THE UNIVERSITY OF
ALABAMA AT BIRMINGHAM
Office of Public Relations and Marketing

February 18, 2005

Ms. Kit King
[redacted]

[redacted]

Please make check payable to the **University of Alabama at Birmingham**.

Remit to: Andrea Davis-Hill
Office of Public Relations & Marketing
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University of Alabama at Birmingham
Institutional Animal Care and Use Committee
Animal Use Application for Noncompetitive Renewal of Extramurally Funded Projects

Revised 11/20/00

Submit the completed form and your most recent progress report and award letter to the IACUC office, VH-B10 (0019); Phone, 934-7692; Fax, 934-1188. Applications received by the last work day of the month will be reviewed in the next month. ***Note: For each third renewal, you must submit the Animal Use Review Form for New Projects.***

Investigator:		**Department:**	
Phone Number:		**Campus Address:**	
FAX Number:		**Email Address:**	

Project Title:	PPG: UAB/Sankyo Program for Rheumatic Diseases and Cancer Research (Koopman); Multimodality Imaging Core		
Fund Source:	DOD		
Previous Year's IACUC APN	021106544	**Project Period**	10/01/02to 09/30/04

Anticipated Animal Use During Renewal Period

Species	Number	Use Category	Preferred Vendor	Housing Site*
Mice	200	B	Jackson Lab/UAB	
Rats	200	B	Harlan, CR, UAB	
Primates	15	B	ARP Approved	

*If non-ARP site, please also submit a completed Outside Housing Request form.

Will there be any changes in animal use procedures or numbers/category of animals used from that described in the previous year's approval? Yes ☐ No ☑

If yes, please attach a description. If the changes involve adding another species then you must also submit a completed Animal Use Review Form for New Projects describing the work with the new species. Please provide justification for any change in animal numbers or use category.

Please check the appropriate box if use of any of the following agents has been added or modified, and attach a description.

Radioisotopes ☐
Highly toxic chemicals or drugs ☐
Recombinant DNA/RNA ☐
Cell or tissue cultures ☐
Microbial agents ☐
Carcinogens, mutagens, teratogens ☐
Toxic products ☐
Blood, fluids, tissues ☐

Please list all non-Animal Resources Program staff with direct animal contact on this project.

Name	SS#	Animal species exposed to during renewal period*	Animal facility card key number (located on top right, back of card)
		All	
		All	

*Note: All personnel who work with or enter rooms where nonhuman primates are housed or used must provide evidence on an annual negative TB test.

I certify that the information furnished is complete and correct to the best of my knowledge.

Investigator Signature

Date OCT - 7

University of Alabama at Birmingham
Institutional Animal Care and Use Committee
Animal Use Application for
Noncompetitive Renewal of Externally Funded Projects

Revised 4/17/03

*Submit the completed form with your most recent **progress report and award letter** to the IACUC Office, VH-B10 (0019); Phone, 934-7692; Fax, 934-1188. Applications received by the last work day of the month will be reviewed in the next month.*

Note: For each third renewal you must submit the Animal Use Review Form for New Projects.

Investigator:		**Department:**	
Phone Number:		**Campus Address:**	
FAX Number:		**Email Address:**	

Project Title:	Cortical Mechanisms of Visual space Perception		
Fund Source:	NEI		
Previous Year's IACUC APN		Project Period	04/01/99 to 03/31/04

Anticipated Animal Use During Renewal Period

Species**	Number	Use Category	Vendor	Housing Site*
M. mulatta	2	B		
M. fascicularis	2	B		

*If non-ARP site, please also submit a completed Outside Housing Request form.

Will there be any changes in animal use procedures or numbers/category of animals used from that described in the previous year's approval? Yes ☐ No ☑

If yes, please attach a description. If the changes involve adding another species then you must also submit a completed Animal Use Review Form for New Projects describing work with the new species. Please provide justification for any change in animal numbers or use category.

****Submit the Environmental Enrichment Form annually for all projects involving nonhuman primates.**

Please check the appropriate box if use of any of the following agents has been added or modified, and attach a description.

Radioisotopes ☐
Highly toxic chemicals or drugs ☐
Recombinant DNA/RNA ☐
Cell or tissue cultures ☐
Microbial agents ☐
Carcinogens, mutagens, teratogens ☐
Toxic products ☐
Blood, fluids, tissues ☐

Please list all non-Animal Resources Program staff with direct animal contact on this project.

