9. The goal of this experiment is to determine the effect of dietary iron concentration on the immune response to oral infection by the Gram-negative bacterial enteropathogen Salmonella enterica serovar Typhimurium (Salmonella Typhimurium). Accordingly, 3 groups of wild-type C57BL/6 mice (Jackson, 15 mice per group, approximately equal numbers of males and females per group) will be placed on ad libitum amounts of standard chow, iron-deficient chow (Harlan-Teklad, 2-6 ppm of elemental iron) or iron-supplemented chow (Harlan-Teklad, 225 mg of elemental iron per kg chow) on day 1. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special chows. On day 14, all mice will be given a single 20 mg dose of streptomycin dissolved in water by oral gavage in order to facilitate Salmonella infection. On day 15, all the mice will be transferred to the BL2 room (CNY114-[redacted] and infected by oral gavage with a single dose of 100 million colony forming units of Salmonella Typhimurium strain SL1344 in 0.2 ml PBS per mouse. The mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. The health status of the mice is expected to remain good, although the animals may experience mild, transient weight loss, softening of the stools and mild to moderate discomfort as a result of the intestinal inflammation induced by the Salmonella infection. The intestinal inflammation is not severe enough to cause bloody diarrhea or dehydration. No analgesics will be provided since they will interfere with our analysis. However, if any animal appears to be in significant distress (as indicated by hunched posture, poor activity, ruffled, ungroomed fur) or experiences body weight loss that is more than 15% relative to the weight of age- and sex-matched control mice on standard mouse growth charts, it will be euthanized immediately by controlled flow carbon dioxide asphyxia. Otherwise, all the mice will be euthanized on day 17 by controlled flow carbon dioxide asphyxia. At necropsy, the ileum, cecum and colon will be excised to evaluate the degree of intestinal inflammation based on histology and analysis of gene expression by ELISA and qRT-PCR. Mesenteric lymph node and spleen will be excised to prepare single cell suspensions for analysis by flow cytometry and to prepare RNA for gene expression analysis. Total number of mice for this experiment: 45 (all category E).

Experiment 10. The goal of this experiment is to determine the effect of dietary iron concentration on the immune response to parenteral infection by

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Salmonella Typhimurium. Accordingly, 3 groups of wild-type C57BL/6 mice (Jackson, 15 mice per group, approximately equal numbers of males and females per group) will be placed on ad libitum amounts of standard chow, iron-deficient chow (Harlan-Teklad, 2-6 ppm of elemental iron) or iron-supplemented chow (Harlan-Teklad, 225 mg of elemental iron per kg chow) on day 1. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special chows. On day 15, all the mice will be transferred to the BL2 room ([redacted] and infected by intraperitoneal injection with a single dose of 1000 colony forming units of Salmonella Typhimurium strain SL1344 in 0.2 ml PBS per mouse. The mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. The health status of the mice is expected to remain good, although the animals may experience mild, transient weight loss and mild to moderate discomfort as a result of the Salmonella infection. No analgesics will be provided since they will interfere with our analysis. However, if any animal appears to be in significant distress (as indicated by hunched posture, poor activity, ruffled, ungroomed fur) or experiences body weight loss that is more than 15% relative to the weight of age- and sex-matched control mice on standard mouse growth charts, it will be euthanized immediately by controlled flow carbon dioxide asphyxia. Otherwise, all the mice will be euthanized on day 19 by controlled flow carbon dioxide asphyxia. At necropsy, the ileum, cecum and colon will be excised to evaluate the degree of intestinal inflammation based on histology and analysis of gene expression by ELISA and qRT-PCR. Mesenteric lymph node and spleen will be excised to prepare single cell suspensions for analysis by flow cytometry and to prepare RNA for gene expression analysis. Total number of mice for this experiment: 45 (all category E).

Experiment 11. The goal of this experiment is to determine the effect of dietary iron concentration on the immune response to intraperitoneal immunization with a model vaccine antigen. Accordingly, 3 groups of wild-type C57BL/6 mice (Jackson, 15 mice per group, approximately equal numbers of males and females per group) will be placed on ad libitum amounts of standard chow, iron-deficient chow (Harlan-Teklad, 2-6 ppm of elemental iron) or iron-supplemented chow (Harlan-Teklad, 225 mg of elemental iron per kg chow) on day 1. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special chows. On day 14, tail vein blood samples will be collected from all the mice to assess pre-immunization antibody levels. On day 15, all the mice will be immunized by intraperitoneal injection with the model vaccine antigen ovalbumin (50 micrograms) mixed with the adjuvant lipopolysaccharide (LPS, 200 ng) in 0.2 ml PBS. A booster immunization will be administered in exactly the same way on day 30. All the mice will be euthanized by controlled flow carbon dioxide

[redacted]

asphyxia on day 45 and blood and splenocyte samples will be collected to assess antibody and T cell responses to ovalbumin. The health status of the mice is expected to remain good during the entire experiment and no adverse effects are expected apart from the transient discomfort of the injections and tail vein blood sampling. The intraperitoneal injection of ovalbumin and LPS does not cause ascites. Nevertheless, the mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. Total number of mice for this experiment: 45 (all category C).

Experiment 12. The goal of this experiment is to determine the effect of dietary iron concentration on the immune response to subcutaneous immunization with a model vaccine antigen. Accordingly, 3 groups of wild-type C57BL/6 mice (Jackson, 15 mice per group, approximately equal numbers of males and females per group) will be placed on ad libitum amounts of standard chow, iron-deficient chow (Harlan-Teklad, 2-6 ppm of elemental iron) or iron-supplemented chow (Harlan-Teklad, 225 mg of elemental iron per kg chow) on day 1. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special chows. On day 14, tail vein blood samples will be collected from all the mice to assess pre-immunization antibody levels. On day 15, all the mice will be immunized by subcutaneous injection (on the back adjacent to the base of the tail) with the model vaccine antigen ovalbumin (50 micrograms) mixed with the adjuvant lipopolysaccharide (LPS, 200 ng) in 0.2 ml PBS. A booster immunization will be administered in exactly the same way on day 30. All the mice will be euthanized by controlled flow carbon dioxide asphyxia on day 45 and blood and splenocyte samples will be collected to assess antibody and T cell responses to ovalbumin. The health status of the mice is expected to remain good during the entire experiment and no adverse effects are expected apart from the transient discomfort of the injections and tail vein blood sampling. Nevertheless, the mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. Total number of mice for this experiment: 45 (all category C).

Experiment 13. The goal of this experiment is to determine the effect of dietary iron concentration on the immune response to intraperitoneal immunization with a model vaccine antigen using alum adjuvant. Accordingly, 3 groups of wild-type C57BL/6 mice (Jackson, 15 mice per group, approximately equal numbers of males and females per group) will be placed

[redacted]

on ad libitum amounts of standard chow, iron-deficient chow (Harlan-Teklad, 2-6 ppm of elemental iron) or iron-supplemented chow (Harlan-Teklad, 225 mg of elemental iron per kg chow) on day 1. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special chows. On day 14, tail vein blood samples will be collected from all the mice to assess pre-immunization antibody levels. On day 15, all the mice will be immunized by intraperitoneal injection with the model vaccine antigen ovalbumin (50 micrograms) mixed with alum adjuvant (100 micrograms). Total volume of the antigen-alum mix will be no more than 200 microliters. A booster immunization will be administered in exactly the same way on day 30. All the mice will be euthanized by controlled flow carbon dioxide asphyxia on day 45 and blood and splenocyte samples will be collected to assess antibody and T cell responses to ovalbumin. The health status of the mice is expected to remain good during the entire experiment and no adverse effects are expected apart from the transient discomfort of the injections and tail vein blood sampling. Nevertheless, the mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. Total number of mice for this experiment: 45 (all category C).

Experiment 14. The goal of this experiment is to determine the effect of dietary iron concentration on the immune response to oral immunization with a model vaccine antigen. Accordingly, 3 groups of wild-type C57BL/6 mice (Jackson, 15 mice per group, approximately equal numbers of males and females per group) will be placed on ad libitum amounts of standard chow, iron-deficient chow (Harlan-Teklad, 2-6 ppm of elemental iron) or iron-supplemented chow (Harlan-Teklad, 225 mg of elemental iron per kg chow) on day 1. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special chows. On day 14, tail vein blood samples will be collected from all the mice to assess pre-immunization antibody levels. On day 15, all the mice will be immunized by oral gavage with the model vaccine antigen ovalbumin (25 mg) mixed with the oral adjuvant cholera toxin (10 micrograms) in 0.2 ml PBS. The oral immunization will be repeated in exactly the same way on days 18, 21 and 35. All the mice will be euthanized by controlled flow carbon dioxide asphyxia on day 50 and blood, mesenteric lymph node and splenocyte samples will be collected to assess antibody and T cell responses to ovalbumin. The health status of the mice is expected to remain good during the entire experiment and no adverse effects are expected apart from the transient discomfort of the oral gavage and tail vein blood sampling. Nevertheless, the mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or

[redacted]

greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. Total number of mice for this experiment: 45 (all category C).

Experiment 15. The goal of this experiment is to determine the effect of dietary iron concentration on the protection induced by a live oral vaccine. Accordingly, 3 groups of wild-type C57BL/6 mice (Jackson, 15 mice per group, approximately equal numbers of males and females per group) will be placed on ad libitum amounts of standard chow, iron-deficient chow (Harlan-Teklad, 2-6 ppm of elemental iron) or iron-supplemented chow (Harlan-Teklad, 225 mg of elemental iron per kg chow) on day 1. The cages will be marked with a Special Husbandry card indicating that the study staff will be responsible for providing the special chows. On day 14, tail vein blood samples will be collected from all the mice to assess pre-immunization antibody levels. On day 15, all mice will be given a single 20 mg dose of streptomycin dissolved in water by oral gavage in order to facilitate Salmonella infection. On day 16, all the mice will be transferred to the BL2 room (CNY114-[redacted] and infected by oral gavage with a single dose of 10 million colony forming units of the attenuated vaccine strain of Salmonella Typhimurium (strain SL3261) in 0.2 ml PBS per mouse. Since SL3261 is an attenuated strain, the infection does not progress and is generally cleared over the course of 2-3 weeks. On day 37, all the mice will be challenged by infecting them by oral gavage with a single dose of 10 million colony forming units of the wild-type Salmonella Typhimurium strain SL1344 in 0.2 ml PBS per mouse. The mice will be monitored closely by study staff during the entire experiment with once daily recording of body weight and clinical condition (activity, grooming, stool consistency). If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to twice daily. The health status of the mice is expected to remain good, although the animals may experience mild, transient weight loss, softening of the stools and mild to moderate discomfort as a result of the Salmonella infection. No analgesics will be provided since they will interfere with our analysis. However, if any animal appears to be in significant distress (as indicated by hunched posture, poor activity, ruffled, ungroomed fur) or experiences body weight loss that is more than 15% relative to the weight of age- and sex-matched control mice on standard mouse growth charts, it will be euthanized immediately by controlled flow carbon dioxide asphyxia. Otherwise, all the mice will be euthanized on day 42 by controlled flow carbon dioxide asphyxia. At necropsy, the ileum, cecum and colon will be excised to evaluate the degree of intestinal inflammation based on histology and analysis of gene expression by ELISA and qRT-PCR. Mesenteric lymph node, liver and spleen will be excised to determine the tissue burden of S. Typhimurium, to prepare single cell suspensions for analysis by flow cytometry and to prepare RNA for gene expression analysis. Serum antibody levels and splenocyte T cell

responses to Salmonella antigens will also be assessed. Total number of mice for this experiment: 45 (all category E).

