JOHNS HOPKINS
UNIVERSITY

Animal Care and Use Committee

1620 McElderry Street
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Baltimore, Maryland 21205-1911
(443) 287-3738 / FAX (443) 287-3747
www.jhu.edu/animalcare

Dr. Abdel Hamad
Department of Pathology

Dear Dr. Hamad:

On 01/04/2017, the Johns Hopkins University Animal Care and Use Committee (ACUC) approved the following research protocol for which you are the Principal Investigator. A copy of the approved protocol is attached.

Protocol Number: MO16M488

TITLE: Inactivation the Fas pathway to T cell autoimmunity (replaces MO14M34)

The approval period is for three (3) years. The ACUC office will send a notice reminding you to submit the 3rd year replacement protocol. This notice will be sent out 90 days prior to the expiration date. Please use this protocol number when placing an order with Research Animal Resources (RAR) (formerly known as Animal Services). They can be contacted by calling 5-3713. Note: Approval of this protocol does not guarantee University space for housing animals.

You may request modifications to this protocol by submitting the appropriate amendment form (i.e., Change in Animal Number, Change in Personnel, or Change in Procedures) to the ACUC office for review and approval. Copies of all our forms can be found on our website www.jhu.edu/animalcare. For guidance on protocol modifications that require amendments, please refer to the reverse side of this letter. If the locations for outside housing or procedures change, please submit a Change in Location Form, also available on the website.

Sincerely,

Nancy A. Ator

Nancy A. Ator, PhD
Chair, Animal Care and Use Committee

Johns Hopkins University Animal Care and Use Committee (ACUC)

PROTOCOL FORM

Release Date: **01/24/2014, minor revision 5/20/2015**

For ACUC Use Only

☐ Original ☑ Revision

PROTOCOL #: M016M488

DATE REC'D: orig 12/1/16; rev 1/3/17

EXPIRATION DATE: 1/4/20

☑ Logged ☑ Database

BSL: 2 3 ABSL: 2 3

New Protocol? _____ Yes _____ No

3rd Year Replacement of Protocol#: ___ M014M34 1/28/17

Do you currently have animals housed on this protocol? ___ Yes ___ No

PROTOCOL TITLE: Inactivation the Fas pathway to prevent T cell autoimmunity

1. PRINCIPAL INVESTIGATOR (PI): Faculty member responsible for design and implementation of the research.

LAST NAME: Hamad **FIRST:** Abdel **M. INITIAL:** **DEGREE (S):** PhD

Title: Associate professor **School:** Medicine **Dep't/Div:** Pathology

Campus Mail Address:

Campus: East **Building:** Ross **Room Number:** 664G

Phone: 410-614-3021 **Fax:** 410-614-3548 **E-mail:** ahamad@jhmi.edu

2. PRIMARY CO-INVESTIGATOR, if applicable: Person who is delegated authority when the PI is unavailable.

LAST NAME: _____ **FIRST:** _____ **M. INITIAL:** ___ **DEGREE (S):** _____

Title: _____ **School:** _____ **Dep't/Div:** _____

Campus Mail Address:

Campus: _____ **Building:** _____ **Room Number:** _____

Phone: _____ **Fax:** _____ **E-mail:** _____

3. ADMINISTRATIVE CONTACT *(to whom the ACUC office may release protocol information if requested)*

Name: Norma Stocker **Phone:** _____ **Fax:** _____ **E-mail:** _____

4a. ANIMAL REQUIREMENTS: A separate protocol form is required for each species. List rodent strains separately. See questions 13 and 17a to list numbers of animals that will be needed.

Genus/Species or Common Name	Strain and/or Stock	Sex	Source (e.g., commercial, another investigator, in-house breeding)	Indicate age and/or weight required
Mouse	NOD-gld/+	F	In-house breeding	4 to 40 weeks
	NOD-LtJ	F	In-house & JAX Lab	2 to 20 weeks
	BDC2.5-NOD-LtJ	F	In-house	4 to 6 weeks
	NOD-IL-10-KO	F	In-house	2 to 40 weeks
	NOD-RAG-KO	F	In-house & JAX Lab	3 to 40 weeks
	NOD-scid	F	JAX Lab	4 to 12 weeks
	NOD-FasL$^{fl/fl}$		In-house	4 to 12 weeks
	NOD-CD19^{cre}		In-house	4 to 12 weeks
	BALB/c	F+M	In-house & JAX Lab	3 to 40 weeks
	BALB/c-gld/gld	F+M	In-house & JAX Lab	3 to 40 weeks
	BALB/c-sdc1-KO	F+M	In-house	3 to 40 weeks
	BALB/c-CD1d-KO	F+M	In-house	3 to 40 weeks
	BALB/c-Ja18-KO	F+M	In-house	3 to 40 weeks
	C3H/Hej		JAX lab	6 to 40 weeks
	C57BL/6	F+M	In house and JAX lab	6 to 40 weeks
		F+M		

The ACUC website, web.jhu.edu/animalcare, contains a formulary for rodent drug doses and ACUC-approved guidelines for many procedures. If you are following these, you can cite them as the method to be used instead of listing all the information in the form.

Mouse	C57BL/6-gld//gld C57BL/6-lpr/lpr C57BL/6-Abi3 bptm1e.1	F+M F+M F+M	In-house In house In-house	2 to 20 weeks 2 to 20 weeks 2 to 20 weeks

4b. Number of rats or mice that might be used from the euthanasia racks per year: __50-100_____
These are cages of animals that have been marked for euthanasia that may be used for training, practice, or tissue harvest using procedures approved in this protocol. The use of these animals must be completed the same day they are taken. Your name will be added to a list of approved users and you will be sent complete instructions if you are not already approved.

5. ANIMAL RESOURCES HOUSING: What is the range (lowest # to highest #) of the number of animals you predict to be housed and cared for in central facilities at any given time?__500- 1000_____

6. SATELLITE HOUSING: Will any animals be housed outside an Animal Resources facility for more than 12 hours if the species is covered by USDA regulations or for more than 24 hours if not? _____ **Yes** __x__ **No**
If **"Yes", complete the Satellite Housing Form (web.jhu.edu/animalcare) and submit as an attachment to this protocol.

7. PROCEDURAL KEYWORD CHECKLIST: *Please read each carefully and check all of the following that apply.* ***Provide requested details in the answer to question 14 unless another question number is indicated.*** (Note: This list is not meant to be exhaustive. It is possible that no items will be checked).

	Antibody production – Indicate if polyclonal or monoclonal antibodies will be produced. Tell whether ascites will be used.*
	Behavioral studies – Methods and apparatus to be used for the purpose of explaining what the animals will experience.*
x	**Breeding colony** - Note: If breeding mice, you will need to complete the "Mouse Breeding Colony Form" that is on the website.*
x	**Caging** – Special caging requirements (e.g., wire bottom, metabolic). Give reasons. High fat diet studies
	Dietary manipulation (i.e., testing the effects of manipulating the content of food or fluid)
	Environmental manipulation – Describe any non-standard environmental manipulation (e.g., altered light cycle, temperature and/or humidity outside normal range)
x	**Euthanize and harvest tissue ONLY—NO other procedures in this protocol, including pre-treatments.**
x	**Food or fluid regulation** – Give durations and information on monitoring weight/hydration status, as applicable.* *Does not apply to pre-anesthetic food restriction.* See question 14f.
	Imaging (X-ray, MR, CT, PET scan, ultrasound, etc.) – Include frequency for individual animals if more than once.
	Infectious disease – See question 19.
x	**Irradiation**– Describe expected effects on animals after the procedure and duration of effects.
	Human embryonic stem cells- Must also submit application to JHU ISCRO (http://eiscro.jhu.edu)
	Non-survival surgery– Describe in 14a
	Paralyzing agents (e.g., during surgery) – Must be used in conjunction with anesthesia and must describe the methods used to determine depth of anesthesia. Also indicate that the animal will be on a ventilator.
	Restraint (other than by hand or briefly) – Indicate the method, rationale, procedures for habituation, durations, and monitoring.*
	Recombinant or synthetic nucleic acids, potentially infectious agents/pathogens, biological toxins or human-derived materials. - See question 19.
	Stress - As the topic of study (e.g. cold exposure, restraint, forced exercise, uncontrollable aversive stimuli). Provide rationale and explain why this particular method is being used.
x	**Survival surgery** - See question 15*.
	Teaching – Type of class(es) or students.
x	**Toxicology or study of effects of chronic drug delivery**– Indicate classes or specific substances to be used; give route(s), sites, and frequencies of administration. Give expected untoward effects and their duration, if known.
	Tumors - Experimentally induced or transplanted – Indicate expected maximum size and plan for monitoring. If xenografts, identify source.*

***See guidelines at:** web.jhu.edu/animalcare

8. LOCATIONS OF PROCEDURES TO BE PERFORMED ON LIVE ANIMALS: If procedures will be performed in an Animal Resources facility, indicate "AR" in place of the room number.