Name	SS#	Animal species exposed to during renewal period***	Animal facility card key number (located on top right, back of card)
		macaque monkeys	
		" "	

***Note: All personnel who work with or enter rooms where nonhuman primates are housed or used must provide evidence on an annual negative TB test.

I certify that the information furnished is complete and correct to the best of my knowledge.

RECEIVED
2 0 2004

Investigator Signature **Date**

University of Alabama at Birmingham
Institutional Animal Care and Use Committee
Animal Use Application for
Noncompetitive Renewal of Externally Funded Projects

Revised 4/17/03

Submit the completed form with your most recent ***progress report and award letter*** *to the IACUC Office, VH-B10 (0019); Phone, 934-7692; Fax, 934-1188. Applications received by the last work day of the month will be reviewed in the next month.*
Note: For each third renewal you must submit the Animal Use Review Form for New Projects.

Investigator:		Department:	
Phone Number:		Campus Address:	
FAX Number:		Email Address:	

Project Title:	Novel VLP-Based Mucosal and Systemic HIV Vaccines; core B: Nonhuman Primate Core		
Fund Source:	NIH		
Previous Year's IACUC APN	031106432	Project Period	02-01-04 to 01-31-05

Anticipated Animal Use During Renewal Period

Species**	Number	Use Category	Vendor	Housing Site*
Macaques	24	A / B	as available	

*If non-ARP site, please also submit a completed Outside Housing Request form.

Will there be any changes in animal use procedures or numbers/category of animals used from that described in the previous year's approval? Yes ☐ No ☑

If yes, please attach a description. If the changes involve adding another species then you must also submit a completed Animal Use Review Form for New Projects describing work with the new species. Please provide justification for any change in animal numbers or use category.

**Submit the Environmental Enrichment Form annually for all projects involving nonhuman primates.

Please check the appropriate box if use of any of the following agents has been added or modified, and attach a description.

Radioisotopes ☐
Highly toxic chemicals or drugs ☐
Recombinant DNA/RNA ☐
Cell or tissue cultures ☐
Microbial agents ☐
Carcinogens, mutagens, teratogens ☐
Toxic products ☐
Blood, fluids, tissues ☐

Please list all non-Animal Resources Program staff with direct animal contact on this project.

Name	SS#	Animal species exposed to during renewal period***	Animal facility card key number (located on top right, back of card)
		Macaques	
		Macaques	

***Note: All personnel who work with or enter rooms where nonhuman primates are housed or used must provide evidence on an annual negative TB test.

I certify that the information furnished is complete and correct to the best of my knowledge.

Investigator Signature

Date

University of Alabama at Birmingham
Institutional Animal Care and Use Committee
Animal Use Application for Noncompetitive Renewal of Extramurally Funded Projects

Revised 11/20/00

Submit the completed form and your most recent progress report and award letter to the IACUC office, VH-B10 (0019); Phone, 934-7692; Fax, 934-1188. Applications received by the last work day of the month will be reviewed in the next month. ***Note: For each third renewal, you must submit the Animal Use Review Form for New Projects.***

Investigator:		**Department:**	
Phone Number:		**Campus Address:**	
FAX Number:		**Email Address:**	

Project Title:	Central Projections of Primate Photoreceptive Retinal Ganglion Cells		
Fund Source:	Eyesight Foundation of Alabama		
Previous Year's IACUC APN	030206761	**Project Period**	8/1/03 to 7/31/04

Anticipated Animal Use During Renewal Period

Species	Number	Use Category	Preferred Vendor	Housing Site*
Monkeys	6	B	Various	
Rats	50	B	Various	

*If non-ARP site, please also submit a completed Outside Housing Request form.

Will there be any changes in animal use procedures or numbers/category of animals used from that described in the previous year's approval? Yes ☐ No ☑

If yes, please attach a description. If the changes involve adding another species then you must also submit a completed Animal Use Review Form for New Projects describing the work with the new species. Please provide justification for any change in animal numbers or use category.

Please check the appropriate box if use of any of the following agents has been added or modified, and attach a description.