Experiment 16. Preparation of peritoneal macrophages. Our research focus is on macrophage function so we have a continuing need to isolate macrophages from mice in order to study various aspects of their inflammatory and anti-microbial responses. When macrophages are required, the mice (wild-type or knockout strains) will be injected intraperitoneally with 1.5 ml of sterile 3% thioglycollate solution using a 27 gauge needle. The thioglycollate is used to increase the yield of macrophages. Only transient discomfort will be experienced during this procedure. The health status of the mice is expected to remain good throughout the experiment and no adverse effects of the thioglycollate injection are expected. The mice do not develop enlarged abdomens and do not display any change in appearance or behavior that would indicate pain or distress. The clinical condition of the mice will be monitored every other day by the study staff. If abnormal clinical signs are observed (slightly disheveled fur, actively moving around the cage but at a reduced rate, weight loss of 5% or greater relative to age- and sex-matched control mice from the same experiment or from standard mouse growth charts), the frequency of monitoring will be increased to once or twice daily as needed. On the fourth day after the injection, the mice will be euthanized by controlled flow carbon dioxide asphyxia. A midline abdominal incision will be made and the skin reflected to expose the peritoneal membrane. About 8 ml of sterile PBS will be injected into the peritoneal cavity and then withdrawn with the peritoneal macrophages. The macrophages will be used to carry out in vitro assays of inflammatory cytokine production and/or anti-microbial function (phagocytosis, bacterial killing, etc.). Each such assay is typically carried out using multiple stimuli as well as control conditions and requires about 15 million macrophages. Since each adult mouse yields about 3 million peritoneal macrophages in our hands, we will require 5 mice for each assay. We usually carry out the assay about once each month based on the needs of our studies. Therefore, we will require 5 x 12 = 60 mice per year, and thus 180 mice over a 3 year period (all category C).

Breeding of mice. Colonies of wild-type (C57BL/6), IL-10 knockout and Rag2 knockout (both on C57BL/6 background) will be maintained by breeding. In addition, IL-10 knockout heterozygote x IL-10 knockout heterozygote breeding pairs will also be maintained in order to obtain wild-type and knockout littermates. To maintain an adequate supply of the required mice, we will use 2 breeding pairs of each of the 4 relevant strains per year, i.e., 16 mice per year for breeding. Therefore, during the 3 year period of the protocol, we will use 48 mice for breeding purposes. Each breeding pair is expected to give birth to litters of 4-6 pups. The pups are not expected to have any phenotypic abnormalities. They will be weaned and separated from the parents at 3 weeks of age. In the case of the IL-10 knockout heterozygote breeders, the pups will be subjected to tail snips at 3 weeks of age for the purpose of genotyping. The genotyping will identify homozygous wild-type, homozygous IL-10 knockout and heterozygous mice. All 3 types will be used and therefore no culling of

[redacted]

pups will be required. In all the other breedings, since we are starting with homozygous wild-type or knockout parents, all the progeny will be homozygotes. Therefore, they will not require tail snips for genotyping or culling. We will use the CCM-provied Labtracks software to keep track of breeding pairs, weaning dates, etc. Breeders will be euthanized by controlled flow carbon dioxide asphyxia once they have reached the age of 1 year. Total number of mice for this experiment: 48 (all category C).

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.***

Varies with experiment (see Research Plan and Flow Charts), but minimum will be 4 days and maximum will be 8 weeks.

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.

As detailed under the Research Plan, the general health status of the mice is expected to remain good throughout the course of the experiments. The animals are not expected to experience significant pain beyond the transient discomfort of injections or oral gavage. The induction of colitis or infection with Salmonella or Citrobacter may be associated with mild discomfort as evidenced

[redacted]

by less vigorous activity, some loss of weight and soft stools. The colitis is not severe enough to cause bloody diarrhea or dehydration. In rare instances, IL-10 knockout mice may experience rectal prolapse, in which case the affected animal will be euthanized immediately by controlled flow carbon dioxide asphyxia. In addition, any animal that experiences body weight loss that is more than 15% relative to control mice of the same age and sex (measured simultaneously from a control group in that experiment or derived from standard mouse growth charts) or that appears to be in more than mild discomfort, as indicated by hunched posture, poor grooming or poor activity, will be euthanized immediately.

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

All experimental procedures involve only transient or mild discomfort, so no analgesics will be used. The use of analgesics would also interfere with the results of the experiments. Analgesia with non-steroidal anti-inflammatory agents cannot be used because of the well known effects of such agents on immunological parameters. Opioid analgesics are also not an option since opioid receptors are expressed on many cells of the immune system, including macrophages, and opioids have been shown to affect the functions of these cells (e.g., [redacted] et al., [redacted]. 2015; 194: 1021, [redacted]i et al., [redacted]. 2015; 1230: 253, [redacted] et al., [redacted]. 2016 Feb 19;6:21094. doi: 10.1038/srep21094). Since our experiments are directed at characterizing immune responses in the various animal models that are used, administering opioids or non-steroidal anti-inflammatory agents would affect the outcome of the experiments. The animals will be assessed as described above.

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.***

The investigators will be responsible for follow-up care. Plans for monitoring the mice have been detailed above in the descriptions of the experimental procedures. In general, the mice will be monitored every other day. In experiments involving induction of colitis or infection with Salmonella or Citrobacter, the mice will be monitored every day. In addition to recording body weight every day, the animals' activity, grooming and consistency of stool will be noted. The frequency of monitoring will be increased to twice a day if any of the following abnormal clinical signs is observed: (i) slightly disheveled fur, (ii) actively moving around the cage but at reduced rate, (iii) weight loss of 5% or greater relative to age- and sex-matched control mice (either from the same experiment or from standard mouse growth charts). Any

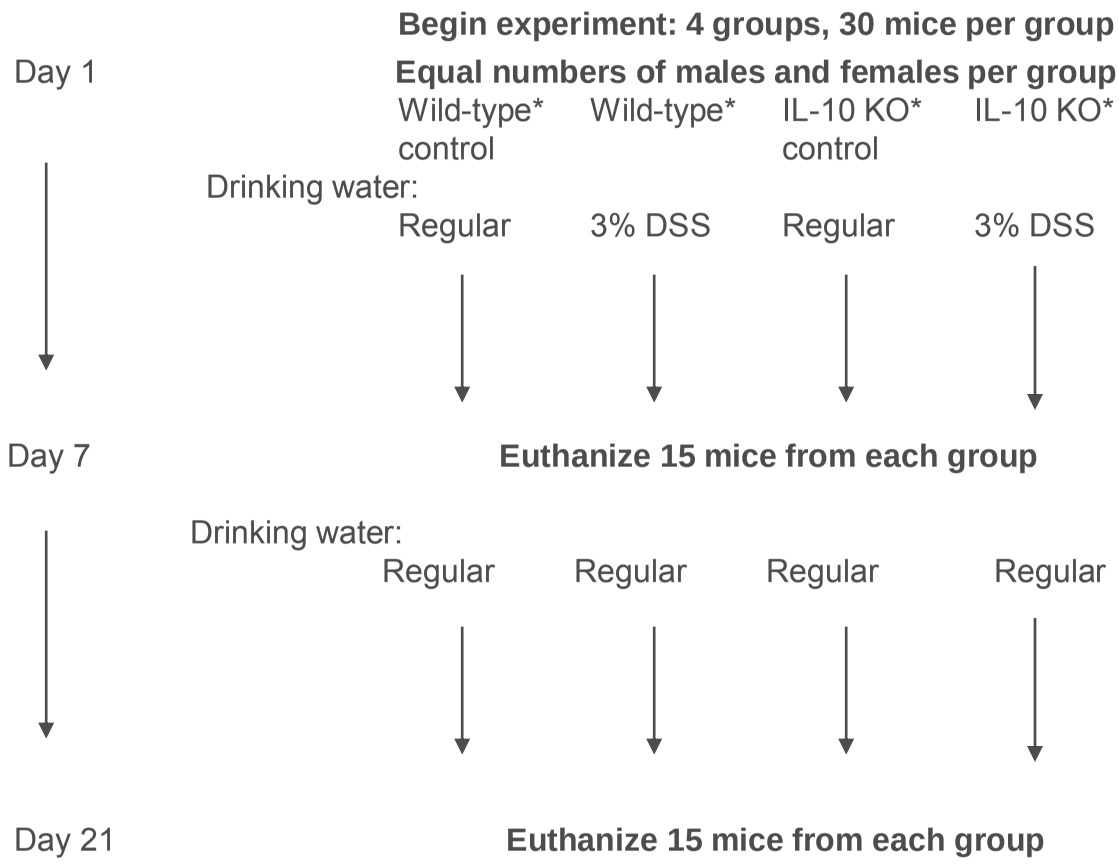
[redacted]

animal that experiences body weight loss that is more than 15% relative to control mice of the same age and sex (measured simultaneously from a control group in that experiment or derived from standard mouse growth charts) or that appears to be in more than mild discomfort, as indicated by hunched posture, poor grooming or poor activity, will be euthanized immediately.

Attachments

Name	Mode
FlowChartsDec2016 (Flowchart)	Electronic

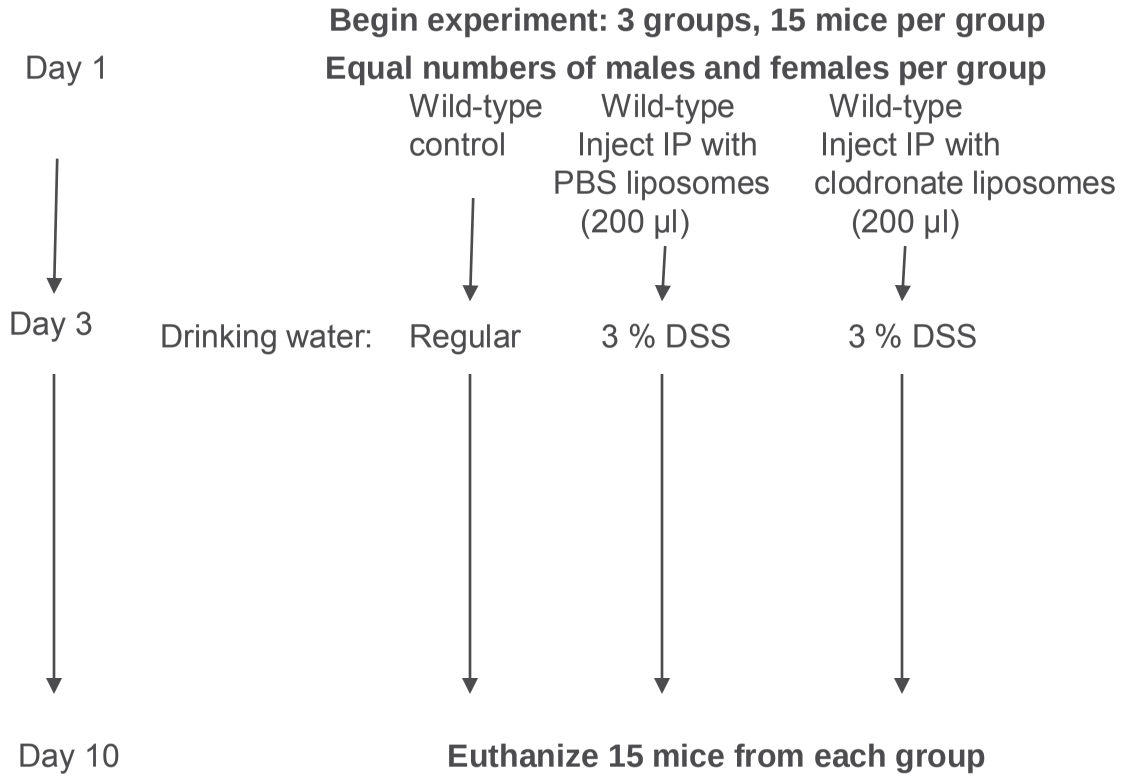
EXPERIMENT 1



*When possible, the wild-type and IL-10 KO mice will be derived from the same litter, i.e., the progeny of the same IL-10 KO heterozygote x IL-10 KO heterozygote breeders. Both homozygous wild-type and heterozygous mice will be used in the wild-type groups since both behave like wild-type. The IL-10 KO mice will be homozygotes.