Procedures performed on live animals:	Building(s)	Room Number(s)	Campus
Survival surgery (Major & Minor)	Ross Bldg	664G	East Baltimore
Non-survival surgery or euthanize and harvest	Ross Bldg, BRBG11 and 17	664G	East Baltimore

The ACUC website web.jhu.edu/animalcare contains a formulary for rodent drug dose and ACUC-approved guidelines for many procedures. If you are following these, you can cite them as the method to be used instead of listing all the information in the form.

Behavioral testing			
Imaging			
CO_2 Euthanasia			
Other (specify): Irradiation	MRB Basement	Suite 14	East Baltimore

In answering the questions below, please use terminology that will be understood by a non-specialist. Spell out abbreviations on first use.

9. **OBJECTIVE(S):** Briefly explain the overall purpose of the project.

We will use established animal models to investigate the following human autoimmune diseases:

a) Investigate the role of the Fas death pathway in regulating pathogenesis of type 1 diabetes (T1D)
b) Investigate the role of Fas pathway in regulating immune homeostasis including the autoimmune lymphoproliferative disease (ALPS) caused by double negative (DN) T cells
c) Investigate the role of the Fas death pathway in regulating pathogenesis of inflammatory bowel disease (IBD)
d) Modulation of FasL activity to promote survival of islet transplants
e) Regulation of diet-induced obesity by the heparan sulfate proteoglycan called syndecan-1.

10. **IMPORTANCE OF RESEARCH:** What is the relevance of this work for human or animal health, the advancement of knowledge, or the good of society?
- Our research is expected to reveal new insights into pathogenesis of the human autoimmune diseases mentioned above (T1D, T2D, Insulin-resistance, ALPS, IBD, and islet transplants). Results of the research can lead to novel therapeutic strategies for these diseases. Because the Fas pathway is not critical for host defense, targeting FasL is not expected to cause immune suppression, which is an advantage over the inherent problem associated with biologics use as immunotherapeutics.

11. **RATIONALE FOR ANIMAL USE:** Why are live animals necessary for this study? *Include the reason a non-animal approach such as mathematical models or computer simulation cannot be used.*
- Autoimmune diseases are highly complex and understanding their pathogenesis, which is critical for developing new strategies to prevent or treat these diseases, requires experimental manipulations that are initially not appropriate in humans. Thus, animal models permit in depth preclinical analysis that should provide proof of concepts, evaluation of potential toxicity and road maps for the best approaches to achieve maximum efficacy for protecting high risk individuals and curing patients.

12. **SPECIES SELECTION:** Explain why you selected this species as opposed to another.
- Mouse is used because their immune system is well characterized and because transgenic and knockout mice for various immunologic molecules are commonly available. The following mouse models are well characterized and provide best opportunities for studying the respective human diseases:

- The non-obese diabetic mouse (NOD) is the well-established and the most widely used model for T1D. Studies in the NOD mouse have continued to provide critical insights into the human disease pathogenesis and are excellent for preclinical analysis to assess new therapeutic strategies.

- BALB/c and C57BL/6 mice bearing the gld mutation are well-characterized models for investigating autoimmune lymphoproliferative disease (APLS) of humans. In addition, availability of various knockout mice are important for functional analysis of the disease mechanism.

- C57BL/6 is established colitis model that is commonly used for investigating pathogenesis of IBD.
- Balb/c-sdc1 deficient mice will be used to study diet-induced obesity and metabolic syndrome including insulin resistance.

NOD and B6 mice and F1 (B6 x NOD) and BALB/c mice will be used as recipients and donors in islet transplant experiments. These mouse strains are commonly used in this procedure.

13. NUMBERS OF ANIMALS AND RATIONALE: Explain how many animals (or range of animals) are needed for each experimental condition (e.g., group size) and estimate the total number of animals for the 3 years covered by this protocol (e.g., numbers of groups or experiments). State how you determined that this number per experimental condition is appropriate (e.g., power analysis, previous studies, FDA request, etc.). Use a table showing experimental and control groups if it aids communication. If the total number of animals will not match up with the total for question 17a, please explain.

Genus/Species or Common Name	Strain and/or Stock	Number of Animals per year projected			
		1	2	3	Total
Mouse	NOD-gld/+	100	100	100	300
	NOD- LtJ	200	200	200	600
	BDC2.5-NOD-LtJ	50	50	50	150
	NOD-IL-10-KO	40	40	40	120
	NOD-RAG-KO	20	20	20	60
	NOD-scid	80	80	60	220
	NOD-FasL$^{fl/fl}$	40	40	40	120
	NOD-CD19^{cre}	40	40	40	120
	BALB/c	150	150	110	410
	BALB/c-gld/gld	25	25	30	80
	BALB/c-sdc1-KO	50	100	50	200
	BALB/c-CD1d-KO	20	20	20	60
	BALB/c-Ja18-KO	50	100	50	200
	BALB/c-sdc1-Ja18-DKO	50	100	50	200
	C3H	30	30	30	90
	C57BL/6	150	125	125	400
	C57BL/6-gld//gld	30	30	30	90
	C57BL/6-lpr/lpr	30	30	30	90
	C57BL/6-Abi3bptm1e.1	30	30	30	90
	Total				**3600**

T1D Experiments:

We anticipate to use 80 to 100 NOD-gld/+,180 to 200 NOD-LtJ, and about 50 BDC2.5-NOD-LtJ mice per year in our diabetes studies. The indicated size of each group is required to assess statistical significance of bearing the gld mutation or antibody blockade of FasL on diabetes incidence and for investigating the underlying mechanisms. The cumulative incidence of diabetes in each group will be plotted using Kaplan-Meier method and data will be analyzed for statistical significance using the x^2 test; p value <0.05 will be considered statistically significant.

Autoimmune lymphoproliferation (APLS) experiments:

Thymectomy experiments

BALB/c-sdc1-KO mice: 10 mice as thymectomized and 10 mice as sham controls per the three year period.
BALB/c mice: 10 mice as thymectomized and 10 mice as sham controls per the three year period.
BALB/c-gld/gld mice: 10 mice as thymectomized and 10 mice as sham controls per 3 years period.
C3H-HeJ mice: 15 mice as thymectomized and 15 mice as sham controls per 3 years period

Adoptive transfer experiments to examine the role of sdc1 on the function and homeostasis of DN T cells.

Adoptive transfer will be performed by intravenous (i.v.) injection of 5- to 10 x10^6 donor cells in 100 µl of PBS into indicated hosts using the following genotypes

BALB/c-sdc1-KO mice: 20 mice as donors of thymocytes per three years period
BALB/c mice: 20 mice as donors of thymocytes per three years period

BALB/c-Ja18KO mice: 20 mice per experiment as recipients of thymocytes from BALB/c mice
BALB/c-Ja18KO mice: 20 mice per experiment as recipients of thymocytes from BALB/c-sdc1-KO mice

We expect to perform total of three experiments.

In vitro analysis:
We anticipate using 20 mice from the BALB/c, BALB/c-sdc1-KO, BALB/c-CD1d-KO, and BALB/c-Ja18 per year for analysis of the role of sdc1 on double negative T cell homeostasis, homing and function.

Islet transplant experiments (C57BL/6 (B6), BALB/c; NOD-LtJ; and NOD-scid):
Use of FasL neutralizing antibody to promote islet transplant in allogeneic hosts
B6 islet transplants into STZ-induced diabetes in NOD mice:
- Total of 200 C57BL/6 donors (approximately 2-3 donors per each recipient) per three year period
- Total of 40 NOD-LtJ recipients that will be treated with FasL neutralizing mAb (MFL4) per three year period
- Total of 40 NOD-LtJ recipients that will receive control IgG per three year period

BALB/c islet transplants into STZ-induced diabetes in C57BL/6 mice:
- Total of 200 BALB/c donors (approximately 2-3 donors per each recipient) per three year period
- Total of 40 C57BL/6 recipients that will receive FasL neutralizing mAb per three year period
- Total of 40 C57BL/6 recipients that will receive IgG control per three year period

Use of FasL neutralizing antibody to promote islet transplants to treat spontaneously diabetic NOD hosts
NOD islet transplants into spontaneously diabetic NOD mice:
- Total of 200 NOD-scid donors (2-3 donors per each recipient) per three year period
- Total of 40 NOD-LtJ recipients that will receive FasL neutralizing mAb per three year period
- Total of 40 NOD-LtJ recipients that will receive control IgG per three year period

In vitro analysis of mechanism(s) of suppression: we will use about 20 mice from each strain (C57BL/6, BALB/c; NOD-LtJ; and NOD-scid) for in vitro experiments to determine whether recipient immune system will become tolerized to donor tissues and mechanisms involved (including cell types and cytokines).

Assessment of metabolic dysregulation using indirect calorimetry

Glucose tolerance test:
We expect to use 40 to 50 of the following genotypes for glucose tolerance experiments: BALB/c, Balb/c-sdc1-ko, Balb/c-sdc1 and Ja18 double knockout.

Insulin tolerance Test:
We expect to use a total of 40 to 50 mice of the following genotypes for insulin tolerance test experiments: BALB/c, Balb/c-sdc1-ko, Balb/c-sdc1 and Ja18 double knockout.

Justification for indicated expected number of mice to be used: The indicated size of each group is required to assess statistical significance of for investigating the underlying mechanisms and our indicated endpoint. The cumulative incidence of diabetes in each group will be plotted using Kaplan-Meier method and data will be analyzed for statistical significance using the x^2 test; p value <0.05 will be considered statistically significant.