Radioisotopes ☐
Highly toxic chemicals or drugs ☐
Recombinant DNA/RNA ☐
Cell or tissue cultures ☐
Microbial agents ☐
Carcinogens, mutagens, teratogens ☐
Toxic products ☐
Blood, fluids, tissues ☐

Please list all non-Animal Resources Program staff with direct animal contact on this project.

Name	SS#	Animal species exposed to during renewal period*	Animal facility card key number (located on top right, back of card)
		Macaca mulatta	
		Macaca mulatta	
		Macaca mulatta and rats	3
		Macaca mulatta	

*Note: All personnel who work with or enter rooms where nonhuman primates are housed or used must provide evidence on an annual negative TB test.

I certify that the information furnished is complete and correct to the best of my knowledge.

Investigator Signature **Date**

RENEWAL

OFFICE USE ONLY (revised 03/14/02)
IACUC Approval Number: 021205386
Funded Date:
Not Funded/Delete Date:
Category

ONLINE, TYPE ONLY IN THE VISIBLE AREA

UAB Animal Use Review Form For New Proposals
General Information
Section A

Principal Investigator _____ Email _____

Department/Division _____ Extension _____

Affiliation:

Graduate School		Medicine	✓
Med. Center Adm.		Nursing	
Med. Center Joint Dept.		Optometry	
S.H.R.P.		Public Health	
Dentistry	✓	Academic Affairs	

Campus Address (Room, Bldg.) _____

Project Title Pathogenic Determinants of SIV Envelope Glycoproteins

Program Project or SCORE Title (If Applicable) _____

Project Period 05/01/01 To 04/30/06

External Supporting Agency NIH Application Deadline --

Internal Supporting Agency _____ Application Deadline _____

Are all individuals having contact with the animals in this project participating in the ARP Personnel Health Program?

Yes ✓ No _____

Species	Number Used Per Year	Approx. No. Days Housed Per Year	Approx. Daily Census Avg. / High	Animal Source Vendor	Housing Site*
1. Macaques	24	365	24 / 24	as available	
2.			/		
3.			/		

* If animals will be housed or undergo procedures outside of ARP animal facilities (in laboratories or other study areas) for more than 12 hours, please indicate the building and room number here: _____

I certify that the information provided on this form is accurate to the best of my knowledge and includes all animal procedures proposed in the corresponding grant or experimental plan.

3

Investigator Signature Date

Investigators responsible for experimental animal procedures other than the principal investigator:

Name(s) _____

Technical staff involved in the experimental procedures (please indicate training and experience):

Name	Hire Date	Length of Experience	Nature of Experience

1. Briefly explain the scientific merit of your proposal to justify the use of animals to any reasonable and well-informed lay person. This explanation should include your project's relevance to human or animal health and/or the advancement of knowledge. Include the rationale for the choice of species. If necessary, attach a separate sheet.

See attached.

2. Briefly justify the number of animals requested for the control and experimental group size and the statistical analysis planned. If necessary, attach a separate sheet.

Experimental and control groups will be composed of 3 or 4 animals. Since animals will be inoculated with virus mutants to evaluate their pathogenicity, large numbers are not required. In our previous evaluation of three of these SIVmac239 variants, reliable conclusions about pathogenicity were made based on the use of three animals per group. Statistical analyses will be used to compare various parameters, including viral burdens and changes in T-cell subsets, and the results of in vitro assays of immune responses, such as proliferative responses to SIV antigens. Tests to be used include Pearson's correlation, linear regression, the parametric Student's t test, ANOVA, and nonparametric Mann-Whitney U test to compare means and medians.