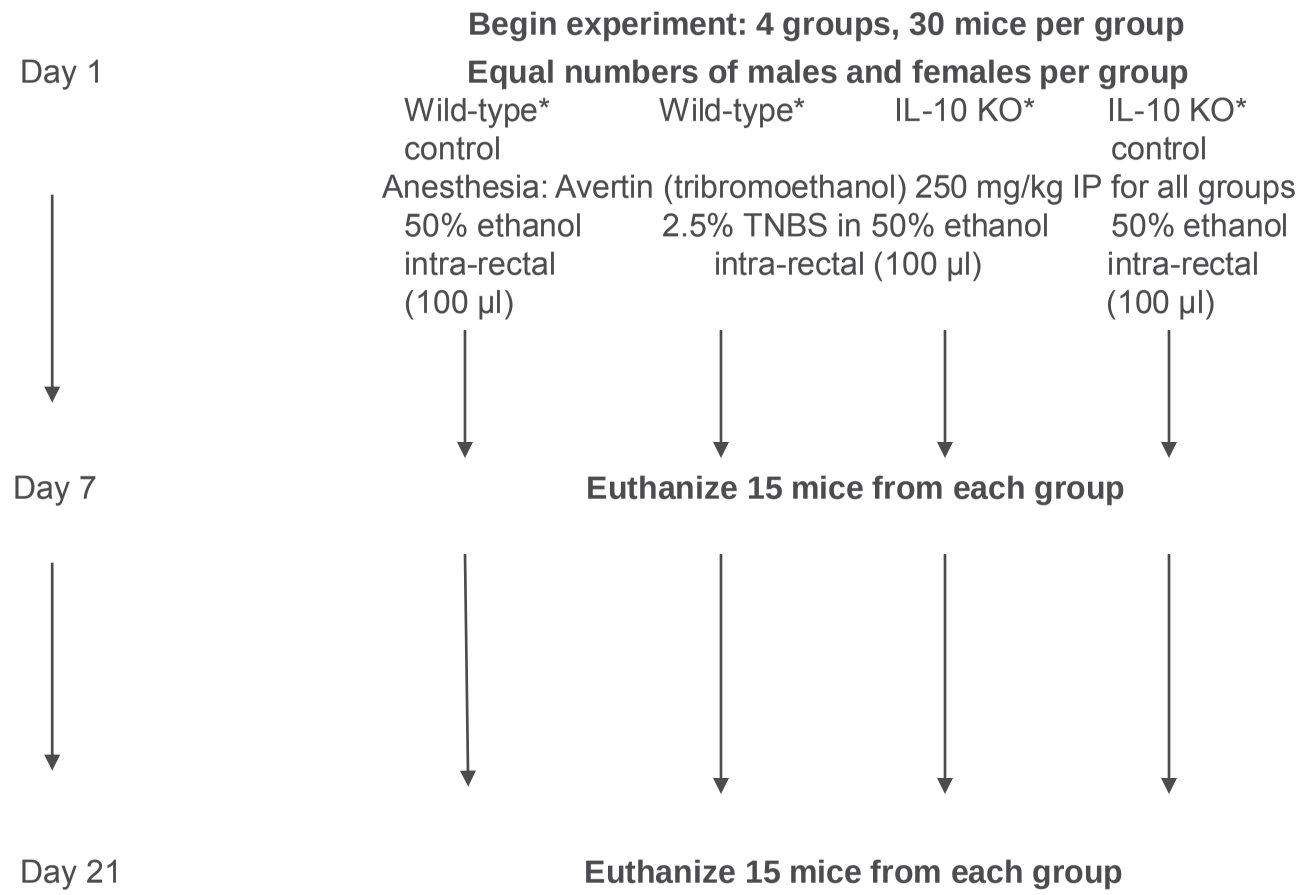
Mice used in this experiment: Category C 60, Category E 60, **Total 120**

EXPERIMENT 2



Mice used in this experiment: Category C 15, Category E 30, **Total 45**

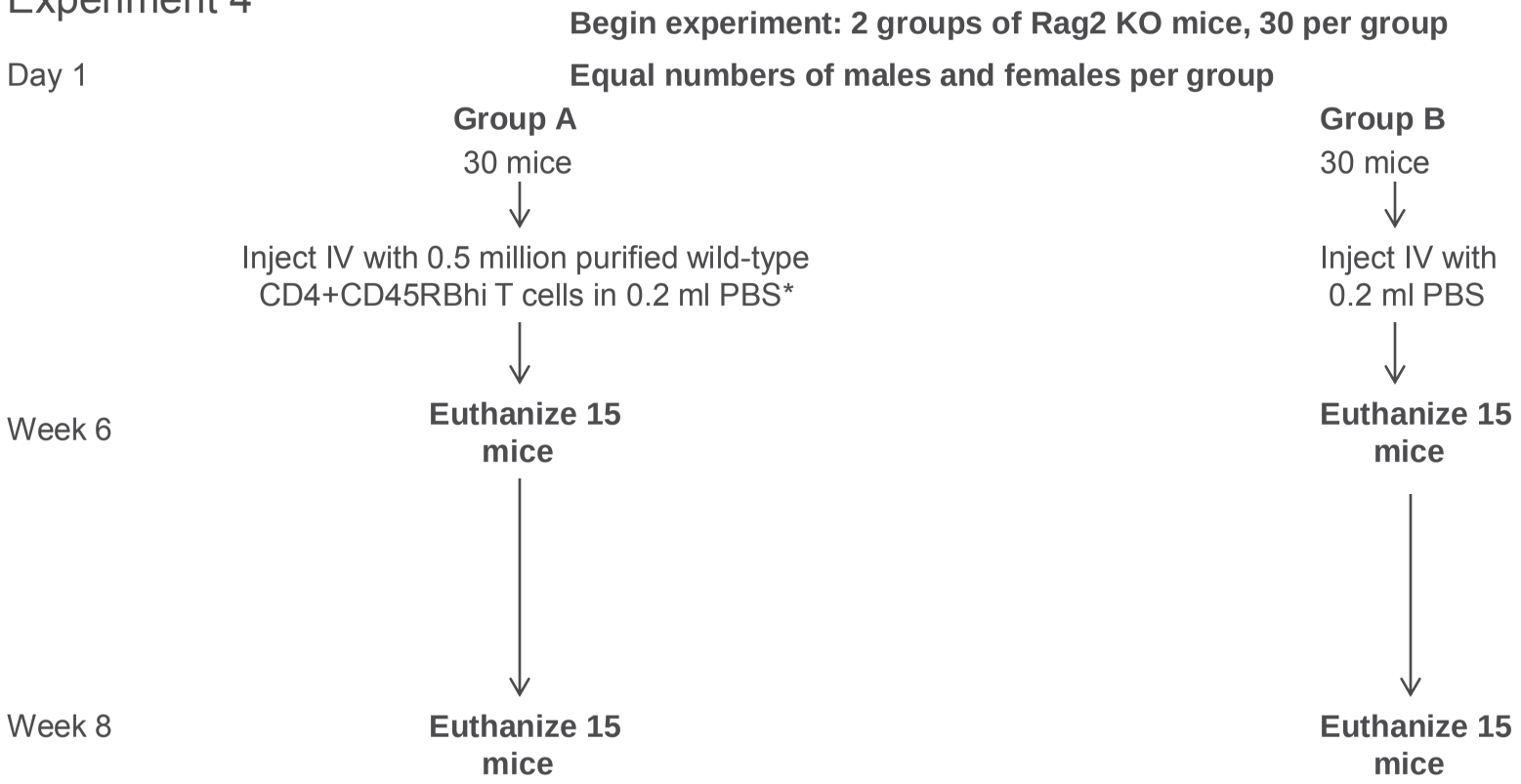
EXPERIMENT 3



*When possible, the wild-type and IL-10 KO mice will be derived from the same litter, i.e., the progeny of the same IL-10 KO heterozygote x IL-10 KO heterozygote breeders. Both homozygous wild-type and heterozygous mice will be used in the wild-type groups since both behave like wild-type. The IL-10 KO mice will be homozygotes.

Mice used in this experiment: Category C 60, Category E 60, **Total 120**

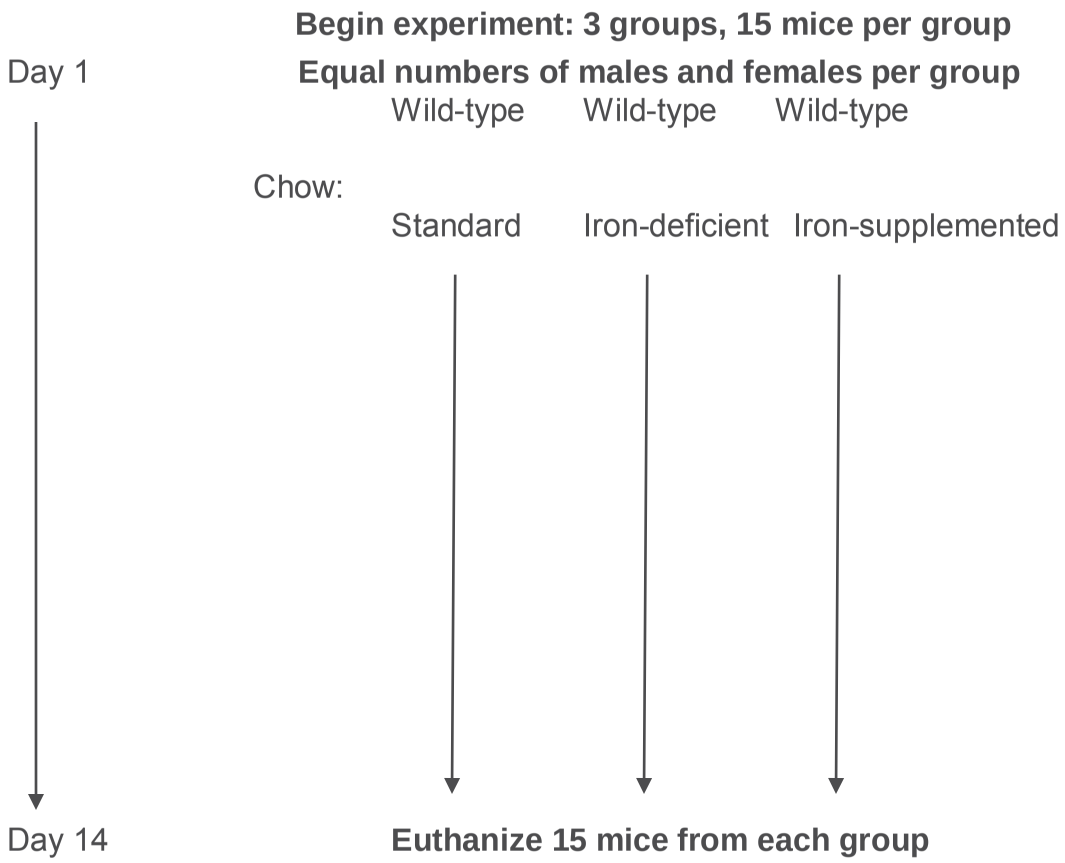
Experiment 4



*The purified T cells will be obtained from a total of 15 wild-type C57BL/6 mice

Mice used in this experiment: Category C 45, Category E 30, **Total 75**

EXPERIMENT 5



Mice used in this experiment: Category C 45, **Total 45**

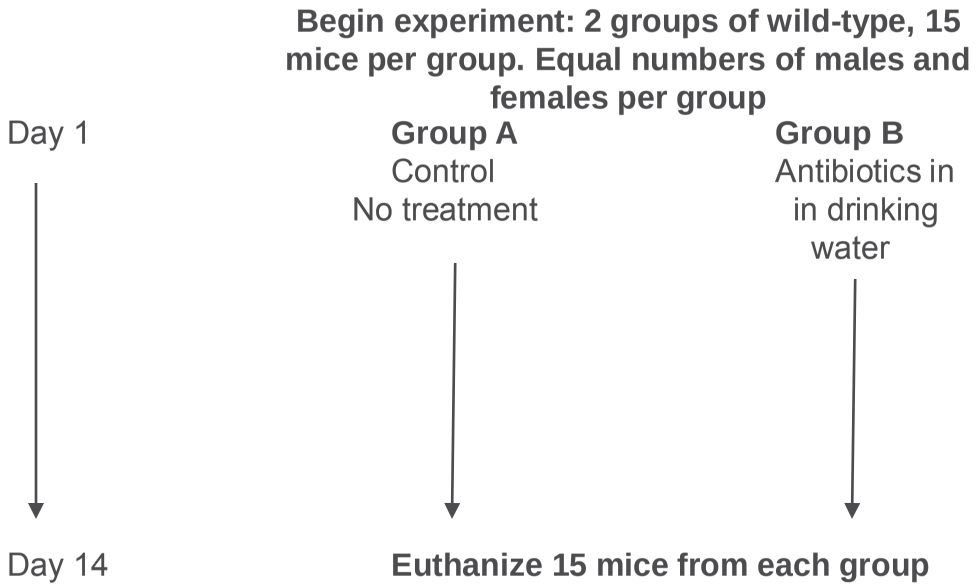
EXPERIMENT 6

Begin experiment: 2 groups, 15 mice per group
Wild-type mice, males and females

	Group A **Standard diet**	**Group B** **Low-protein, low-fat diet**
Day 1	Start diet	Start diet
		