14a. DESCRIBE THE PROPOSED PROCEDURES: Provide a description of all procedures to be carried out on living animals. **See prompts in question 7 for details of specific procedures to include. For survival surgeries, state which surgeries will be performed but save details of surgery and post-operative care for question 15. For non-survival surgeries, include details here. Save method of euthanasia for question 16d.** ***Further Guidance: a) Blood sampling-*** *include sites, quantities and frequencies of collection.* ***b) Pharmacological assessments –*** *include names of drugs or identify groups by chemical class and/or mechanism of action. Provide the basis on which dose ranges will be determined if specific doses are not known. Indicate possible routes of administration.* ***c)*** *Use ranges for the durations of experimental conditions and other parameters unless these are not likely to change.* ***d)*** *Think ahead to minimize the need to submit amendments later if the possibility of other procedures or modifications of parameters is likely.* **Make sure it will be clear which procedures are being done to each animal or group of animals and the likely sequence.**

Blood collection from the tail vein of mouse. Blood samples will be used to analyze various hemopoietic cells. The blood collection will be performed following the procedure approved by ACUC as described at: www.jhu.edu/animalcare.
In other instances, the tail vein will be pricked with a needle and used oozed blood (~ 5 ul) to measure blood glucose levels by using using Contur test strips. For assessing diabetes development, blood sugar will be assessed **on weekly basis for at least two consecutive weeks** or until we rule out diabetes development **(for a total number of 6 to 10 collections over w 6- to 10 weeks period**.

Irradiation. In adoptive transfer experiments, mice will receive two consecutive doses of 450 rads, 24 hrs apart to eliminate hemopoietic cells, no anesthesia will be used. We use the Gamma cell 40 located on the MRB basement Suite 14 as approved by the imaging and biosafety department.

Survival surgery. Thymectomy, intrathymic injection, and islet transplantation (see 15a for details).

Recombinant DNA and/or biohazardous. We will infect bone marrow with lentivirus encoding siRNA for FasL to inactivate FasL in hemopoietic cells (Biosafety registration# DN0506090208). Using lentiviral vector to silence FasL allows us to analyze the impact of experimental abrogation of FasL in proliferating and non-proliferating tissues. Bone marrow cells from 4- to 6- week-old NOD-wt mice will be infected with the siRNA lentivirus vectors. Transduced cells will be selected using ampicillin and then sorted out using GFP reporter by flow cytometry and then adoptively transferred intraperitoneally into lethally irradiated 2- to 3-week-old NOD-wt mice.

Toxicology or chronic drug administration studies. We will treat NOD-LtJ mice with Fas ligand-neutralizing antibody to assess the potential of blocking the Fas pathway on interrupting the pathogenic process and protecting mice from developing diabetes and/or to reverse disease in new-onset cases. Mice will be injected i.p. on a weekly basis with antibody in 100 μl of PBS for a maximum of 6 weeks.

T1D Experiments:

The purpose of these adoptive transfer experiments is to define the role of different immune cell subsets and the IL-10 cytokine in the protection against autoimmune disease in NOD mice bearing the gld mutation.

Adoptive transfer will be performed by intravenous (i.v.) injection in the tail vein of 5- to 1 x10^7 cells in 100 μl of PBS into indicated hosts; and their activation, proliferation and homeostasis will be analyzed four days after transfer when the mice will be euthanized and tissues (pancreata, pancreatic lymph nodes and spleens) will be isolated and characterized in vitro. To assess statistical significance, each experiment will be repeated three times. No anesthetic will be used.

We anticipate to use 150 NOD-gld/+, 300 NOD-LtJ, and 90 BDC2.5-NOD-LtJ mice per year in our diabetes studies. The indicated size of each group is required to assess statistical significance of bearing the gld mutation or antibody blockade of FasL on diabetes incidence and for investigating the underlying mechanisms. The cumulative incidence of diabetes in each group will be plotted using Kaplan-Meier method and data will be analyzed for statistical significance using the x^2 test; p value <0.05 will be considered statistically significant. We propose to perform the following in vivo and in vitro experiments:

***In vivo analysis* (NOD-gld/+; NOD-LtJ; and BDC2.5-NOD mice):**

Adoptive transfer experiments to examine the effect of gld on the fate and functions of islet-reactive BDC2.5 TCR transgenic cells in NOD-gld/+ host. Adoptive transfer will be performed by intravenous (i.v.) injection in the tail vein (no anesthetic to be used) of 5- to 10 x10^6 donor cells in 100 μl of PBS into indicated hosts using the following genotypes:
NOD-BDC2.5 mice (donors of T cells).
NOD-gld/+ mice (recipients).
NOD-LtJ mice (recipients - controls).
We expect to perform total of three experiments.

Adoptive transfer experiments to examine the role of APCs in gld-mediated protection from diabetes. ***Adoptive transfer will be performed by intravenous (i.v.) injection of 5- to 10 x10^6 donor cells in 100 µl of PBS into tail vein in the indicated hosts using the following genotypes:***

NOD-BDC2.5 (donors of diabetogenic T cells.
NOD-gld/+ mice (donors of gld APCs).
NOD-LtJ mice (donors of wt APCs)

NOD-gld/+ mice (Recipients of wt APCs)
NOD-gld/+ mice (recipients of gld APCs - controls)

NOD-LtJ mice (recipients of gld APCs).
NOD-LtJ mice (recipients of wt APCs - controls)
We expect to perform total of three experiments.

Adoptive transfer experiments to examine the role of B cells in gld-mediated protection from diabetes ***Adoptive transfer will be performed by intravenous (i.v.) injection of 5- to 10 x10^6 donor cells in 100 µl of PBS into tail vein of the indicated hosts:***

NOD-BDC2.5: (donors of diabetogenic T cells).
NOD-RAG-KO mice: (recipients of BDC2.5 T cells and gld B cells).
NOD-RAG-KO: (recipients of BDC.25 T cells and wt B cells).
We expect to perform total of three experiments.

Analysis of the role of IL-10 in protection from diabetes induced by FasL neutralizing mAb.
NOD-IL-10KO mice (recipients of MFL-4 FasL neutralizing antibody).
NOD-IL-10KO (control recipients of hamster IgG).
We expect to perform total of three experiments.

Use of FasL neutralizing antibody to cure or prevent diabetes in NOD-wt mice
NOD-LtJ mice (recipients of MFL-4 FasL neutralizing antibody).
NOD-LtJ mice (recipients of hamster IgG).

Use of Lentivirus to block FasL and prevent diabetes in NOD-wt mice
NOD-LtJ recipients: Total of 20 mice as recipients of siRNA of FasL-lentivirus transuduced with bone marrow per three year period.
NOD-LtJ recipients: Total of 20 mice as control recipients of empty vector-transuduced bone marrow per three year period

In vitro analysis:
We anticipate using about 60 mice from the BDC2.5-NOD, NOD-gld/+ and NOD-LtJ genotypes and about 20 NOD-IL-10-KO and NOD-RAG-KO mice per year for: (*i*) analysis of gld APC stimulatory capacity in vitro (*ii*) analysis of pancreatic islet chemotactic capacity.

Autoimmune lymphoproliferation (APLS) experiments:
The purpose of these experiments is to assess the role of syndecan-1 (sdc1) in regulating homeostasis and function of DN and NKT cells using thymectomy and adoptive transfer. We will use thymecotmized and mice lacking all lymphocytes (RAG-1 deficient) and mice deficient in NKT cells. Adoptive transfer will be done by intravenous injection of 5 to 10 x 10^6 cells. Mice will be euthanized at different time periods of 1 to 4 weeks when various tissues and cell types will be isolated and analyzed.

Thymectomy experiments
BALB/c-sdc1-KO mice: To be used as thymectomized and sham controls.
BALB/c mice: To be used as thymectomized and sham controls).
BALB/c-gld/gld mice: To be used thymectomized and sham controls).
C3H-HeJ mice: To be used as thymectomized and sham controls)

*Adoptive transfer experiments to examine the role of sdc1 on the function and homeostasis of DN T cells. Adoptive transfer will be performed by intravenous (i.v.) injection of 5- to 10 x10^6 donor cells in 100 µl of PBS per **tail vein** into indicated hosts using the following genotypes*
BALB/c-sdc1-KO mice: (donors of thymocytes)
BALB/c mice: (donors of thymocytes)

BALB/c-Ja18KO mice: (recipients of thymocytes from BALB/c mice).
BALB/c-Ja18KO mice: (recipients of thymocytes from BALB/c-sdc1-KO mice).
We expect to perform total of three experiments.

Intrathymic injection of FITC/biotin
Swab the chest area with 70% EtOH. Using a clean fine scissors, start at the sterna notch; make a 3-5 mm incision down through the sternum. Using two curved forceps gently open the ribcage, removing any tissue that may be above the thymus. The tip of the thymus should be visible. Inject 10 µl of 1-3 mg/ml FITC in PBS. Using a curved forceps hold the two flaps of skin together and apply 2-3 sutures. Inject Buprenorphine (0.05 mg/Kg), SC, immediately after surgery. Lay the mouse on its side in a clean cage on a heading pad to recover.