Persistent infection by HIV-1 is characterized by continual virus replication despite the presence of vigorous humoral and cellular immune responses by the host. Recent findings indicate that the structure of the HIV Env glycoprotein might contribute to this failure to clear virus. A variant of SIV, which expresses high levels of Env on the surface of infected cells and stabilizes the interaction of the two Env subunits (gp120 and gp41-TM), has been identified. In a preliminary study, we showed that changing one Tyr residue in the cytoplasmic tail attenuates the pathogenicity of SIVmac239. Because high levels of Env on the surface of an infected cell might either elicit a more rapid immune response or facilitate killing of infected cells, it is important to distinguish between these two possibilities. We have generated additional SIVmac239 mutants, some with changes in the TM ectodomain that stabilize the gp120/TM interaction. We will also generate a chimeric SHIV (SIVmac239 genome in which the SIV Env has been replaced by an HIV Env) encoding these same mutations. These various mutants will be inoculated into macaques and a detailed analysis of both the rate of induction and the functional activities of SIV-specific immune responses will be done. By determining whether a particular SIV or SHIV variant is pathogenic or attenuated and characterizing the immune responses elicited by that virus, we should be able to identify factors important for attenuation. Since the innate immune system, characterized by dendritic cells (DC), interferon (IFN)-α and -β, and natural killer (NK) cells, is the first line of defense, we will also assess these responses. Virus infections lead to IFN-α/β production by plasmacytoid DC (pDCs), which comprise less than 1% of peripheral blood cells; however, pDC can be mobilized from progenitor cells in bone marrow by Flt3 ligand (Flt3L), which has been administered to mice, macaques, baboons, and humans with no ill effects. Therefore, we will also inoculate Flt3L, either purified protein or as a DNA vector, to normal or SIV-infected macaques to determine whether increased numbers of pDC, IFN-α/β, and NK cells are observed. Bone marrow biopsies from these animals will also be obtained to evaluate these cells in *in vitro* experiments.

Macaques will be used because this species can be infected with various SIV and SHIV strains, which establish long-term persistent infections and disease ranging in severity from asymptomatic to AIDS. These studies will provide valuable information about both viral and host determinants of pathogenicity and, in particular, how the structure of the viral envelope glycoprotein influences induction of immunity and attenuates disease.

3. Please categorize the level of pain or discomfort associated with all procedures. Provide numbers of animals for each species and category (A, B, or C) as needed. (Please see instructions for explanation of categories.) If groups of animals fall into more than one category, fill out a line for each category. The total number for each species should be equal to the total number on page 1.

Category (A, B, or C)	Species	Number Per Year
A	macaques	3
B	macaques	21

If any animals fall into Category C, justify the need to perform painful experiments without the use of anesthetics or analgesics. If necessary, attach a separate sheet.

4. The Animal Welfare Act (P.L. 99/158) requires that the principal investigator provide the following information:

a. Please provide assurance that alternatives to the use of animals were considered in planning these research activities:

Studies of pathogenesis and therapeutic approaches can be done only in live animals.

b. Please provide assurance that these research activities do not *unnecessarily* duplicate previous experiments:

These experiments do not duplicate any previously published studies.

c. Please describe the methods and sources used to determine that alternatives are not available and that unnecessary duplication of experiments will not occur. **If the sources include a database search, please include the databases searched, the date of the search, the years covered by the search, and the key words and/or search strategy used. If other sources are consulted, please include appropriate documentation.**

P.I. is familiar with all experimental studies involving the SIV/SHIV-macaque model through attending meetings on HIV/AIDS, reviewing NIH grant applications and manuscripts submitted to major journals, and scanning Tables of Contents monthly of more than 40 major journals.

Check all that apply:

_____ Index Medicus (Medline, etc.)
_____ Biological Abstracts
_____ Current Research Information Service
_____ National Agricultural Library
_✓_ Other (describe)

SPECIFICS OF PROCEDURES INVOLVING ANIMALS
SECTION B

1. Euthanasia Methods: **(This question must be completed for all protocols)**:

   A. Inhalant agents (ether, halothane, methoxyflurane, CO_2)

   Species _____ Drug / Gas _____

   Method of Administration _____

   B. Injectable Agents (Barbiturates, KCl*. . .)

   Species Macaques Drug Pentabarbitol Dose 50 mg/kg Route IV

   C. Physical Methods

   Cervical dislocation (poultry, mice, rats <200g, rabbits <1kg)

   Species _____

   Decapitation with guillotine – Species _____

   Will the animal be sedated / anesthetized during cervical dislocation and/or decapitation?

   ( ) Yes – Fill out section C.

   ( ) No – Provide scientific justification for performing this procedure without sedation/anesthesia (e.g. interference with specific experimental parameters).

   D. Exsanguination* - Species _____

   E. Other Method (Describe) _____

   Species _____

   *Method to be used only in anesthetized animals – fill out Section C.