Day 28	**Euthanize all mice**	**Euthanize all mice**

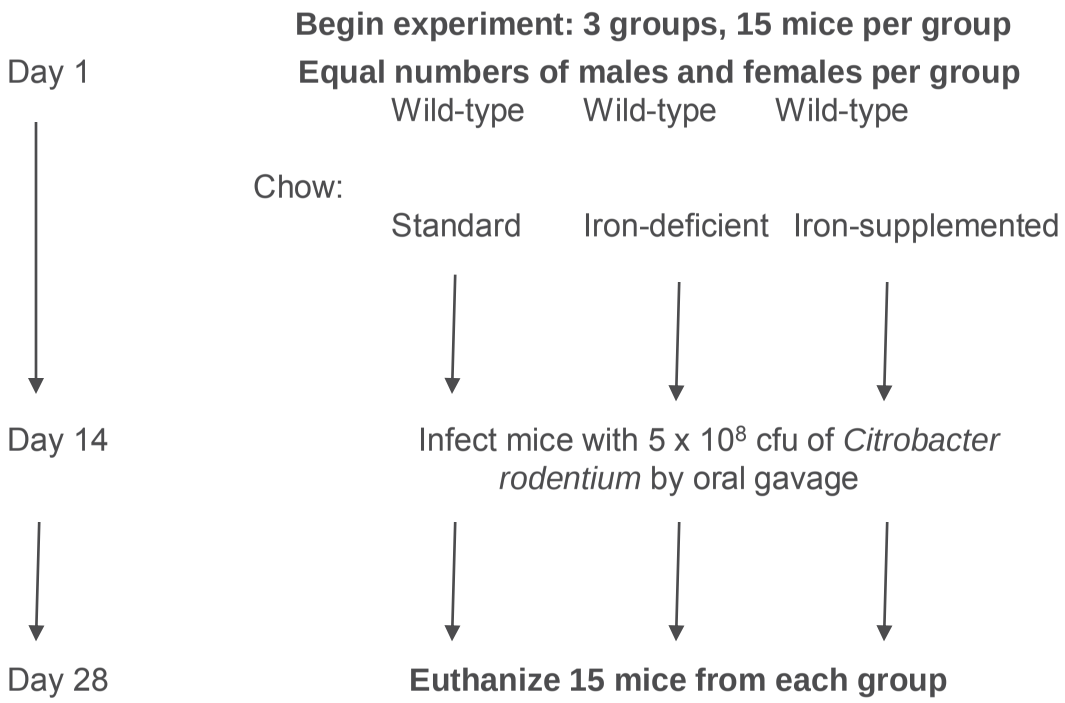
Mice used in this experiment: Category C 30, **Total 30**

EXPERIMENT 7



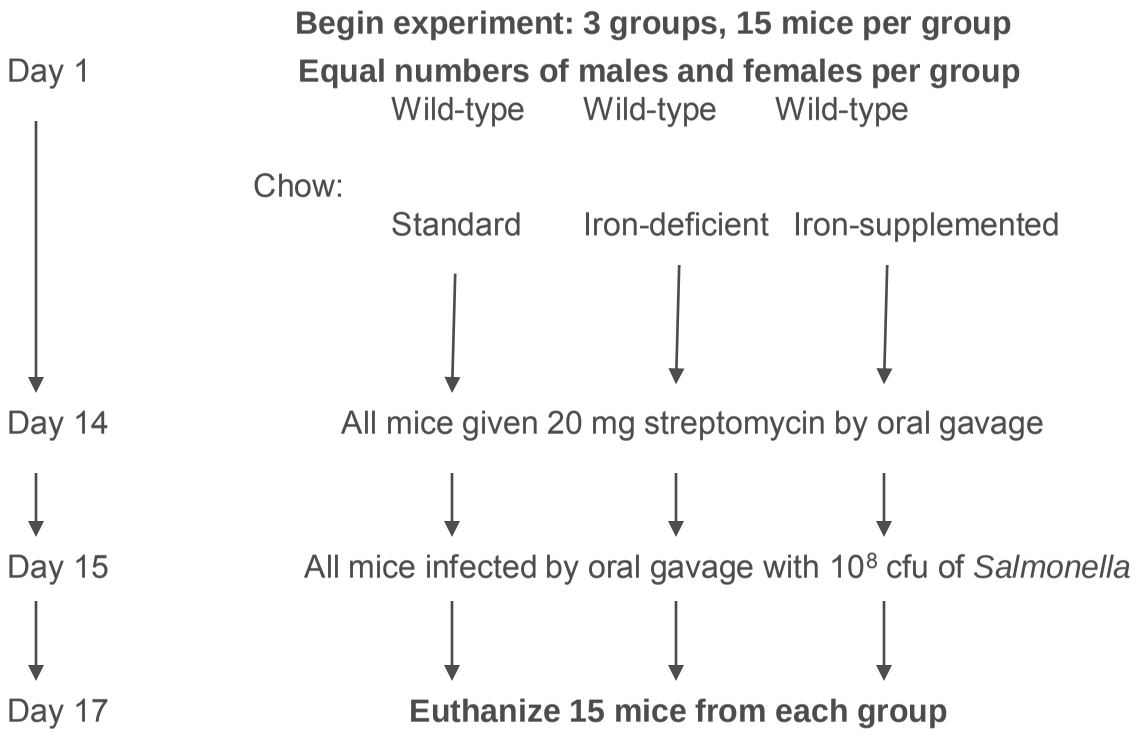
Mice used in this experiment: Category C 30, **Total 30**

EXPERIMENT 8



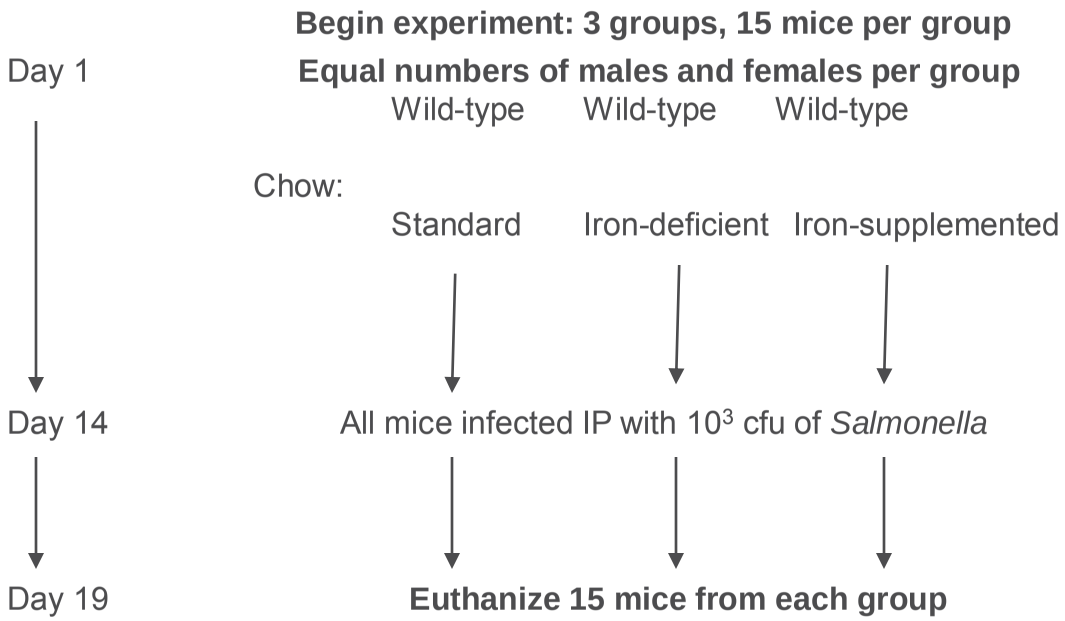
Mice used in this experiment: Category E 45, **Total 45**

EXPERIMENT 9



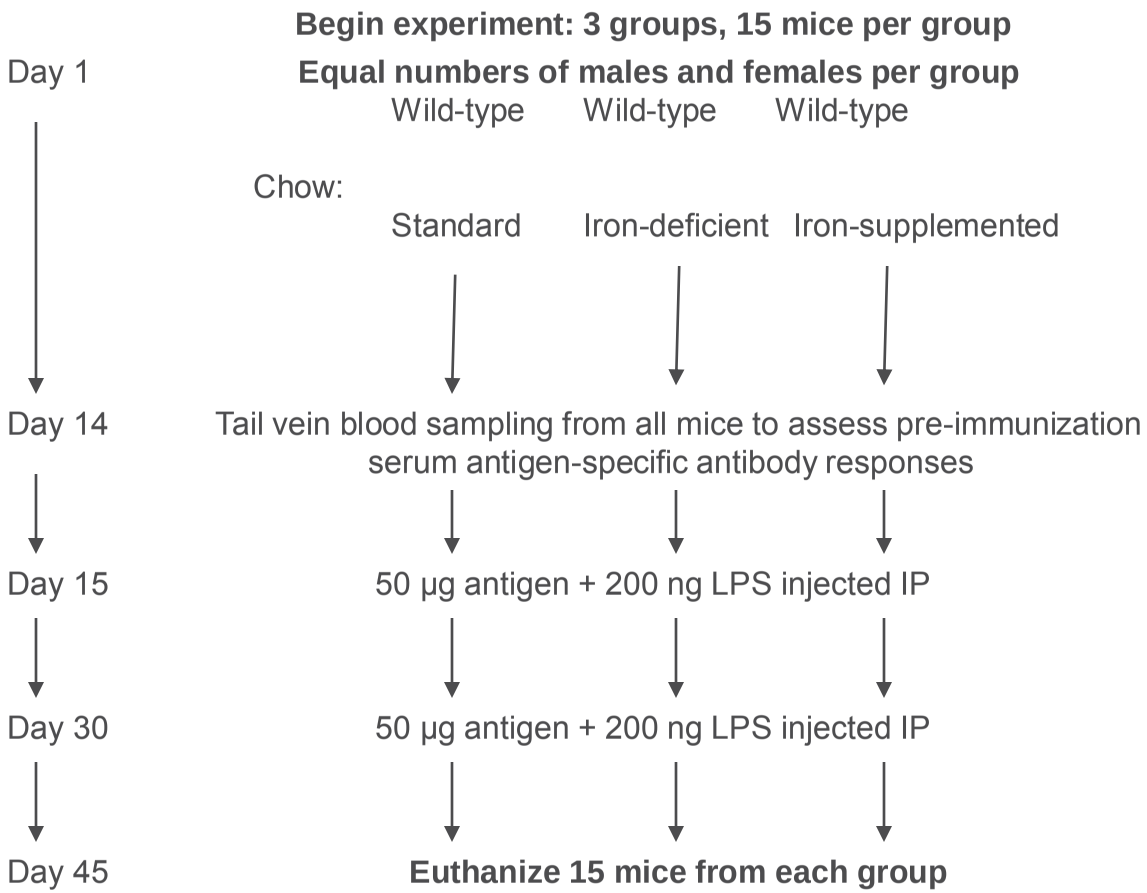
Mice used in this experiment: Category E 45, **Total 45**