In vitro analysis:
(*i*) analysis of the role of sdc1 on double negative T cell homeostasis, homing and function.

***Islet transplant experiments* (C57BL/6 (*B6*), *BALB/c; NOD-LtJ; and NOD-scid*):**
The purpose of these experiments is to assess efficacy of FasL neutralizing mAb (MFL4 mAb) to promote acceptance of islet transplants. We will use spontaneously diabetic NOD mice and mice that are induced to become diabetic by injection Streptozotocin (STZ). To make 4- to 6-week-old B6 or NOD mice diabetic, single dose of STZ i.p. injection will be used (200 mg STZ per kg body weight, which equals 10µl STZ solution per 1g body weight). We will use donors and recipients of varied backgrounds with the purpose of finding how different recipient and host can be and still promote tolerance using FasL blocking mAb.

Use of FasL neutralizing antibody to promote islet transplant in allogeneic hosts
B6 islet transplants into STZ-induced diabetes in NOD mice:
- C57BL/6 mice will be used donors
- NOD-LtJ recipients will be treated with FasL neutralizing mAb (MFL4) or control IgG

BALB/c islet transplants into STZ-induced diabetes in C57BL/6 mice:
- BALB/c donors will be used as donors.
- C57BL/6 recipients will receive FasL neutralizing mAb or IgG isotype control.

Use of FasL neutralizing antibody to promote islet transplants to treat spontaneously diabetic NOD hosts
NOD islet transplants into spontaneously diabetic NOD mice:
- NOD-scid will be used as donors.
- NOD-LtJ recipients will receive FasL neutralizing mAb or control.

In vitro analysis of mechanism(s) of suppression: we will use about 20 mice from each strain (C57BL/6, BALB/c; NOD-LtJ; and NOD-scid) for in vitro experiments to determine whether recipient immune system will become tolerized to donor tissues and mechanisms involved (including cell types and cytokines).

Regulation of diet-induced obesity by syndecan-1
Objective. To determine whether mice genetically deficient in sdc1 gene are resistant to DIO.
Procedure. Mice will be weighed and then administered a high fat diet (60% fat) for up to 18 weeks. Body weight will be recorded on weekly basis and food consumed measured. We will subject the mice to glucose tolerance test and insulin resistance test (described below). For retro-orbital bleeding, we will use topical ophthalmic anesthetic e.g. proparacaine or tetracaine drops as general anesthesia will not be compatible with our experiment goal. We will collect 100 to 150 ul with at least 15 days between collections to measure insulin and adipokines. As alternative option, we will use lateral vein and ventral dorsal artery sampling to measure insulin and adipokines.

Assessment of metabolic dysregulation using indirect calorimetry

We will use the service center at the Center for Metabolism and Obesity Research (CMOR, IBBS) to perform indirect calorimetry assessment under supervision of Dr. Susan Aja and using her SOP described in her protocol SP12M338.

Insulin tolerance test: Mice will be maintained in a normal light/dark cycle according to the standard protocols of JHU Animal Care and Use Committee. Mice are tested with age matched or litter mate controls.

On the day of the test, animals are weighed, the tail is nicked with a fresh razor blade by a horizontal cut of the very end, ~35 to 50 microliters of blood is very gently massaged from the tail to an eppendorf tube which is immediately placed on ice, baseline blood glucose is measured by the glucose oxidase method using a Glucometer Elite glucometer, and 1.0 units per kg body weight of diluted Regular Human Insulin (Lily) is drawn up in a 29 gauge 1/2" insulin syringe (insulin is diluted to 1:1000 (0.1 units/ml) with regular insulin diluent) (1 of a unit of 1:1000 insulin for every gram of body weight). Animals are transferred to individually labeled 1000cc cardboard soup cups with the lid liners removed. We will use least amount possible and no more than 50 ul total blood will be collected from a single mouse within a 24 hr-time-period.

When all mice have been prepared the test is begun. Insulin is injected into the intraperitoneal cavity (i.p.). At 15, 30, and 60 minutes, the blood glucose is sampled from the tail of each mouse by gently massaging a small drop of blood onto the glucometer strip (contour TS Glucometer). Insulin injections and blood glucose sampling is timed to take approximately the same amount of time per animal (i.e. 25 animals are injected in 12 minutes and blood glucose sampling of those same 25 animals should also take about 12 minutes) so that the sample times are accurate for each animal.

Random fed immunoreactive insulin levels: whole blood samples are spun in a refrigerated microfuge at 14,000 rpm for 10 minutes and transferred to a clean tube. 6 microliters of serum is tested using an ELISA assay (Crystal Chem) with mouse insulin as a standard according to the standard protocol.

Glucose tolerance Test (GTT): Mice are fasted overnight for 6 h or overnight 1(6-18 h) with free access to water in clean cages and with new clean bottles. Each mouse is then weighed, and a baseline fasted blood glucose measurement is taken by applying tail blood to a contour TS Glucometer (Lifescan Canada Ltd.). Each mouse is injected i.p. with a filter-sterilized solution of 25 mg/ml D-glucose, with the size of the bolus determined by animal weight (different doses ranging from 1.5 to 5 mg glucose/g body weight will be used). Blood glucose measurements will be taken as described above for each animal at 15, 30, 45, 60, 90, and 120 minutes. We will use least amount possible and no more than 50 ul total blood will be collected from a single mouse within a 24 hr time period. The data are plotted as blood glucose concentration (mg/dL) over time (minutes).

14b. WILL ALL DRUGS USED FOR SEDATION, ANESTHESIA, ANALGESIA OR EUTHANASIA NAMED IN THIS PROTOCOL (Questions 14a, 15, 16d) BE "PHARMACEUTICAL GRADE"? ****See "Non-pharmaceutical Grade Drug Policy" at web.jhu.edu/animalcare for full definitions of "pharmaceutical grade" and exceptions and for JHU requirements on preparation and storage.**** ___ **Yes** ___ **No** ____x___ **Not applicable.** Formulations used for these purposes must be those sold for clinical use (i.e., "pharmaceutical grade") unless an alternative formulation is necessary due to (1) scientific considerations or (2) non-availability of the preferred compound in a clinical use formulation that can be used unaltered (e.g., without dilution). **If the answer above is "No", state below the name of any drugs for these purposes that may not be pharmaceutical grade and your reason for using the non-pharmaceutical grade version.**

Drugs are mixed to reduce the number of injection for each animal.

14c. WILL ALL DRUGS/CHEMICALS USED AS RESEARCH TOOLS OR THE SUBJECT OF INVESTIGATION BE "PHARMACEUTICAL GRADE"? __x___ **Yes** _____ **No** ____ **Not applicable.** If **"No"**, state below the name or class of the compound and the reason that non-pharmaceutical grade is necessary. **Reasons could include: (1) non-availability in a clinical use formulation or in one that could be used unaltered; or clinical use formulation is not suitable for desired mode of delivery (e.g., is a pill); (2) scientific considerations (e.g., lack of suitable vehicle control, presence of preservatives, necessity of manipulating concentration, comparability with previous studies), (3) use of drugs provided by NIH drug supply program.** ****See "Non-pharmaceutical Grade Drug Policy" at** web.jhu.edu/animalcare *for full policy and requirements for preparation and storage.****

14d. MULTIPLE SURVIVAL SURGERIES: If you indicated in 14a that a survival surgery (i.e., any procedure involving an incision) will occur, state whether any animal will undergo more than one survival surgery (i.e., involving separate periods of anesthesia and recovery). This includes any surgeries yet to be fully described in #15. **_____ Yes __x__ No _____ Not applicable**. If **"Yes"**, the order and the interval (or range of intervals) between them should be stated. Below, provide the scientific justification for multiple survival surgeries in the same animal. (Note: Neither cost savings alone nor reduction of the number of animals needed is considered adequate.)

14e. BREEDING: Will **animals** be bred at JHU under this protocol? **__x____ Yes _____ No**

If **"Yes"** provide a rationale below for why breeding is needed for this protocol and for the planned number of breeders indicated in Question 17a.

If breeding **mice,** complete the **Mouse Breeding Colony Form** (web.jhu.edu/animalcare).

If breeding **species other than mice,** provide details below (i.e., projected number of offspring per year; breeding age range; weaning age; genotyping, if applicable; special care; etc.):

14f. FOOD OR FLUID REGULATION: Refers to regulation of food/fluid as a necessary component of an experimental design (*e.g., "scheduled access" where the animal is given unrestricted access to food/fluid for one or more periods of time each day or "restricted access" where amount of food/fluid per day is controlled).* This does not refer to restriction before anesthesia (i.e., pre-operative). Will food or fluid be regulated? **__x__ Yes ____ No.**
If **"Yes",** a justification and description will need to be provided below if not already included in 14a. See **"Food and Fluid Regulation"** guideline (web.jhu.edu/animalcare) for a list of the specific information you will need to include when answering this question.
Provided above

15. SURVIVAL SURGERY: ***(If more than one type will be performed, fill out a separate question 15 for each one.)***
Name of Surgical Procedure: Intrathymic injection of biotin (8- to 12-week-old)

15a. MAJOR OPERATIVE PROCEDURE? Will this surgery penetrate and expose a major body cavity or cause substantial impairment of physical or physiologic function? ___x_ Yes ____ No

15b. Pre-anesthetic agents (e.g., to permit handling, intubation). ***Name, dose, and route.***

Buprenorphine (0.05 mg/Kg), SC, 30 min before surgery and immediately after surgery.