2. Immunizations / Antibody Production

Complete the following for *each species and immunogen.*

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses

B. Will adjuvants be used? Yes _____ No _____

Type of adjuvant:

Primary injection _____

Booster injection(s) _____

3. Other Injections:

If drugs or chemicals (other than anesthetics) are to be given, complete this section *for each species and agent.*

If anesthetics will be used, compete Section C.

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses
Macaques	Flt3L-DNA	IM	thigh	0.5 ml	4	4-7 days
	Flt3L protein	SC	thigh	0.5 ml	8	daily

B. If toxic or other deleterious reactions may occur in animals. state the possible reaction(s) and procedures to deal with these reactions. If LD_{50} studies are planned, state the number of animals per dosage group (see instructions).

None

4. Are physical restraints used? Yes _____ No ✓
If yes, describe the restraint system and indicate the approximate time in the restraint for each experiment.

System _____

Time _____

5. Blood Samples (Note: Some blood collection methods such as intracardiac or retro-orbital techniques should be performed only in anesthetized animals -- fill out Section C.

Route	Amount	Freq.
IV	20 ml	biweekly to monthly

6. Pain Threshold Tests

Type	Freq.
None	

7. Special Diets/Food Deprivation

Type	Freq.
None	

8. Tumor inoculations / implantations (including hybridoma ascites tumors).

A.

Species	Tumor Type	Site
None		

B. Describe procedures to monitor tumor size and ascitic fluid accumulation and frequency of tapping ascitic fluids. Also, describe criteria for euthanasia of animals if they become ill due to tumor growth:

NA

9. Other procedures: (inhalation, infectious agents, inoculations, etc.).

IV inoculation of macaques with SIV or SHIV can result in an AIDS-like disease. Animals will be monitored daily for signs of disease and antibiotics and analgesics will be given as needed.

ANESTHESIA
SECTION C

1. List procedure(s) requiring anesthesia by species

Species	Procedure
Macaques	Blood collection; virus and Flt3L inoculations;
	bone marrow biopsy

2. Describe the pre-anesthesia protocol, including any fasting or pre-anesthetic drugs:

None

3. Anesthetic agent(s). If more than one agent is to be used (e.g. for induction and maintenance), list all agents by species:

Species	Anesthetic Agent	Dosage	Route
Macaques	ketamine	10 mg/kg	IM

4. Describe procedures to monitor the depth of anesthesia: (e.g. respiratory rate, toe-pinch reflex, palpebral reflex)

Respiratory rate; toe-pinch reflex

5. Will a paralytic agent be used? Yes _____ No ✓

If yes, please specify.
Note: paralytic agents can only be administered to anesthetized animals, and animals must be monitored appropriately (i.e., blood pressure, ECG, etc.) to assure adequate anesthesia

Species	Agent	Dose	Route

SURGICAL PROCEDURES
SECTION D

1. Indicate where surgery will be performed, the person performing the surgery, and the qualifications and experience of that individual to perform the techniques involved: (Note: Major survival surgery on non-rodent mammalian species must be performed in a facility approved by the IACUC.)

   Bldg. _____ Room

   Name of Surgeon(s) _____

   Experience _____

2. Non-Survival Surgery (Animal will not recover from anesthesia) _____

   Survival Surgery (Animal will recover from anesthesia) X (fill out Section 4)

3. Describe in detail the surgical procedure including the surgical approach, closure, support care, and monitoring during the procedure. If necessary, attach a separate sheet.

   Bone marrow biospy (not considered surgery)
   BM biopsies can be done via the tubercle of the iliac crest or on the cranio-lateral side of the proximal femur or humerus. After shaving the site, the area will be cleaned with betadine. A pediatric biopsy needle will be inserted through a small incision and, when the cortex of the iliac crest or humerous is reached, forced through the bone (with rotation). After the medullary cavity is entered, a syringe will be attached to the needle, approximately 2 ml of BM will be withdrawn and immediately transferred to a heparinized blood collection tube. The incision site will be sutured, if needed.

4. Fill out this section if survival surgery is to be performed.

   A. Describe post-operative care, including supportive care, post-operative monitoring, analgesia, antibiotic therapy, arrangements for after hours, weekend, and holiday care. If necessary, attach a separate sheet.

      Animals will be monitored until conscious and the site of incision monitored for several days for evidence of infection.