EXPERIMENT 10



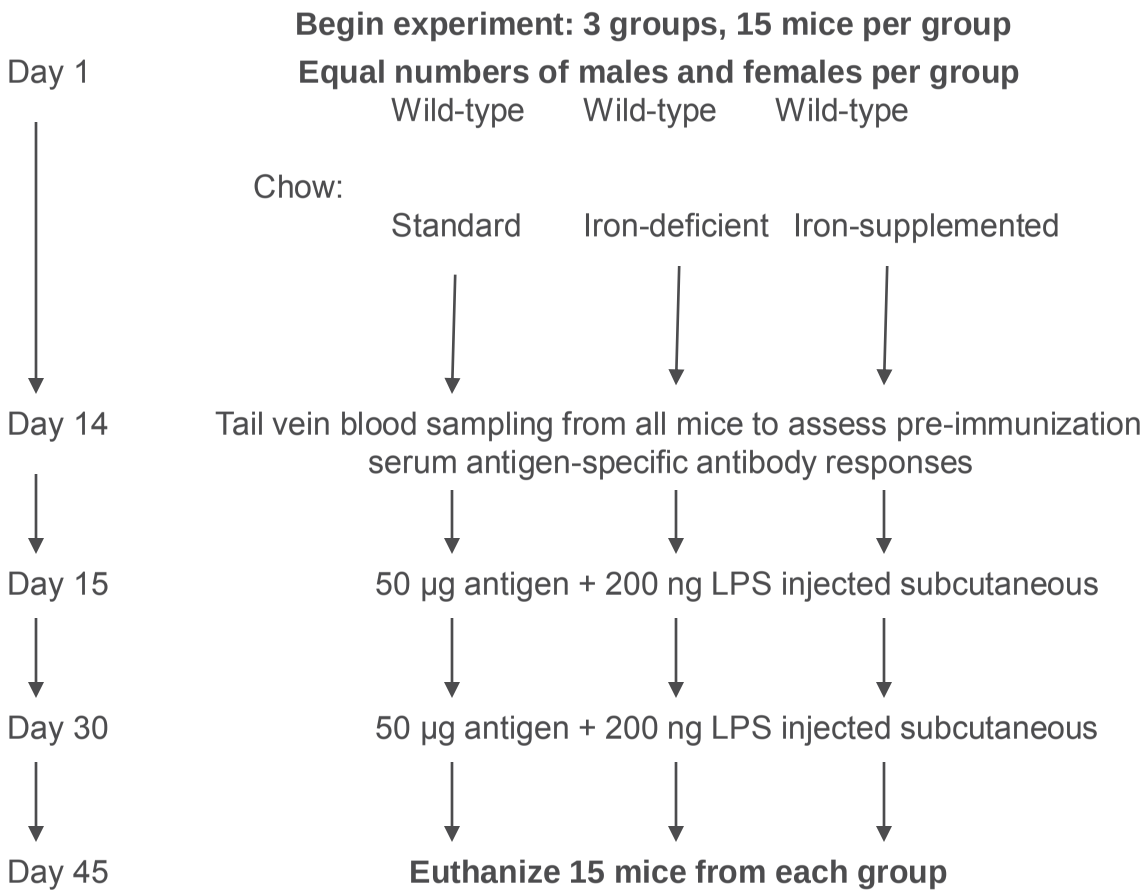
Mice used in this experiment: Category E 45, **Total 45**

EXPERIMENT 11



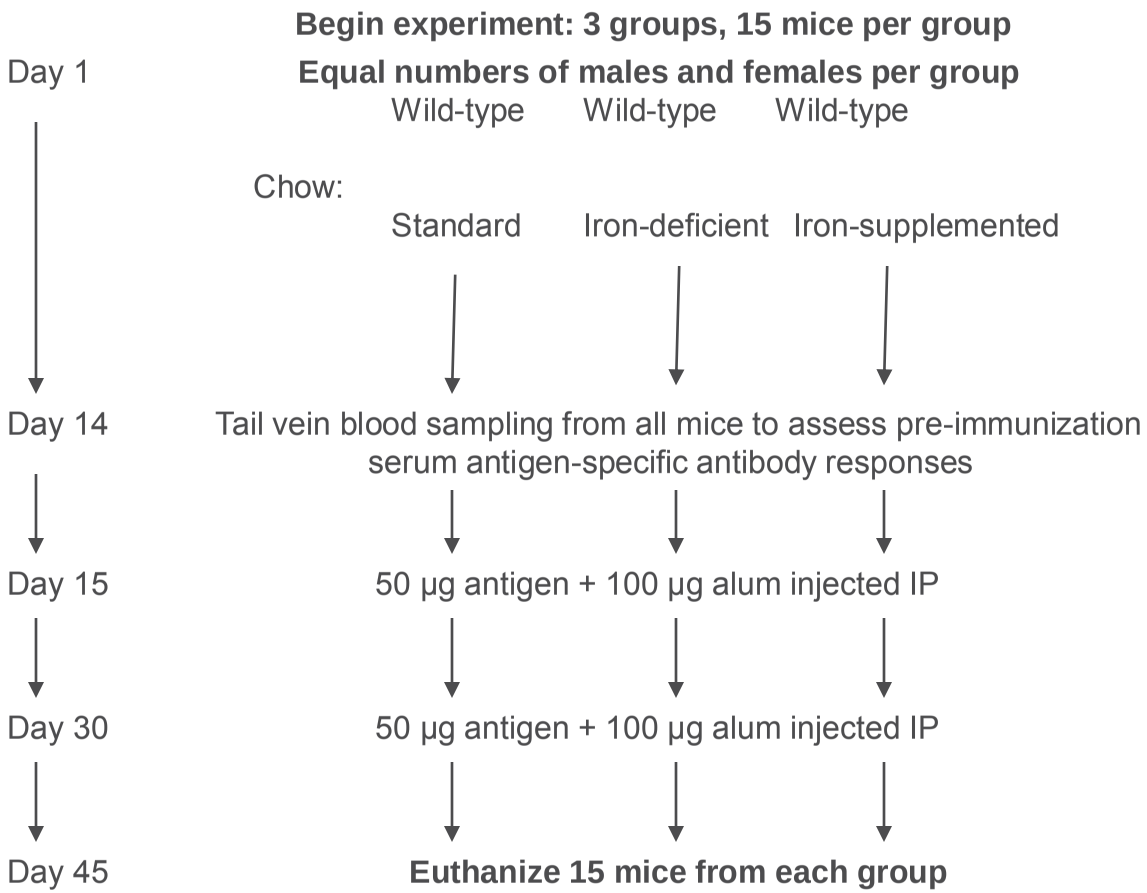
Mice used in this experiment: Category C 45, **Total 45**

EXPERIMENT 12



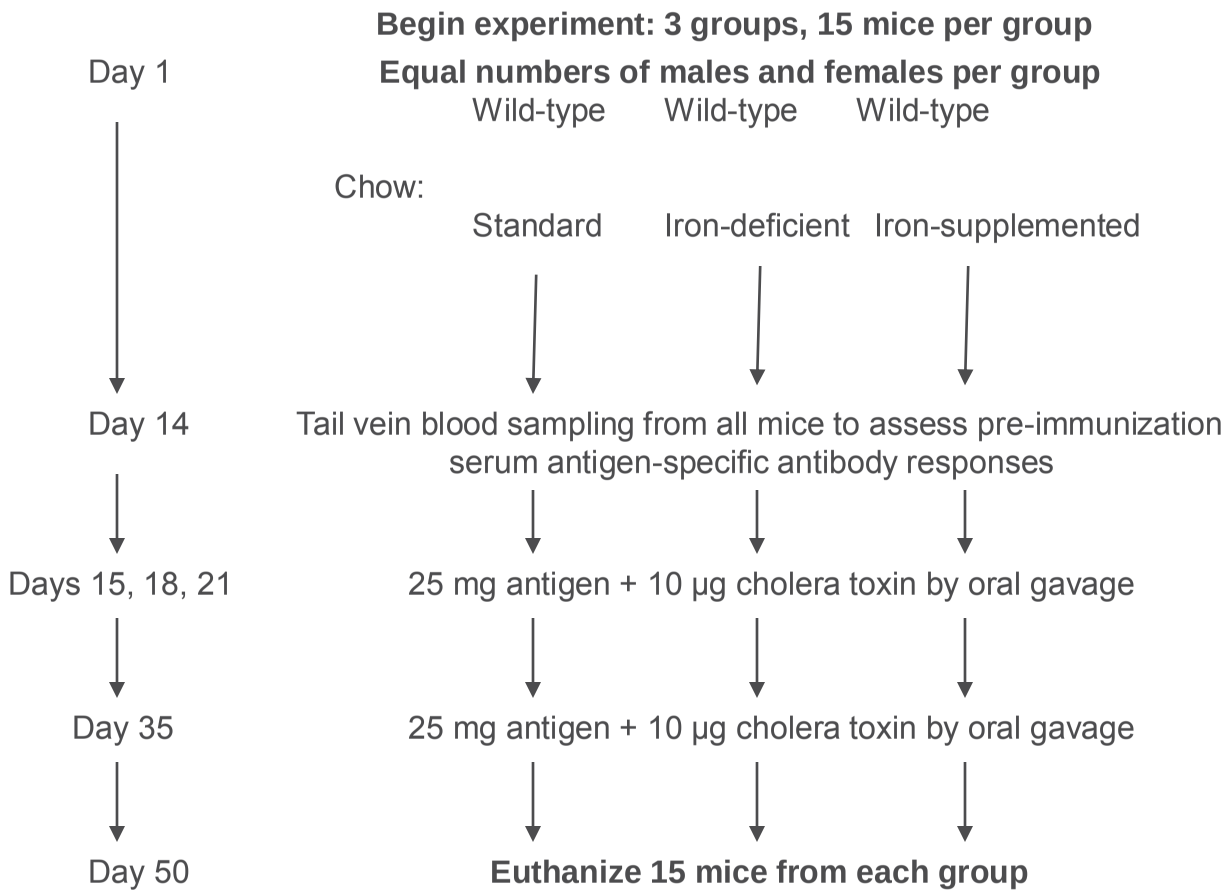
Mice used in this experiment: Category C 45, **Total 45**