15c. Pre-emptive analgesia (i.e., analgesia given prior to the surgical procedure). ***Name, dose, and route.***
Buprenorphine (0.05 mg/Kg), SC, 30 min before surgery

15d. Anesthesia. ***Name, dose, and route.*** **Also state the method that will be used to assure the animal is anesthetized prior to incision.**

15e. Will a neuromuscular blocking agent (paralytic) (i.e., one that prevents respiration) be used at any point in the procedure? ____ Yes __x___ No If "Yes", state name, dose, route and frequency of administration of paralytic agent(s). What parameter(s) will be used to determine that the animal remains anesthetized after the paralytic has been administered?

15f. Describe intra-operative procedures including: monitoring, surgical procedure, method of wound closure, etc. Must be aseptic technique including assurance regarding the use of gown, gloves, mask, and sterilized instruments. Include name, dose, and route of any intra-operative analgesia.

Intra-operative procedures. Aseptic technique (gloves, mask, autoclaved instruments etc) will be used throughout the procedure, which consists of the following steps. Buprenorphine is used as a pre-operative analgesic (0.05 mg/Kg, SC) just prior to surgery and then as needed up to every 12 hrs. The mice are then anesthetized using 65 mg/kg ketamine, 13 mg/kg xylazine and 2 mg/kg acepromazine I. Surgery will be performed after surgical plane of anesthesia is verified by the lack of reaction to firm hind limb toe pinch. The surgical area of the mouse is shaved and cleaned with iodine or chlorhexidine solution and aseptic techniques practiced. Using a clean fine scissors, start at the sterna notch; make a 3-5 mm incision down through the sternum. Using two curved forceps gently open the ribcage, removing any tissue that may be above the thymus. The tip of the thymus should be visible. Inject 10 ul of 1-3 mg/ml FITC in PBS. Using a curved forceps hold the two flaps of skin together and apply wound clips. Inject Buprenorphine (0.05 mg/Kg), SC, immediately after surgery. Lay the mouse on its side in a clean cage on a heading pad to recover. Buprenorphine (0.05 mg/Kg, SC), is injected immediately after surgery. Mice will be monitored continuously after anesthesia until ambulatory. The mouse is allowed to recover in a cage with a heat lamp or heat pad, under close supervision. The wound clips are removed after two weeks.

15g. Describe immediate post-operative care (first 24 hours). Include monitoring, supportive care, and analgesia.

Buprenorphine (0.05 mg/Kg), SC, ~ 6 hrs after surgery. The mice will be monitored twice daily for the first 2 days and then daily.
Mice are evaluated for general appearance, ability to move normally around cage and reach food and water, ability to eat and drink, grooming habits, skin color, lethargy, and aggression (animals will be housed individually if aggressive behavior is shown).

15h. Describe post-operative care procedures after the first 24 hours, including plan for monitoring, suture removal, further analgesia, special feeding, etc:

When surgical clips are used, and they will be removed 10-14 days post surgery.
Transplanted mice will be followed until rejection by measuring blood glucose level for 100 days at which the mice will be euthanized. Mice will be monitored daily for the first 3-5 days and weekly thereafter. Mice will be evaluated twice a week by using Body condition Score Index (BCS) that ranges from 1 (emaciated) to 5 (obesity). Mice with BC1 (skeletal structure extremely prominent; little or no flesh cover, vertebrae distinctly segmented) or BC2 (mouse is under-conditioned with evident vertebral column segmentation and dorsal pelvic bones readily palpable) will be euthanized.

15. SURVIVAL SURGERY: ***(If more than one type will be performed, fill out a separate question 15 for each one.)***
Name of Surgical Procedure: __Islet transplantation (12- to 14-week-old)

15a. MAJOR OPERATIVE PROCEDURE? Will this surgery penetrate and expose a major body cavity or cause substantial impairment of physical or physiologic function? ___x_ Yes ____ No

15b. Pre-anesthetic agents (e.g., to permit handling, intubation). ***Name, dose, and route.***

Same as above

15c. Pre-emptive analgesia (i.e., analgesia given prior to the surgical procedure). ***Name, dose, and route.***
Same as above

15d. Anesthesia. ***Name, dose, and route.*** **Also state the method that will be used to assure the animal is anesthetized prior to incision.**

The ACUC website web.jhu.edu/animalcare contains a formulary for rodent drug dose and ACUC-approved guidelines for many procedures. If you are following these, you can cite them as the method to be used instead of listing all the information in the form.

15e. Will a neuromuscular blocking agent (paralytic) (i.e., one that prevents respiration) be used at any point in the procedure? ____ Yes __x___ No If "Yes", state name, dose, route and frequency of administration of paralytic agent(s). What parameter(s) will be used to determine that the animal remains anesthetized after the paralytic has been administered?

15f. Describe intra-operative procedures including: monitoring, surgical procedure, method of wound closure, etc. Must be aseptic technique including assurance regarding the use of gown, gloves, mask, and sterilized instruments. Include name, dose, and route of any intra-operative analgesia.

Intra-operative procedures. Aseptic technique (use of gloves, mask, autoclaved instruments etc.) will be used throughout the procedure. The procedure consists of the following steps. Buprenorphine is used as a pre-operative analgesic (0.05 mg/Kg, SC) just prior to surgery and then as needed up to every 12 hrs. The mice are then anesthetized using 65 mg/kg ketamine, 13 mg/kg xylazine and 2 mg/kg acepromazine I. The surgical area of the mouse is shaved and cleaned with iodine or chlorhexidine solution and aseptic techniques practiced. With aid of dissecting microscope, a small incision is made in the left flank and the left kidney is exposed. Approximately 500-1000 islets are transplanted under the kidney capsule using a Hamilton syringe with P-150 polypropylene tubing. Homeostasis is maintained, the tissue is moistened with saline and the kidney is placed back into the abdominal cavity. The peritoneum is then closed with 5-0 silk sutures and the skin is closed with wound clips. Buprenorphine (0.05 mg/Kg, SC), is injected immediately after surgery. Mice will be monitored continuously after anesthesia until ambulatory. The mouse is allowed to recover in the cage with a heat lamp or heat pad, under close supervision. The wound clips are removed after two weeks.

15g. Describe immediate post-operative care (first 24 hours). Include monitoring, supportive care, and analgesia.

Same as above

15h. Describe post-operative care procedures after the first 24 hours, including plan for monitoring, suture removal, further analgesia, special feeding, etc:

When surgical clips are used, they will be removed 10-14 days post surgery.
Transplanted mice will be followed until rejection or 100 days at which time the mice will be euthanized. Mice will be monitored twice a week using BCS and those with BC1 or BC2 will be immediately euthanized.

15. SURVIVAL SURGERY: *(If more than one type will be performed fill out a separate question 15 for each one.)*
Name of Surgical Procedure: _Thymectomy (8- to 12-week-old)_____

15a. MAJOR OPERATIVE PROCEDURE? Will this surgery penetrate and expose a major body cavity or cause substantial impairment of physical or physiologic function? __x__ Yes ____ No

15b. Pre-anesthetic agents (e.g. sedatives to permit handling, intubation). *Name, dose, and route.*
Same as described above

15c. Pre-emptive analgesia (i.e., analgesia given prior to the surgical procedure). *Name, dose, and route.*
Buprenorphine (0.05 mg/Kg), SC, 30 min before surgery

15d. Anesthesia. *Name, dose, and route.* Also state the method that will be used to assure the animal is anesthetized prior to initial incision and during the surgical procedure.

15e. Will a neuromuscular blocking agent (paralytic) (i.e., one that prevents respiration) be used at any point in the procedure? ____ Yes __x___ No If **"Yes"**, state name, dose, route and frequency of administration of paralytic agent(s). What parameter(s) will be used to determine that the animal remains anesthetized?

15f. Describe intra-operative procedures including: intubation, IV fluid delivery, monitoring, surgical procedure, method of wound closure, etc. Must be aseptic technique including assurance regarding the use of gown, gloves, mask, and sterilized instruments. Include name, dose, and route of any intra-operative analgesia, antibiotic or other drug. DO NOT REPEAT INFORMATION ALREADY PROVIDED ABOVE.