   B. Comments regarding potential post-operative complications and / or pain:
      None

   C. Postoperative analgesic therapy:

Species	Agent	Dosage	Route	Frequency

   D. Anticipated post-operative survival time: Indefinitely

   E. Will multiple survival surgeries be performed on a single animal? Yes _____ No ✔
      If yes, explain and justify why this cannot be avoided. Attach a separate sheet if necessary.

UAB ANIMAL USE SAFETY INFORMATION

This project must be registered and authorized by UAB OH&S if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal or animal facility.

OH&S Administrative Use Only
Project #
Authorization Date

PI INFO

Name _____ Phone _____ Emergency # _____

Department _____ Alternate Contact _____ Alt. Phone _____

PROJECT

Project Title _____ Species Macaques

Pathogenic Determinants of SIV Envelope Glycoproteins

Funding Source NIH

IACUC Administrative Use Only
APN:

POTENTIALLY HAZARDOUS MATERIALS (Excluding Anesthetics)

OH&S Use Only | OH&S Use Only | OH&S Use Only

Agent/Material is potentially hazardous for:
- ☑ Humans
- ☑ Animals (Species macaques)

Agent(s)	Route of Administration	Excretion (e.g., urine/feces)	Human Health Risks or Other Concerns
SIV	IV	urine / feces / saliva	Risk of infection for personnel is low unless direct transfer of blood via cuts.
SHIV	IV	urine / feces / saliva	
		(but negligible)	

SPECIAL PRECAUTIONS/INSTRUCTIONS (check all that apply)

- ☐ The PI or his/her technicians are responsible for the feeding and care of these animals (must receive IACUC approval)
- ☑ The following may be contaminated with potentially hazardous material and must be handled only by authorized personnel:
  - ☑ Cage ☐ Pen ☐ Cage/pen accessories ☐ Water Bottle ☑ Animal Carcasses ☐ Bedding ☐ Other _____
- ☐ Cages/Pen must be decontaminated before cagewash (Method _____)
- ☐ Cages/Pen/Bedding must be autoclaved before cleaning or disposal
- ☑ Animal carcasses must be disposed of as follows:
  - ☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures)
  - ☑ Chem. And/or Bio. Contaminated (Red barrel incineration) ☐ Other _____
- ☐ All contaminated waste (soiled bedding and other animal waste) must be properly labeled and disposed of as follows:
  - ☐ Chem. Contaminated (Yellow barrel incinerate) ☐ Bio. Contaminated (Autoclave/ Red barrel)
  - ☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures) ☐ Other _____
- ☑ Other (incl. special tests or immunizations) Annual TB test

REQUIRED PERSONAL PROTECTIVE EQUIPMENT (PPE) (check all that apply)

☑ The following Personal Protective Equipment (PPE) must be worn/used in the room:

☐ Lab Coat	☑ Disposable Gloves	☐ Face Shield	☑ Safety Glasses	☐ Goggles
☑ NIOSH Certified Dust Mask	☐ Head/hair (beard) Cover	☐ Closed front gown with long sleeves and elastic cuffs		
☑ Shoe Covers/Booties	☐ Disinfectant Foot Spray	☑ H_2O Repellant Coveralls/Jumpsuit		
☐ Biosafety Cabinet req.	☐ N-95 or Equivalent Fitted Respirator			
☐ Other				

- ☑ PPE must be removed before leaving the room.
- ☑ PPE must be discarded or decontaminated after each use.
- ☐ Other _____

☐ Check here if additional information is attached.

Form Must Be Posted On Animal Room Door Where Animal is Used or Housed

IACUC Revised 11/10/00

OFFICE USE ONLY (revised 05/16/01)
IACUC Approval Number: 5620
Funded Date:
Not Funded/Delete Date:
Category

ONLINE, TYPE ONLY IN THE VISIBLE AREA

UAB Animal Use Review Form For New Proposals
General Information
Section A

Principal Investigator _____ Email _____

Department/Division _____ Extension _____

Affiliation:

Graduate School		Medicine	
Med. Center Adm.		Nursing	
Med. Center Joint Dept.		Optometry	✓
S.H.R.P.		Public Health	
Dentistry		Academic Affairs	

Campus Address (Room, Bldg.) _____

Project Title FMR Imaging of Eye Stabilization Processes (APN 030405620:

Program Project or SCORE Title (If Applicable) _____

Project Period 07/01/02 To 06/30/05

External Supporting Agency NIH/NEI Application Deadline 05/01/04

Internal Supporting Agency _____ Application Deadline _____

Does this proposal recover indirect costs? Yes ✓ No _____

RECEIVED
MAR 16 2004
IACUC

Are all individuals having contact with the animals in this project participating in the:
(1) ARP Personnel Health Program? Yes ✓ No _____
(2) ARP Animal Care and Use Training Program? Yes ✓ No _____

Species	Number Used Per Year	Approx. No. Days Housed Per Year	Approx. Daily Census Avg. / High	Animal Source Vendor	Housing Site*
1. Macaca Mulatta	2	365	4 /4	Various	
2.			/		
3.			/		

* If animals will be housed or undergo procedures outside of ARP animal facilities (in laboratories or other study areas) for more than 12 hours, please indicate the building and room number here: _____

I certify that the information furnished herein is complete and correct to the best of my knowledge.

Investigator Signature _____ Date _____

Investigators responsible for experimental animal procedures other than the principal investigator:

Name(s) _____

_____

Technical staff involved in the experimental procedures (please indicate training and experience):

Name	Hire Date	Length of Experience	Nature of Experience

1. Briefly explain the scientific merit of your proposal to justify the use of animals to any reasonable and well-informed lay person. This explanation should include your project's relevance to human or animal health and/or the advancement of knowledge. Include the rationale for the choice of species. If necessary, attach a separate sheet.

Functional magnetic resonance imaging (fMRI) is becoming the most used, and abused, tool for the study of the human brain. An objective validation of the technique is long overdue, with the need of a systematic comparison of the highlighted areas with the underlying neural activation. This can be done only on animal models, requiring invasive anatomical and electrophysiological techniques. At the same time, fMR imaging in animal models can be a powerful tool in expanding our knowledge of brain function per se, being able to obtain activation maps of large areas of the animal brain, on which much more localized studies can then be planned.

Short-latency cortical eye stabilization mechanisms are a group of sensorimotor processes specific of humans and non-human primates and extensive parallel studies have shown almost astonishing similarities between the two species. Their machine-like repeatability, together with their richness and complexity involving major cortical areas directly linked to perception, make them the best behavioral and electrophysiological tools for the validation of the fMRI technique as well as for our further understanding of vision and oculomotor physiology and pathology.

This is the third and last year of a pilot project to develop techniques and experimental protocols using the 4.7T UAB primate fMRI system located in the Center for the Development of Functional Imaging (CDFI) with the aim of developing, on the basis of preliminary results, a fully integrated fMRI/electrophysiological project. These results will be critical in understanding what those colorful images actually say as well as in the guidance of the fMRI exploration of the eye stabilization processes in humans.

2. Briefly justify the number of animals requested for the control and experimental group size and the statistical analysis planned. If necessary, attach a separate sheet.

The Macaca monkey has visual and oculomotor capabilities remarkably similar to those of the human. This is particularly true for the cortical eye stabilization processes object of this study. These systems are not present in lower animals and their study requires alert and cooperating subjects. Thus, it is the necessary experimental choice, giving the possibility of direct extrapolation of the results to humans. This species does not appear to be in short supply. Being that this project is a pilot study, two animals per year is considered a sufficient (as well as minimal) number of animals.

3. Please categorize the level of pain or discomfort associated with all procedures. Provide numbers of animals for each species and category (A, B, or C) as needed. (Please see instructions for explanation of categories.) If groups of animals fall into more than one category, fill out a line for each category. The total number for each species should be equal to the total number on page 1.

Category (A, B, or C)	Species	Number Per Year
B	Macaca mulatta	2

If any animals fall into Category C, justify the need to perform painful experiments without the use of anesthetics or analgesics. If necessary, attach a separate sheet.