EXPERIMENT 13



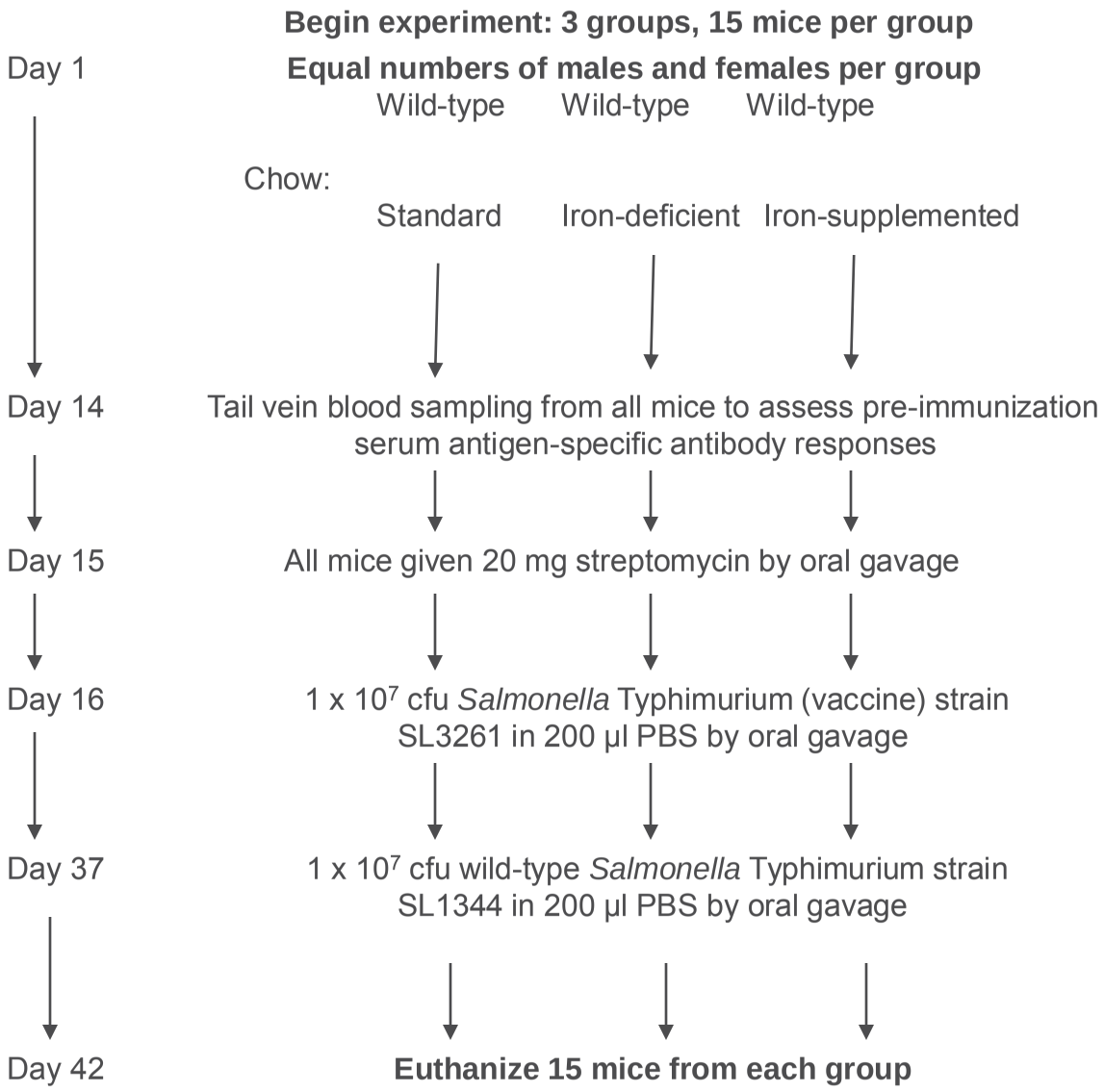
Mice used in this experiment: Category C 45, **Total 45**

EXPERIMENT 14



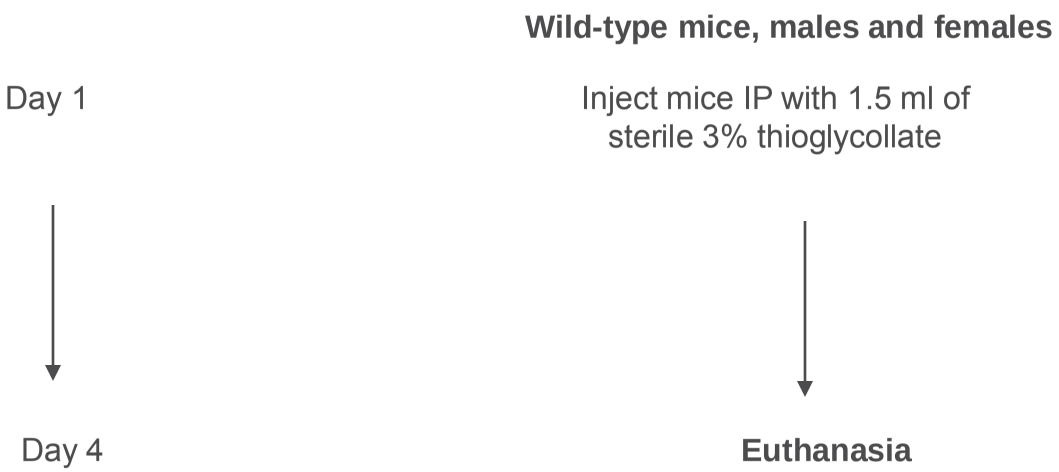
Mice used in this experiment: Category C 45, **Total 45**

EXPERIMENT 15



Mice used in this experiment: Category E 45, **Total 45**

EXPERIMENT 16



Mice used in this experiment: Category C 60 per year X 3 years, **Total 180**

BREEDING

2 breeding pairs per year per strain:

Wild-type C57BL/6 x Wild-type C57BL/6
IL-10 knockout x IL-10 knockout
Rag2 knockout x Rag2 knockout
IL-10 knockout heterozygote x IL-10 knockout heterozygote

We will maintain 2 breeding pairs of each type.
Number of breeders used per year: 8 pairs (16 mice)
Total number of breeders used over 3 years = **48 mice** (category C)

[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.

Wild-type mice (C57BL/6, 129/Sv). Normal phenotype.

IL-10 knockout (C57BL/6 background). Phenotype: mice may develop spontaneous colitis starting at 5-6 weeks of age.

IL-10 heterozygote. Normal phenotype.

Rag2 knockout (C57BL/6 background). Phenotype: these mice are lymphocyte deficient and are more susceptible to infection than wild-type animals. However, they generally remain healthy under specific pathogen-free conditions.

---

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 knockout (B6 background): The mice may develop spontaneous colitis starting at 5-6 weeks of age.

Rag2 knockout (B6 background): The mice are lymphocyte deficient and are more susceptible to infection but generally remain healthy under specific pathogen-free conditions.

[redacted]

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

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4. Sex

O Male O Female ◉ Both

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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.

[redacted]

- 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

TOTAL NUMBER OF ANIMALS REQUESTED
USDA Pain & Distress Category (See FAQ for information)

Animals	B	C	D	E	Total
Research Objective 1		693		360	
Other (e.g. breeding, training):					
Total requested	0	693	0	360	1053
Animals currently in house					
Total approved for purchase	0	693	0	360	1053

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

Please describe:

[redacted]

For experiments 1 through 15, the group size was based on the number of mice per group needed to study the differences in the mean between groups. The intra-group variability of the experimental parameters being measured is expected to be 20-25%. Given this variability, to obtain statistical significance between groups at the 5% confidence level ($p<0.05$), we expect that 7 or 8 mice per group per time point will be required. Allowing for repeating each experiment once to confirm reproducibility, we have proposed using 15 mice per group per time point. The numbers of experimental groups and time points analyzed for each experiment are detailed in the flow charts, which also provide the total number of mice in each experiment.

For experiment 4, in addition to the calculation described above, we will also require 15 mice that will be used to prepare purified CD4+CD45RBhi T cells. This number is based on the fact that we can prepare 1 million of these cells from each mouse and that we will require a total of 15 million cells for the experiment.

For experiment 16, the number of mice requested is based on the number of peritoneal macrophages required to carry out functional analyses of inflammatory and anti-microbial responses. Based on our experience, we are able to obtain about 3 million peritoneal macrophages from each adult mouse. Thus, we will need a total of 60 mice per year and therefore 180 mice over the 3 year duration of the protocol.

For breeding purposes, we will require 2 breeding pairs per year for each of 4 different matings (as indicated in the flow chart), giving rise to 16 breeders per year and therefore 48 mice during the 3 year duration of the protocol.

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?

○ 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.

[redacted]

DSS-induced colitis. Administration of 3% (w/v) dextran sulfate sodium (DSS) in drinking water for 7 days induces inflammation of the colon. This is used as a model of inflammatory bowel disease and may cause the mice to feel mild to moderate discomfort or pain.

TNBS-induced colits. Intra-rectal administration of 2.5% (w/v) trinitrobenzene sulfonate (TNBS) induces inflammation of the colon. This is used as a model of inflammatory bowel disease and may cause the mice to feel mild to moderate discomfort or pain.

Transfer of CD4+CD45RBhi T cells to Rag2 knockout mice leads to the induction of inflammation in the colon over the course of 5-6 weeks. This is used as a model of inflammatory bowel disease and may cause the mice to feel mild to moderate discomfort or pain.

Oral infection with Citrobacter rodentium is used as a model of human enteropathogenic E. coli (EPEC) infection. It cause a mild inflammation of the colon, which may cause the mice to feel mild to moderate discomfort or pain.

Oral or parenteral infection with Salmonella Typhimurium is used as a model of human Salmonella gastroenteritis and human typhoid. The infection can induce inflammation in the cecum and also systemically. As a result, the mice may feel mild to moderate 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.

The experiments described above are directed at evaluating intestinal and systemic inflammatory and immune responses. Anesthetics, analgesics and other drugs used for pain relief can interfere with these responses and influence the results of the experiment. Thus, these agents cannot be used in our experiments.

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

The mice will be monitored once daily or more frequently as indicated by the clinical condition. Body weight will be recorded once daily. No analgesics will be administered since they will interfere with our analysis. However, if any animal appears to be in significant distress (as indicated by hunched posture, poor activity, poor grooming) or experiences body weight loss that is more than 15% relative to the age- and sex-matched control mice, it will be euthanized immediately by controlled flow carbon dioxide asphyxia.

360

Replacement, Reduction, and Refinement

[redacted]

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

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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 [redacted] Office of Laboratory Animal Welfare, and the USDA Animal Welfare Information Center.

☐ Medline (http://library.massgeneral.org/)
☑ Pubmed (http://library.massgeneral.org/)
☐ Agricola (https://agricola.nal.usda.gov/)
☐ ALTBIB (https://toxnet.nlm.[redacted]gov/altbib.html)
☐ ALTWEB (http://altweb.jhsph.edu/)
☐ Animal Welfare Institute (https://awionline.org/)
☐ Google Scholar (http://scholar.google.com/)
☐ Other databases (please list):

[redacted]

☐ 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.***

November 30, 2016

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

1970-2018

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 8: Oral gavage	Keywords: Oral gavage, mice, alternatives
Procedure 2: Tail snip	Keywords: Tail snip, mice, genotyping alternatives
Procedure 9: Thioglycollate-induced peritonitis	Keywords: Thioglycollate, peritonitis, macrophages, mice, alternatives
Procedure 13: T cell transfer colitis	Keywords: CD4+ T cells, Rag knockout, colitis, mice, alternatives
Procedure 7: Oral administration of DSS	Keywords: Dextran sulfate sodium, colitis, mice, alternatives
Procedure 6: Intravenous (IV) injection	Keywords: Intravenous injection, mice, alternatives
Procedure 10: Salmonella-induced intestinal inflammation	Keywords: Salmonella typhimurium, intestinal inflammation, mice, alternatives
Procedure 3: Tail vein blood sampling	Keywords: Tail vein blood sampling, mice, alternatives
Procedure 12: Salmonella typhimurium infection	Keywords: Salmonella typhimurium, infection, mice, alternatives
Procedure 4: Intraperitoneal (IP) injection	Keywords: Intraperitoneal injection, mice, alternatives
Procedure 1: Subcutanous injection	Keywords: Subcutaneous injection, mice, alternatives
Procedure 11: Citrobacter-induced intestinal inflammation	Keywords: Citrobacter rodentium, intestinal inflammation, mice, alternatives
Procedure 5: Intra-rectal injection of TNBS	Keywords: Intra-rectal injection, trinitrobenzene sulfuric acid, mice, alternatives

[redacted]

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 1: Subcutanous injection

Keywords: Subcutaneous injection, mice, alternatives

Procedure 2: Tail snip

Keywords: Tail snip, mice, genotyping alternatives

Procedure 3: Tail vein blood sampling

Keywords: Tail vein blood sampling, mice, alternatives

Procedure 4: Intraperitoneal (IP) injection

Keywords: Intraperitoneal injection, mice, alternatives

Procedure 5: Intra-rectal injection of TNBS

Keywords: Intra-rectal injection, trinitrobenzene sulfuric acid, mice, alternatives

Procedure 6: Intravenous (IV) injection

Keywords: Intravenous injection, mice, alternatives

Procedure 7: Oral administration of DSS

Keywords: Dextran sulfate sodium, colitis, mice, alternatives

Procedure 8: Oral gavage

Keywords: Oral gavage, mice, alternatives

Procedure 9: Thioglycollate-induced peritonitis

Keywords: Thioglycollate, peritonitis, macrophages, mice, alternatives

Procedure 10: Salmonella-induced intestinal inflammation

Keywords: Salmonella typhimurium, intestinal inflammation, mice, alternatives

Procedure 11: Citrobacter-induced intestinal inflammation

[redacted]

Keywords: Citrobacter rodentium, intestinal inflammation, mice, alternatives

Procedure 12: Salmonella typhimurium infection

Keywords: Salmonella typhimurium, infection, mice, alternatives

Procedure 13: T cell transfer colitis

Keywords: CD4+ T cells, Rag knockout, colitis, mice, alternatives

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:

◉ Animals will be removed from the study and euthanized if any of the above clinical signs/conditions are found

O Some or all of the criteria listed above cannot apply to this study. Animals will be euthanized if the following criteria are met.