Intra-operative procedures. Aseptic technique (use of gloves, mask, autoclaved instruments etc.) will be used throughout the procedure. The procedure consists of the following steps. Buprenorphine is used as a pre-operative analgesic (0.05 mg/Kg, SC) just prior to surgery and then as needed up to every 12 hrs. The mice are then anesthetized using 65 mg/kg ketamine, 13 mg/kg xylazine and 2 mg/kg acepromazine I. The surgical area of the mouse is shaved and cleaned with iodine or chlorhexidine solution and aseptic techniques practiced. The following steps are then used: (1) Make a midline longitudinal skin incision over the suprasternal notch, using a scissor. Extend the incision, 1 cm down the chest); (2) Loosen the skin from underlying muscle using the blunt end of forceps, reflect the skin to expose the thoracic cage; (3) Stabilize the thoracic cage, insert scissors under the sternum to cut the second and third rib; (4) Insert the tips of closed caudal forceps into the incision, expose the chest by allowing the forceps to open, using the cranial forceps, retract the strap muscle, remove the thymus by suction; (5) Insert the suction cannula and place the tip over the lower pole of one thymus lobe; (6) Apply suction by mouth or vacuum and simultaneously lift the lobe up and toward the head so the thymic lobe lifts off the heart and with gentle manipulation is aspirated into the cannula; (7) Repeat the procedure for the other lobe; (8) Inspect the thymus to make sure both lobes are intact; (9) Remove forceps and use tissue glue to seal and close the chest; (10) Wipe excess blood from the incision site and place the mouse on its side in a clean cage. Buprenorphine (0.05 mg/Kg, SC), is injected immediately after surgery. Mice will be monitored continuously after anesthesia until ambulatory. The mouse is allowed to recover in the cage with a heat lamp or heat pad, under close supervision. The wound clips are removed after two weeks.

15g. Describe first 24 hours of post-operative care. Include frequency of monitoring, supportive care, and analgesia.

Same as described above. Surgical clips will be removed 10-14 days post surgery.

15h. Describe post-operative care procedures after the first 24 hours, including plan for frequency of monitoring by laboratory personnel, suture removal, further analgesia, special feeding, etc.

If surgical clips are used, they will be removed 10-14 days post surgery.
Transplanted mice will be followed until rejection or 100 days at which time the mice will be euthanized. **Blood glucose levels in each mouse were observed during the ensuing period and mice that become diabetic will be euthanized and tissue processed for flow cytometry, cytokine production and histology. In addition,** mice will be monitored twice a week using BCS and those with BC1 or BC2 will be immediately euthanized.

16a. PLANNED ENDPOINT/EUTHANASIA: State the time point or other criterion in the experiment at which euthanasia will occur for each animal or experimental group if the study goes as planned. If this has been provided in 14a, please refer reader back to that section. Save method of euthanasia for 16d.

- Experimental animals will be euthanized to collect tissues for various immunological analyses. The time of euthanasia will be determined based on the purpose of the experiments as indicated for each procedure. Although experimental animals are expected to remain healthy throughout the duration of experiments, early euthanasia will be performed if any of the conditions described below occurs.

- We will use an overdose of isoflurane as the method of euthanasia in the laboratory. Authorized and trained postdoctoral fellows and technicians in the laboratory will perform euthanasia. Death will be verified by cardiac and respiratory arrest and by fixed and dilated pupil of the animal.
- For routine thinning of the mouse colony we will request Animal Resources personnel to carry out the euthanasia.

16b. If euthanasia is not required by the study, or for particular animals, indicate the possible disposition of the animals (e.g., adoption, transfer to another study). Note: At the discretion of the Attending Veterinarian, animals may be adopted out without specific inclusion of that option in the protocol.

Retired breeders and pups of undesired sex or genotype will be euthanized and Animal Resources personnel will be requested to carry out the euthanasia.

16c. Give the health conditions and/or criteria under which early euthanasia or withdrawal of an animal from the study will be considered. These include, but are not limited to, general signs of distress such as hunched posture, lethargy, anorexia, dehydration, rough hair coat etc and/or those that are directly related to the experimental procedures (e.g. tumor ulceration, dislodged/unrepairable headcap, etc.).

In case of gamma irradiation, if 48 hrs after bone marrow transplantation, the mice appear extremely lethargic, are not eating and show signs of piloerection or distress (hunched posture, lethargy, anorexia, dehydration, rough hair coat), these animals will be removed from the study and euthanized. In the colitis studies if weight loss in the mice is ≥ 25% of the start body weight, the mice will be withdrawn from the experiment and euthanized. In case of islet transplant, recipient mice that remain diabetic 24 hr after islet transplant (blood sugar of >250 mg/dl) will be euthanized.

16d. Which method(s) of euthanasia will be used by laboratory personnel? State how death will be verified before disposal (give two methods if euthanasia is other than decapitation). *See JHU's Euthanasia Guideline at web.jhu.edu/animalcare for suggested methods.*

CO2 inhalation followed by decapitation using a sharp blade, alternatively an over dose of isoflurane will be used.

17a. PAIN/DISTRESS: Indicate in the table below the number of animals that will fall in each Pain/Distress category. In *addressing pain and distress, please consider all aspects of the study (e.g., surgery, phenotype of the animal, induced disease, tumor burden, behavioral procedures).* For protocols that involve more than one procedure, place animals in the category that pertains to the greatest degree of pain, distress and/or discomfort to which the animal will be exposed. *Do not count an animal more than once*. The total number of animals entered must agree with the total animals in question 13 unless the discrepancy is explained there. The total should not include the number in answer to question 4b (euthanasia rack). ***Use of anesthesia for rodent tail snips or purely for restraint (e.g., for imaging) does not require placement in Category D.***

Categories	Examples	Total Number of Animals for 3 Years (Entered into ACUC database at time of protocol approval)
B—Breeding - number of males and females to be used for breeding	e.g., breeding only, no other procedures	200-300 mice
C—Procedures cause momentary, slight, or no pain/distress in absence of analgesia or anesthesia	e.g., injections, euthanasia, blood collection, brief restraint, imaging	3040 mice
D—Procedures potentially are painful but anesthetics and/or analgesics are given	e.g., surgery, blood collection by invasive routes	360 mice
E—Procedures involve pain/distress that will not be alleviated by drugs	e.g., toxicity studies, pain or stress studies, some disease models	0 mice

****** Answer 17b and c below only if any animals fall into Category D or E. ******

17b. Which procedure(s) or other elements in this protocol fit the definition of Category D and/or E as given in the chart above?
Thymectomy (category D); islet transplant (category D).

17c. Are there alternative methods to those named in 17b that could be used that would produce less pain and distress and achieve the same experimental purpose? EITHER state approaches that might seem to be reasonable alternatives to the ones in 17b and explain why they will not accomplish the experimental objective with less pain/distress OR carry out a Keyword/Literature Search. DO NOT DO BOTH.

The ACUC website web.jhu.edu/animalcare contains a formulary for rodent drug dose and ACUC-approved guidelines for many procedures. If you are following these, you can cite them as the method to be used instead of listing all the information in the form.

If you choose the first approach, state the reasons you can be confident that you have relevant and up-to-date information on the topic. Reasons could include: 1) consultation with an expert in the research area (give name/qualifications), 2) regular attendance at scientific meetings (names of organizations), 3) regular attention to the scientific literature on the topic (cite sample journal names), and/or 4) personal experience with the alternative method.

The goal of thymectomy is to identify origin of T cells that cause lymphoproliferation in gld mutant mice. Therefore, we plan to stop generation of new T cells by removing the thymus and analyze the capacity of existing T cells to cause lymphoproliferation in thymectomized mice. **Unfortunately, there is no alternative to this surgical procedure: Online search of the literature using PubMed (1917 up todate) under the keywords: procedure, non-animal model, in vitro, and thymectomy yielded no alternative approach for thymectomy, whereas search of the PubMed using only the key word "thymectomy" produced 8062 articles.**

Islet transplant is increasingly used for treating T1D patients who do not respond well to insulin therapy. However, still there are many unknowns about the mechanism that require significant improvements. In addition scarcity of compatible donors necessitates use islets from allogeneic or incompatible donors. Therefore, finding therapeutic approach to facilitate survival of transplanted islets is highly beneficial to T1D patients and the proposed studies this protocol are expected to provide mechanistic and preclinical insights that will help reaching this goal. **Up to now, there is no alternative to islet transplant in mice too assess mechanisms and efficacy: Online search of literature using PubMed (1959 up todate) using the keywords: procedure, non-animal model, in vitro, and islet transplants yielded no article that describes an alternative non-animal approach; on the other hand, search of PubMed with the key word "islet transplant" alone produced 6833 articles.**

If you do a Keyword/Literature search provide the following information:

Date (day, month, year) search was performed: __11/30/2016_____

Years covered by search: __1959-present_____

Keywords used in search: These must be in relation to alternatives to the procedure(s) named in 17b, not in relation to use of animals.
Thymectomy, islet transplantation, intrathymic injection

Number of hits: Thymectony (9043); islet transplantation (11256); intrathymic injection (416)_____

Databases searched (check all that apply):
__x__ MEDLINE /PUBMED __x__ AWIC _x___ TOXLINE _x__ AGRICOLA ____ Other (describe)

Did the literature search reveal one or more alternatives that cause less pain/distress than the methods proposed but accomplish the same scientific purpose? ____ **Yes** _x__ **No** If **"Yes"**, explain why the alternative is not being used.

18. ENVIRONMENTAL ENRICHMENT: Requests for exemption from the JHU program. See *http://web.jhu.edu/animalcare/links.html* for detailed information on the JHU Environmental Enrichment/Social Housing program for all species. *Unless an exemption is approved, the animals under this protocol will be included in the campus-wide plan for this species.*

ALL SPECIES:

a. **Most animals will be provided toys and/or other non-food enrichment items (such as nesting material for mice). Are you seeking an exemption for scientific reasons from such enrichment for any portion of the work covered in this protocol? _____ Yes ___x__ No If "Yes", explain below.**

b. **Most animals (other than mice and rats) are periodically provided edible enrichment. Are you seeking an exemption for scientific reasons for any portion of the work covered in this protocol? _____Yes _____No ___x__ Not Applicable. If "Yes", explain below.**

The ACUC website web.jhu.edu/animalcare contains a formulary for rodent drug dose and ACUC-approved guidelines for many procedures. If you are following these, you can cite them as the method to be used instead of listing all the information in the form.

c. **Will you be providing edible or other enrichment beyond or in place of that provided by central facilities for your animals? _____ Yes __x___ No If "Yes", state how. NOTE: Nylabones and plastic cylinders and huts for rats or mice may be chosen at any time as per the JHU Enrichment Program without explicit approval in this protocol.**

d. **Most animals will be routinely housed in pairs or groups with others of their species. Are you seeking an exemption for scientific reasons from JHU's social housing plan for this species for any portion of the work covered in this protocol? _____ Yes _____ No If "Yes", explain below.** Note: The Attending Veterinarian may exempt your animals from social housing for health or other well-being concerns even if you do not seek an exemption for scientific reasons.

DOGS:

e. **If this protocol covers dogs, are you seeking an exemption for scientific reasons from JHU's exercise program? _____ Yes ___x__ No If "Yes", state reason.**

19. BIOHAZARDOUS, RADIOACTIVE, OR CHEMICALLY HAZARDOUS AGENTS USED IN ANIMALS.
Use of certain recombinant or synthetic nucleic acids, infectious or other biohazardous agents requires approval from Health, Safety & Environment's Institutional Biosafety Office/Committee (410-955-5918).

Type of Hazardous Agent	Yes	No	Name of Agent	Current Approval Date*	Biosafety Regis. # or Radiation Safety #
BIOHAZARDS:	x		siRNA for FasL to inactivate FasL in hemopoietic cells	6/1/2016	**DN0506090212**
Recombinant or synthetic nucleic acids	X		Lentivirus	6/13/2016	**P0506090212**
Bacteria, parasites virus or viral-based vectors or other pathogens		x			
Human tissues or cell lines		x			
RADIOACTIVE COMPOUNDS		x			
CHEMICALS (e.g., lead, MPTP) that render the animal's waste toxic		x		Not applicable	Not applicable

***If Biosafety Committee approval is pending, attach a copy of the IBC application.**

NOTE: If it is determined that an animal given the agent(s) named above has the potential to be hazardous to people (e.g., through exposure to its urine or feces), you are required to label the cage, rack, or room door with an appropriate card at the time of initial exposure and for the duration of hazardous effect. Instructions for biohazards will be included with the Approval Letter for this protocol.

20. TRAINING & QUALIFICATIONS: Provide specific information on training and/or experience that qualifies each person to perform the procedures involving the species of animal in this protocol. Make additional copies of these pages as necessary.

If any individuals listed below have not previously been included on an approved protocol at JHU they must complete the online Animal Care and Use training course (link is found on our website web.jhu.edu/animalcare or through *My Learning* (http://www.hopkinsmedicine.org/interactive_learning/my_learning/) and enroll in the Animal Exposure Surveillance Program (AESP) before the protocol can be approved (contact Occupational Health at 410-955-6211). Or the person can be removed and added after protocol approval via personnel amendment.

The ACUC website web.jhu.edu/animalcare contains a formulary for rodent drug dose and ACUC-approved guidelines for many procedures. If you are following these, you can cite them as the method to be used instead of listing all the information in the form.

The AESP certificate of enrollment for these individuals must be supplied to the ACUC Office before final approval of the protocol may be granted.

Note: For protocols used only for Teaching/Training trainees do not need to be listed in question 20.

Role: **Principal Investigator**

Last Name: Hamad First Name: Abdel M. Initial:

1) ***Degree(s) held.*** *Ph.D*

2) ***Specialty and/or major for each degree listed above.*** *Immunology*

3) ***List procedures this person will be performing (can state "all" if appropriate).*** *Supervisor*

4) ***Describe the person's experience with the procedures and the species in this protocol. Significant***

5) ***If training and/or supervision of this person is necessary, who will be providing it?***

ACUC office use: AESP ✓ OLT ✓ VIG

Role: **Primary Co-investigator (person delegated authority when PI is unavailable)**

Last Name: First Name: M. Initial:

1) ***Degree(s) held.***

2) ***Specialty and/or major for each degree listed above.***

3) ***List procedures this person will be performing (can state "all" if appropriate).***

4) ***Describe the person's experience with the procedures and the species in this protocol.***

5) ***If training and/or supervision of this person is necessary, who will be providing it?***

ACUC office use: AESP __ OLT __

Role: ☐ Co-Investigator ✕ Fellow ☐ Student ☐ Faculty ☐ Staff ☐ Outside Collaborator (Check all that apply.)

Last Name:	Jaiswal	First Name:	Anil	M. Initial:
Department:	Pathology	Phone Number:	2-2832	
JHU Address:	720 Rutland	Bldg & Room:	Ross 664G	
Email Address:	ajaiswa2@jhmi.edu			

1) ***Degree(s) held.*** *Ph.D*

2) ***Specialty and/or major for each degree listed above.*** *Immunology*

3) ***List procedures this person will be performing (can state "all" if appropriate).*** *All*

4) ***Describe the person's experience with the procedures and the species in this protocol. 5 years***

5) ***If training and/or supervision of this person is necessary, who will be providing it?***

ACUC office use: AESP ✓ OLT ✓

Role: ☐ Co-Investigator ✕ Fellow ☐ Student ☐ Faculty ☐ Staff ☐ Outside Collaborator (Check all that apply.)

Last Name:	Ahmed	First Name:	Rizwan	M. Initial:
Department:	Pathology	Phone Number:	2-2832	
JHU Address:	720 Rutland Ave	Bldg & Room:	Ross 664G	
Email Address:	rahmed14@jhu.edu			

1) ***Degree(s) held.*** *Ph.D*

2) ***Specialty and/or major for each degree listed above.*** *Immunology*

3) ***List procedures this person will be performing (can state "all" if appropriate).*** *Diabetes*

4) ***Describe the person's experience with the procedures and the species in this protocol.*** *2 years*

5) ***If training and/or supervision of this person is necessary, who will be providing it?***

ACUC office use: AESP ✓ OLT ✓

ACUC office use: AESP __ OLT __

The ACUC website web.jhu.edu/animalcare contains a formulary for rodent drug dose and ACUC-approved guidelines for many procedures. If you are following these, you can cite them as the method to be used instead of listing all the information in the form.

Role: ☐ Co-Investigator ☒ Fellow ☐ Student ☐ Faculty ☐ Staff ☐ Outside Collaborator (Check all that apply.)

Last Name:	Sadasivam	First Name:	Mohan	M. Initial:
Department:	Pathology	Phone Number:	2-2832	
JHU Address:	720 Rutland Ave	Bldg & Room:	Ross 664G	
Email Address:	smohanr1@jhu.edu			

1) ***Degree(s) held.*** *Ph.D*

2) ***Specialty and/or major for each degree listed above.*** *Immunology*

3) ***List procedures this person will be performing (can state "all" if appropriate).*** *DN T cell studies*

4) ***Describe the person's experience with the procedures and the species in this protocol.*** *5 years*

5) ***If training and/or supervision of this person is necessary, who will be providing it?***

ACUC office use: AESP ✓ OLT ✓ ✓M

ACUC office use: AESP ___ OLT ___

Role: ☐ Co-Investigator ☒ Fellow ☐ Student ☐ Faculty ☐ Staff ☐ Outside Collaborator (Check all that apply.)

Last Name:	Shen	First Name:	Yang	M. Initial:
Department:	Pathology	Phone Number:	2-2832	
JHU Address:	720 Rutland Ave	Bldg & Room:	Ross 664G	
Email Address:	yshen44@jhmi.edu			

1) ***Degree(s) held. Ph.D***

2) ***Specialty and/or major for each degree listed above. Immunology***

3) ***List procedures this person will be performing (can state "all" if appropriate). Diabetes studies***

4) ***Describe the person's experience with the procedures and the species in this protocol.*** *4 years*

5) ***If training and/or supervision of this person is necessary, who will be providing it?***

ACUC office use: AESP ✓ OLT ✓

Role: ☐ Co-Investigator ☒ Fellow ☐ Student ☐ Faculty ☐ Staff ☐ Outside Collaborator (Check all that apply.)

Last Name:	Wu	First Name:	Wai-hong	M. Initial:
Department:	Pathology	Phone Number:	2-2832	
JHU Address:	720 Rutland Ave	Bldg & Room:	Ross 664G	
Email Address:	wwu.whw@gmail.com			

1) ***Degree(s) held. PhD***

2) ***Specialty and/or major for each degree listed above. Tissue collection***

3) ***List procedures this person will be performing (can state "all" if appropriate).*** *All*

4) ***Describe the person's experience with the procedures and the species in this protocol.*** *>10 years*

5) ***If training and/or supervision of this person is necessary, who will be providing it?***

ACUC office use: AESP ✓ OLT ✓

Role: ☐ Co-Investigator ☒ Fellow ☐ Student ☐ Faculty ☐ Staff ☐ Outside Collaborator (Check all that apply.)