[redacted]

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.***

The end points of each experiment have been detailed along with the experimental procedures. In general, they involve specific times after the start of specific experimental interventions. Significant morbiditiy or death are not expected prior to the specified end points. However, in all experiments, the animals will be monitored carefully, and any animal that loses more than 15% of the starting body weight or whose body weight at any time during the experiment is more than 15% lower than the body weight of a control mouse of the same age and sex (measured simultaneously from a control group in that experiment or derived from standard mouse growth charts) or that appears to be in more than mild discomfort, as indicated by hunched posture, poor grooming or poor activity, will be euthanized immediately.

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 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

[redacted]

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)

☐ 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)
☐ Cervical dislocation (without anesthesia) - animals

[redacted]

☐ 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]

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]

☐ Other IACUC approved satellite/laboratory housing area

[redacted]

☐ 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.

☐ 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

[redacted]

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

☑ Other

Describe and scientifically justify the nature of the exemption.

In some of the experiments, the animals will be placed on medicated drinking water containing either antibiotics or dextran sulfate sodium (DSS). In such experiments, "Special Husbandry" cards will be placed on the relevant cages to indicate that the study staff will be responsible for providing the water. Based on discussions with CCM staff, 200 ml of the medicated water will be provided in special red colored bottles and the water level will be monitored by the study staff on alternate days and will be replenished as needed. The condition of the mice (body weight, activity, grooming) will also be monitored to ensure that the animals are getting an adequate fluid intake. If the mice appear dehydrated (decreased activity, poor grooming, 15% or greater loss of body weight compared to control mice), the medicated water will be terminated and replaced with regular water.

In some of the other experiments, the mice will be placed on special powdered chow (obtained from Harlan-Teklad) that will be formulated to contain different concentrations of iron (standard, iron-deficient or iron-supplemented). In such experiments, "Special Husbandry" cards will be placed on the relevant cages to indicate that the study staff will be responsible for providing the special chow. Based on discussions with CCM staff, the powdered chow will be provided in autoclaved stainless steel dispensers that are inserted into the standard cages. Study staff will monitor the amount of chow on alternate days and will replenish as required. The condition of the mice (body weight, activity, grooming) will also be monitored to ensure that the animals are getting an adequate food intake. If the condition of the mice deteriorates (decreased activity, poor grooming, 15% or greater loss of body weight compared to controls), the medicated chow will be terminated and replaced with regular chow.

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

[redacted]

☐ Exemption from canine exercise program

If there are any relevant citations or other documents that are needed to support these special housing, husbandry, or handling requirements, please attach them to this form.

Anesthesia Regimen: Avertin Option

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.

Avertin Option

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
Tribromoethanol	250 mg/kg	IP	once

---

2. Are any of the agents listed paralytics?

○ 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 ◉ No

All non-pharmaceutical grade agents must be included on the **Controlled and Non-pharmaceutical Grade Substances** form.

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

[redacted]

☑ Other (please describe)

As per IACUC guidelines (http://is.partners.org/aniweb/PDF files/small animal anesthesia.pdf), we will use visual observation to monitor mucous membrane color and capillary refill time as indicators of cardiovascular status, and respiratory rate, rhythm and estimated tidal volume as indicators of respiratory status.

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.

Every minute during the period of anesthesia (total duration 4-5 minutes).

Procedures: Intraperitoneal injection of thioglycollate

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 of thioglycollate

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, Irritant

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 restrained manually for 30-60 seconds with the abdomen facing up. A 27 gauge needle connected to a 1 ml tuberculin syringe will be inserted into the peritoneal cavity by puncturing the abdominal wall in the left lower quadrant. Sterile 3% thioglycollate solution (1.5 ml) will be injected and the needle then withdrawn. The mice will be returned to their cages and observed for a few minutes to ensure that they resume normal activity. Only transient discomfort will be experienced during this procedure. No sedatives, anesthetics or analgesics will be used. No special post-procedure care is required. The animals will be monitored every other day until euthanasia 4 days after the injection.

---

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

Mice will be monitored every other day.

---

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

[redacted]

Provide scientific justification for not using analgesia for this painful procedure

The pain associated with intraperitoneal injection is very mild and transient, so analgesics are not required. In addition, analgesics can interfere with the inflammatory response that is being elicited and evaluated in the experiment.

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

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

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

[redacted]

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

[redacted]

☐ 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 will be subjected to tail-snip at 3 weeks of age. The animal will be immobilized by placing it in a small, perspex rodent restrainer. The tail will be held gently and extended. About 0.3 cm of the tail tip will be excised using a sterile, disposable scalpel blade. Light manual pressure will be applied to the tail to stop bleeding. No sedative or anesthetic will be used since the procedure will cause only momentary pain. The mouse will be observed until hemostasis is assured and then returned to its cage.

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

[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

Observe until bleeding ceases.

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

The pain associated with tail snip is very transient and mild and do not require analgesics.

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

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 during the course of this procedure be of pharmaceutical grade?

◉ Yes O No

Procedures: Intravenous 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:
Intravenous 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.)

[redacted]

Injection intravenous

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

---

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)

Each mouse to be injected will be placed in a small plastic rodent restrainer. The tail will be grasped gently and the lateral tail vein visualized. A 30 gauge

[redacted]

needle attached to a 1 ml tuberculin syringe will be used to penetrate the skin at the base of the tail and the tail vein entered. One hundred microliters of phosphate buffered saline containing 0.5 million purified CD4+CD45RBhi T cells will be injected into the vein and the needle then withdrawn. Gentle pressure with a sterile gauze will be applied to the puncture site for 10-15 seconds to ensure hemostasis. The mouse will then be removed from the restrainer and returned to its cage. The procedure will result in transient, minimal distress to the animal. No sedatives, anesthetics or analgesics will be used. No specific post-procedure care will be required but the laboratory staff will observe the mice after the procedure to ensure that there is no bleeding from the injection site and that the animals return to their normal level of activity.

---

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

Once daily.

---

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

[redacted]

The pain associated with intravenous injection is very mild and transient so analgesics are not required. In addition, analgesics can interfere with the inflammatory and immune responses that are being evaluated in the experiment.

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

Procedures: Tail vein blood collection

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

[redacted]

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 blood collection

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.)
Blood collection, tail vein

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?

[redacted]

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 mouse will be placed in a small perspex rodent restrainer. The tail will be grasped, gently extended, and the lateral vein visualized. The vein will be nicked gently using a sterile scalpel blade. A few drops of blood will be collected into a serum tube and then gentle pressure applied to the nick site using a sterile gauze. Once hemostasis has been ensured, the mouse will be removed from the restrainer and returned to its cage. The procedure will cause minimal, transient distress. No anesthetics, sedatives or analgesics will be used. No specific post-procedure care will be required. However, the study staff will observe the animal after the procedure to ensure that there is no bleeding from the nick site.

---

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

Once daily.

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

The pain of tail vein blood collection is very transient and mild and does not require analgesia. In addition, analgesics can alter the inflammatory response that is being evaluated in the experiments.

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 during the course of this procedure be of pharmaceutical grade?

◉ Yes O No

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

---

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

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

---

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)

The mouse will be placed in a small, perspex rodent restrainer with its caudal end towards the operator. The tail will be held steady with one hand. A 27 gauge needle attached to a 1 ml tuberculin syringe will be inserted under the skin at the base of the tail and used to inject 50-100 microliters of antigen mixed with LPS. After the injection, the needle will be withdrawn and the

[redacted]

injection site observed to ensure that there is no bleeding. Brief pressure will be applied to the injection site to stop bleeding if required. The mouse will then be returned to its cage and will be observed for a few minutes to ensure that it resumes normal activity. The total duration of the procedure is 1-2 minutes and the animal will experience only transient pain as a result of the injection. No adverse effects are expected. No analgesics, anesthetics or antibiotics will be required.

---

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

Every other day.

---

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

The pain associated with subcutaneous injection is very mild and transient so analgesics are not required. In addition, analgesics can interfere with the inflammatory and immune responses that are being evaluated in the experiment.

[redacted]

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

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

[redacted]

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?

○ 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.)

Gavage, 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?

[redacted]

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 will be restrained manually for a few seconds in the vertical position with the head held steady by grasping the scruff of the neck. A 21 gauge, ball-tipped feeding needle connected to a 1 ml tuberculin syringe will be inserted gently into the mouth and advanced into the esophagus via the pharynx. Drug (streptomycin) or bacteria (Salmonella or Citrobacter) will be injected in a volume of 200 microliters. The needle will then be withdrawn, the mouse returned to its cage and observed for a few minutes to ensure that it resumes normal activity. Only transient discomfort will be experienced during this procedure. No sedatives, anesthetics or analgesics will be used. No special post-procedure care is required. The animals will be monitored every day after the oral gavage.

---

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

Every other day.

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

There is no significant pain associated with oral gavage. The discomfort associated with the procedure is very mild and transient and does not require analgesics. In addition, analgesics can interfere with inflammatory and immune responses that are being evaluated in the experiment.

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

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 during the course of this procedure be of pharmaceutical grade?

◉ Yes O No

Procedures: DSS colitis

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:
DSS colitis

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.)

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)

Dextran sulphate sodium (DSS) will be dissolved in the animals' drinking water at a concentration of 3% (w/v). The mice will be allowed to drink the water ad

libitum for 7 days, after which regular drinking water will be provided in some experiments. No sedatives or anesthetics will be used for the procedure and the procedure itself will not cause any pain or discomfort. The administration of DSS induces a mild to moderate colitis and the mice may experience mild to moderate discomfort, some transient weight loss and softening of stools as a result of the intestinal inflammation. Accordingly, the mice will be observed every day and their body weight and clinical condition recorded. Any animal that appears to be in significant distress (as indicated by hunched posture, poor activity and disheveled appearance) or experiences body weight loss greater than 15% of the simultaneously measured weight of the age- and sex-matched control mice, will be euthanized immediately by controlled flow carbon dioxide asphyxia.

---

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

Once daily.

---

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

[redacted]

The experiment requires assessment of DSS-induced intestinal inflammation. Since analgesics can alter the inflammatory response, they cannot be used for pain relief.

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

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

Procedures: Intraperitoneal injection of drugs

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

[redacted]

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 of drugs

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, 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

[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)

Mice will be restrained manually for a few seconds with the abdomen facing up. A 27 gauge needle connected to a 1 ml tuberculin syringe will be introduced into the peritoneal cavity by puncturing the abdominal wall in the left lower quadrant. The drugs (liposomal PBS and clodronate, antigen + adjuvant, doses will vary with experiment) will be injected in a volume of 200 microliters and the needle then withdrawn. The mice will be returned to their cage and observed for a few minutes to ensure that they resume their normal level of activity. Only transient discomfort will be experienced during this procedure. No sedatives, anesthetics or analgesics will be used. No special post-procedure care is required. The animals will be monitored every other day until euthanasia.

---

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

[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

Every other day.

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

The pain associated with intra-peritoneal injection is very mild and transient so analgesics are not required. In addition. analgesics can interfere with the inflammatory and immune responses that are being evaluated in the experiment.