Last Name:	Cornwell	First Name:	Ben	M. Initial:
Department:	Pathology	Phone Number:	2-2832	
JHU Address:	720 Rutland Ave	Bldg & Room:	Ross 664G	
Email Address:	bcornwe4@jhmi.edu			

6) ***Degree(s) held. BSC***

7) ***Specialty and/or major for each degree listed above.***

8) ***List procedures this person will be performing (can state "all" if appropriate).*** *Diabetes*

9) ***Describe the person's experience with the procedures and the species in this protocol.*** *6 months*

10) ***If training and/or supervision of this person is necessary, who will be providing it?*** *Rizwan Ahmed*

ACUC office use: AESP ✓ OLT ✓

The ACUC website web.jhu.edu/animalcare contains a formulary for rodent drug dose and ACUC-approved guidelines for many procedures. If you are following these, you can cite them as the method to be used instead of listing all the information in the form.

21. ASSURANCES: Signature verifies that you accept these responsibilities.

a) I assume responsibility for compliance with Johns Hopkins University, United States Department of Agriculture and/or Public Health Service requirements, as applicable, for work carried out under this protocol.

b) I assume responsibility for providing each member of the laboratory who will perform procedures under this protocol with a final version of the approved protocol and require that they follow the procedures described.

c) I assure that all individuals carrying out procedures described in this protocol will be experienced or trained appropriately in the procedures each will perform.

d) I understand that amendments for significant changes, as defined in the JHU ACUC Guidelines, must be approved by the JHU ACUC prior to their implementation. Exceptions may be authorized for clinical reasons on a one-time basis by a Research Animal Resources (RAR) veterinarian.

e) I certify that I have determined that the research to be conducted under this protocol is not unnecessarily duplicative of previously reported research.

f) I understand that no work can begin unless I have received approval from the Animal Care and Use Committee.

Principal Investigator's Signature: _____ **Date:** 1/3/2017

IACUC Chair's Signature: Nancy A. Aton **Date:** 1/4/17

Please submit to the JHU ACUC office (please do not staple):
Reed Hall B122, East Baltimore Campus
If you prefer to fax, please use only the last five numbers if dialing within Hopkins (7-3747). Use the entire number only if faxing from outside of Hopkins (443-287-3747).

To email send to: acuc@jhmi.edu

If you fax the protocol and choose also to mail the original, please put a cover sheet on the original indicating that the protocol previously was faxed so that it is not logged in as a new submission.
Thank you.

The ACUC website web.jhu.edu/animalcare contains a formulary for rodent drug dose and ACUC-approved guidelines for many procedures. If you are following these, you can cite them as the method to be used instead of listing all the information in the form.

Johns Hopkins University Animal Care and Use Committee

MOUSE BREEDING COLONY FORM

Release Date: 5/12, revised 11/12, 3/14

For ACUC office use only	
3rd Year Renewal:	MO16M488
Date Received:	12/1/16
Compliance Approval:	KJD
☑ Logged	☑ Database

Protocol Number (if assigned): M014M34

Protocol Title: Inactivation the Fas pathway to prevent T cell autoimmunity

Principal Investigator: Abdel Hamad

Office Phone: 4-3021 Fax: 4-3548 E-mail: ahamad@jhmi.edu

The purpose of this form is to identify investigator mouse production plans.

- ***It does not guarantee additional cage space.***
- ***See the Mouse Breeding Guidelines at the ACUC website, www.jhu.edu/animalcare***
- ***For help filling out this form, contact the ACUC Office at 443-287-3738***

1. Strain and/or Stock and Estimated Numbers Per Year: *(fill in the table, one form may be used for multiple strains/stocks)*

Strain and/or stock	Estimated # male breeders	Estimated # female breeders	Estimated # of offspring born	Estimated # of offspring to be used or % based on genotype
NOD-gld/+	10-15	20-30	200-300	50% (only females)
NOD- LtJ	10-15	20-30	200-300	50% (only females)
NOD-IL-10-KO	3-6	3-6	70-100	50%
NOD-FasL$^{fl/fl}$	3-6	3-6	70-100	100%
NOD-CD19^{cre}	3-6	3-6	70-100	100%
NOD-CD19^{cre} X NOD-FasL$^{fl/fl}$	3-6	3-6	70-100	50%
BALB/c	10-15	20-30	200-300	50% (only females)

BALB/c-gld/gld	3-6	3-6	70-100	100%
BALB/c-sdc1-KO	10-15	20-30	200-300	50% (only females)
BALB/c-CD1d-KO	3-6	3-6	70-100	50%
BALB/c-Ja18-KO	10-15	20-30	200-300	50% (only females)
BALB/c-sdc1;Ja18-DKO	10-15	20-30	200-300	50% (only females)
C57BL/6	10-15	20-30	200-300	100% (only females)
C57BL/6-gld//gld	3-6	3-6	70-100	100%
C57BL/6-Abi3bptm1e.1	3-6	3-6	70-100	100%

2. Indicate what will be done with Retired Breeders and Excess Offspring: *(check all that apply)* **Do not leave dependent pups marked for euthanasia without a lactating dam.**

_x___ Euthanized as described in question 16 of the parent protocol

__x__ Marked for euthanasia by Animal Resources using the Technical Request for Euthanasia Form

__x__ Transferred to another protocol using the Transfer Request Form

3. Breeding Plan(s): What is the age range during which the animals will be bred? Six to 24 weeks

a. BREEDING SCHEME: (*check all that apply*)

__x__ Monogamous (one male and one female per cage)

__x__ Trio (one male and two females per cage)*

__x__ Polygamous (one male and more than two more females per cage)*

*In question 3b. indicate how the cage population will be managed if there could be 2 litters in one cage. Options 3., 6., and 7. address this.

b. BREEDING MANAGEMENT - to prevent overcrowding or welfare concerns: *(check all that apply)*

1. _x__ Females pregnant from postpartum estrus will have their weanling age pups weaned concurrent with the delivery of the next litter
2. ___ Males will be removed from the cage once females are confirmed pregnant
3. _x__ Pregnant females will be moved to their own cages prior to parturition
4. ___ Males will be rotated through the females cages
5. ___ Females will not be bred again until their offspring are weaned
6. _x__ When 2 females, each with a litter are in 1 cage, one female & litter will be moved to her own cage
7. _x__ Offspring will be used before weaning
8. ___ Other breeding cage management options, include special conditions due to atypical breeding/offspring production: ***(describe)***

4. IDENTIFICATION: *(check all that apply)*

__x__ Some or all offspring will be individually identified _x__ Individual identification is not necessary

___x_ Ear punch __x__ Ear tag _____ Toe tattoo _____ Other***(describe)***

Toe clip _____ ***(provide justification)***
Toe-clipping should be used only when no other identification method is feasible. It may be an acceptable method for neonatal mice up to 7 days of age if the identification and genotyping can be combined. For additional requirements see the ACUC *Toe Clipping Policy* on the ACUC website.

5. GENOTYPING (*recommended* before weaning): *(check one)*

__x__ Genotyping will be performed for some or all _____ Genotyping is not necessary

Tissue will be obtained from the following source: __x__ **Tail*** _____ **Ear** _____ **Other:** ***(describe)***

*Will the amount of tail tissue exceed 5 mm? _____ **Yes** __x__ **No**

If **"Yes"**, ***(indicate amount needed and provide justification)***

*When the tail tissue is collected, could animals be <u>greater than</u> 21 days old? _x_ **Yes** __ **No**

If" **Yes"**, even occasionally, the mice <u>must be anesthetized or receive topical analgesic</u>. This does not require placement in Pain Category D. See the JHU Guidelines *for Tail Biopsy* at the ACUC website
(indicate the approximate age and what anesthesia will be used)

This will rarely be used. In these instances, we will use local anesthesia - Ethyl chloride spray or ice-cold Alcohol (isopropyl or ethyl)

6. WEANING SCHEDULE: *(check all that apply)*

Litters of females that are pregnant due to post-partum estrus *<u>must</u>* be weaned at 21 days of age unless "Other breeding cage management" is described in section 3**b** of this form.

_x__ 21 days of age

__x_ >21 – 28 days of age

___ >28 days of age: ***(justify delayed weaning and maximum age)***

7. SPECIAL HUSBANDRY REQUIREMENTS:

___ Special care is not required

___ Special care is required for some or all: ***(describe: e.g., food on floor, alternate water source etc.).***

8. CONSIDERATION OF PHENOTYPE:

If using genetically engineered animals, might the phenotype result in a change in appearance or a functional deficit? __x__ **Yes** ____ **No** ____ **Unknown**

If **"Yes"**, ***(describe) Gld mice develop benign lymphoproliferation that could be mistaken for tumors***

If **"Unknown"**, ***(describe plan for monitoring animals to detect possible impairments)***

PI Signature: _____ Date: 01/10/2016

ACUC Chair Signature: Stacey A. Acton Date: 1/4/17