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

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

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

Procedures: Intrarectal TNBS

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:
Intrarectal TNBS

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.)
Drug Delivery, Intrarectal

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 O 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.

Avertin Option

---

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

[redacted]

approach used, the method(s) of wound closure, and intra-operative suppo
fluids, mechanical ventilation)

Using brief manual restraint, the mice will be anesthetized by intraperitoneal injection of Avertin (tribromoethanol) 250 mg/kg. Once adequate anesthesia has been achieved, as assessed by lack of limb withdrawal on toe pinch (usually within a few minutes), the mouse will be placed on its back and an epidural catheter (FlexTip Plus, Arrow International) will be inserted into the rectum to a distance of 4 cm. One hundred microliters of 2.5% TNBS in 50% ethanol will be instilled through the catheter. The control group will receive 100 microliters of 50% ethanol without the TNBS. After instillation, the catheter will be withdrawn and the mice will be held in an inverted (head down) position for 1 minute to minimize loss of the TNBS. During the period of anesthesia, as per IACUC guidelines (http;//is.partners.org/aniweb/PDF files/small animal anesthesia.pdf), we will monitor mucous membrane color and capillary refill time as indicators of cardiovascular status, and respiratory rate, rhythm and estimated tidal volume as indicators of respiratory status. Once the procedure is completed, the mice will be returned to their cages and monitored until they recover the righting reflex (usually 30-40 minutes).

---

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

During the period of anesthesia, as per IACUC guidelines (http;//is.partners.org/aniweb/PDF files/small animal anesthesia.pdf), we will monitor mucous membrane color and capillary refill time as indicators of cardiovascular status, and respiratory rate, rhythm and estimated tidal volume as indicators of respiratory status. Once the procedure is completed, the mice will be returned to their cages and monitored until they recover the righting reflex (usually 30-40 minutes). The clinical condition of the animals will be monitored at least once daily after the procedure.

[redacted]

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 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

Every day

---

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

Analgesics will interfere with the intestinal inflammation that is being induced by intra-rectal administration of TNBS and so 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?

[redacted]

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

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

Procedures: Ear punch

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:

Ear punch

[redacted]

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

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

[redacted]

anesthesia cannot be used

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)

This procedure will be carried out on mice that are 3 weeks of age or older. The mouse will be restrained manually for a few seconds by grasping the scruff of the neck. An ear punch device will be disinfected with alcohol wipes (at the start of the procedure and between animals) and used to punch holes in the left, right or both ears according to a predetermined code and using the minimum number of holes required for unambiguous identification. Care will be taken to apply the punch to the thin part of the ear pinna, avoiding blood vessels. After the procedure, the mice will be returned to their cage and observed for a few minutes to ensure that they resume their normal level of activity. Only transient discomfort will be experienced during this procedure. No sedatives, anesthetics or analgesics will be used. No special post-procedure care is required. The animals will be monitored every other day until euthanasia.

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

Every other day.

[redacted]

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

The pain associated with ear punch is very mild and transient, so analgesics are not required.

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

[redacted]

Hazardous Agent Administration and Use: Citrobacter rodentium

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
- ○ Chemical
- ○ 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] - Regulation of Innate Imm...

2. Please select the biological agent that will be used

   Citrobacter rodentium

3. Indicate the classification for the selected biological agent

[redacted]

☑ 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 rodentium

b. Enter the specific strain of the agent to be used
DBS100

☐ 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]

Indicate the room number(s):
Room [redacted] (BL2)

- 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?

500 million colony forming units 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.

14 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

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

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

[redacted]

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 ◉ Chemical ○ Radioisotope/ Radionuclide

---

1. Please select the chemical hazard to be used.

cholera toxin

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 ○ 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]

[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 per mouse

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

4

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

Once every 3 days for 3 doses, followed by a 4th dose 14 days later.

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

15 days after the last dose

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: Dextran sulphate sodium (DSS)

[redacted]

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 ◉ Chemical ○ Radioisotope/ Radionuclide

---

1. Please select the chemical hazard to be used.

   Dextran sulphate 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 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 ○ 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?

Dissolved in drinking water. Mice will drink ad libitum.

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

Available in drinking water continuously for 7 days.

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

Available in drinking water continuously.

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

7-21 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

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: Trinitrobenzene sulphonic acid (TNBS)

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 ◉ Chemical ○ Radioisotope/ Radionuclide

---

1. Please select the chemical hazard to be used.

Trinitrobenzene sulphonic acid (TNBS)

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 [redacted] 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?

100 microliters per animal

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.

7 or 21 days

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

◉ Yes

[redacted]

○ No

○ 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: Salmonella enterica

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 ○ Chemical ○ Radioisotope/ Radionuclide

[redacted]

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] - [redacted] - Regulation of Innate Imm...

2. Please select the biological agent that will be used

Salmonella enterica

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

Salmonella

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

SL1344, SL3261 (attenuated aroA mutant vaccine strain derived from SL1344)

☐ Recombinant and Synthetic Nucleic Acid Molecules

☐ Biological Toxin

☐ Nonhuman Primate Tissue/Sample

☐ Human Tissue/Sample

[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] (BL2)

- 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?

100 million colony forming units per mouse (oral) or 1000 colony forming units per mouse (IP).

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

One (Expts. 9 & 10) or two (Expt. 15).

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

Once (Expts. 9 & 10) or twice, the 2 doses being separated by 3 weeks (Expt. 15).

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

2 or 5 days after administering SL1344. 26 days after administering SL3261.

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.**

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?

◉ Yes O No

a. Indicate where DEA/Federally Controlled Substances will be stored. If your license is pending or if a secure location is not yet identified, please enter "TBD" in the Room field.

Building:

[redacted]

b. Room:

[redacted]

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:

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

[redacted]

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
Dextran sulfate sodium (DSS)	3% (w/v)	Oral	Continuous administration in drinking water	7 days
Lipopolysaccharide	200 nanograms	Subcutaneous or intra-peritoneal	One dose every 15 days x 2 doses	30 days
Clodronate liposomes	0.2 ml	Intra-peritoneal	One dose	10 days
Trinitrobenzenesulfonic acid (TNBS)	2.5% (w/v)	Intra-rectal	One dose	7 or 21 days
Tribromoethanol	250 mg/kg body weight	Intra-peritoneal	One dose	7 or 21 days
Cholera toxin	10 micrograms	Oral	One dose every 3 days x 9 days followed by 1 dose 14 days later	23 days
Alum	100 micrograms	Intra-peritoneal	Once every 15 days x 2 doses	30 days

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.

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

Please describe:

Alum, cholera toxin, clodronate liposomes, lipopolysaccharide, DSS, TNBS and tribromoethanol are not available in pharmaceutical grade. Therefore, they will be used as non-pharmaceutical grade reagents of the highest purity available.

We cannot use isoflurane or ketamine/xylazine (which are available in pharmaceutical grade) for anesthesia for intra-rectal administration of trinitrobenzene sulfuric acid (TNBS, Experiment 3) because isoflurane has been reported to reduce gut motility (e.g., et al., 2005; 294: 65, et al., . 2014; 26: 1477) and both isoflurane and ketamine have been shown to have significant attenuating effects on inflammation in various tissues, including the intestine (et al., 2004; 10: 1028, et al., . 2010; 111: 1051, et al., . 2012; 178: et al., 2013; 119: 901, et al., . 2015; 194: 599). These potential side effects of isoflurane and ketamine/xylazine anesthesia could adversely affect the outcome of our experiment.

☑ 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.

Brief manual restraint is required during intra-peritoneal injections and oral gavage.

Brief mechanical restraint is required for tail vein blood collection, subcutaneous injections and intravenous injections.

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

[redacted]

◉ Conscious ○ 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.

Brief manual restraint lasting 5-10 seconds will be used during intraperitoneal injections and oral gavage.

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

Restraint will be applied with the use of minimum force and for the minimum time needed for the procedure. The animals will be monitored after the procedure to ensure that they have normal appearance and activity.

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

Restraint will be applied with the use of minimum force and for the minimum time needed for the procedure. The animals will be monitored after the procedure to ensure that they have normal appearance and activity.

☑ 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

[redacted]

- ☐ Restraint duration longer than 15 minutes, but less than 4 hours
- ☐ Restraint duration longer than 4 hours

b. Indicate the frequency of mechanical restraint.

The period of restraint will be a minimum of 1 minute and a maximum of 3 minutes.

The frequency of restraint will be just once in most experiments. In some of the experiments, restraint will be required more than once, as detailed below:

Experiment 9: 2 periods of brief manual restraint on days 14 and 15 in order to administer streptomycin and Salmonella, respectively, by oral gavage.

Experiments 11 and 13: 1 period of brief mechanical restraint to collect tail vein blood on day 14 and 2 periods of brief manual restraint for IP injection on days 15 and 30.

Experiment 12: 1 period of brief mechanical restraint on day 14 to collect tail vein blood and 2 periods of brief mechanical restraint on days 15 and 30 for subcutaneous injection.

Experiments 14: 1 period of brief mechanical restraint on day 14 to collect tail vein blood and 4 periods of brief manual restraint on days 15, 18, 21 and 35 for oral gavage.

Experiment 15: 1 period of brief mechanical restraint on day 14 to collect tail vein blood and 3 periods of brief manual restraint on days 15, 16 and 37 for oral gavage.

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

Restraint will be applied with the use of minimum force and for the minimum time needed for the procedure. The animals will be monitored after the procedure to ensure that they have normal appearance and activity.

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

Restraint will be applied with the use of minimum force and for the minimum time needed for the procedure. The animals will be monitored after the procedure to ensure that they have normal appearance and activity.

[redacted]

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

☑ Monogamous pair - ONE (1) adult male and ONE (01) adult female
☐ 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

[redacted]

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

Colonies of the wild-type C57BL/6 and knockout (Rag2, IL-10, both on C57BL/6 background) strains will be maintained by breeding. In addition, IL-10 knockout x wild-type heterozygote breeding pairs will also be maintained in order to obtain wild-type and knockout littermates. To maintain an adequate supply of the required mice, we will use 2 breeding pairs of each of the 4 relevant strains per year, i.e., 16 mice per year for breeding. Therefore, during the 3 year period of the protocol, we will use 48 mice for breeding purposes. Each breeding pair is expected to give birth to litters of 4-6 pups. The pups are not expected to have any phenotypic abnormalities. They will be weaned and separated from the parents at 3 weeks of age. In the case of the IL-10 knockout heterozygote breeders, the pups will be subjected to tail snips at 3 weeks of age for the purpose of genotyping. The genotyping will identify homozygous wild-type, homozygous IL-10 knockout and heterozygous mice. All 3 types will be used and therefore no culling of pups will be required. In all the other breedings, since we are starting with homozygous wild-type or knockout parents, all the progeny will be homozygotes. Therefore, they will not require tail snips for genotyping or culling. The breeders will be euthanized by carbon dioxide asphyxia once they have reached 1 year of age.

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

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

Please describe.

Ear-tag

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?

[redacted]

◉ Yes ○ 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

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

[redacted]

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.

Genetic control of inflammatory responses

A. At which Institution will the research be conducted?

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

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B. The proposed research project will involve the following:

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

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]

[redacted] **The proposed study involves the use of:**

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

[redacted]

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
2016InspectionReport (Chemical Hygiene Plan)	Electronic
TNBSMSDS (MSDS Sheet)	Electronic
DSSMSDS (MSDS Sheet)	Electronic
[redacted] Resubmission Prereview (Other)	Electronic
PointByPointResponse12-23-16 (Point by Point Response)	Electronic
SalmonellaentericaPSDS (Safety Info)	Electronic
LPSFormulation&MSDS (Safety Info)	Electronic
CholeraToxinFormulation&MSDS (Safety Info)	Electronic
CitrobacterPSDS (Safety Info)	Electronic
ClodronateLiposomes (Safety Info)	Electronic
AlumFormulation&MSDS (Safety Info)	Electronic