No

4. The Animal Welfare Act (P.L. 99/158) requires that the principal investigator provide the following information:

a. Please provide assurance that alternatives to the use of animals were considered in planning these research activities:

These oculomotor stabilization processes are high-level cortical circuits present only in humans and non-human primates. While fMR imaging is a technique commonly used in humans, the electrophysiological recordings can be performed only on alert cooperating non-human primates.

b. Please provide assurance that these research activities do not *unnecessarily* duplicate previous experiments:

Extensive research of scientific literature has shown that the planned experiments are not duplicates of previous studies.

c. Please describe the methods and sources used to determine that alternatives are not available and that unnecessary duplication of experiments will not occur. **If the sources include a database search, please include the databases searched, the date of the search, the years covered by the search, and the key words and/or search strategy used. If other sources are consulted, please include appropriate documentation.**

UAB Ovid System Access of Medline 1996 to current
Date search December 2000 through January 2001 and February 2004 through March 2004.
Keywords: (MRI or fMRI) and (Macaca or primates or monkey or Haplorhini)

Check all that apply:

- ✓ Index Medicus (Medline, etc.)
- _____ Biological Abstracts
- _____ Current Research Information Service
- _____ National Agricultural Library
- _____ Other (describe)

SPECIFICS OF PROCEDURES INVOLVING ANIMALS
SECTION B

1. Euthanasia Methods: **(This question must be completed for all protocols)**:

   A. Inhalant agents (ether, halothane, methoxyflurane, CO_2)

   Species NA Drug / Gas _____

   Method of Administration _____

   B. Injectable Agents (Barbiturates, KCl*. . .)

   Species Macaca mulatta Drug Pentobarbital Dose 30mg/kg Route IV

   C. Physical Methods

   Cervical dislocation (poultry, mice, rats <200g, rabbits <1kg)

   Species NA

   Decapitation with guillotine – Species _____

   Will the animal be sedated / anesthetized during cervical dislocation and/or decapitation?

   ( ) Yes – Fill out section C.

   ( ) No – Provide scientific justification for performing this procedure without sedation/anesthesia (e.g. interference with specific experimental parameters).

   Exsanguination is necessary to clear blood from brain histology.

   D. Exsanguination* - Species Macaca mulatta (anesthetized)

   E. Other Method (Describe) NA

   Species _____

   *Method to be used only in anesthetized animals – fill out Section C.

2. Immunizations / Antibody Production

Complete the following for *each species and immunogen.*

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses
NA						

B. Will adjuvants be used? Yes _____ No _____

Type of adjuvant:

Primary injection _____

Booster injection(s) _____

3. Other Injections:

If drugs or chemicals (other than anesthetics) are to be given, complete this section *for each species and agent.*

If anesthetics will be used, compete Section C.

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses
NA						

B. If toxic or other deleterious reactions may occur in animals, state the possible reaction(s) and procedures to deal with these reactions. If LD_{50} studies are planned, state the number of animals per dosage group (see instructions).

NA

4. Are physical restraints used? Yes ✓ No _____
If yes, describe the restraint system and indicate the approximate time in the restraint for each experiment.

System Primate chair with head restraint (WORB room 402 or magnet room, outside or inside the magnet bore).

Time 3-5 h/day

5. Blood Samples (Note: Some blood collection methods such as intracardiac or retro-orbital techniques should be performed only in anesthetized animals – fill out Section C.

Route NA Amount _____ Freq. _____

6. Pain Threshold Tests

Type NA Freq. NA

7. Special Diets/Food Deprivation

Type Water Intake (see page 7, additional sheet) Freq. Daily (see page 7, additional sheet)

8. Tumor inoculations / implantations (including hybridoma ascites tumors).

A. Species Tumor Type Site

NA

B. Describe procedures to monitor tumor size and ascitic fluid accumulation and frequency of tapping ascitic fluids. Also, describe criteria for euthanasia of animals if they become ill due to tumor growth:

NA

9. Other procedures: (inhalation, infectious agents, inoculations, etc.).

NA

USE FOR ADDITIONAL INFORMATION IF NECESSARY

Additional technical staff which may be involved in the experimental procedures (cont):

a programmer/analyst. will assist during recording sessions and will develop imaging protocols.

-Research Technician will assist during recording sessions, and with animal handling and training.

Water intake (cont):

Water access will be restricted to 3-5 hours per day in the lab. Water and juice will be used as positive reinforcement for correctly performing the behavioral tasks. Animals will be closely monitored during the periods of water restriction to prevent dehydration and loss of health status. Daily weights and water intake after each training/recording session will be chartered and sent to the veterinarian each month to be included in the animal's USDA record. After each session the animal is returned to its home cage. The animal will have at least 24 hrs of free access to water at least every 7 days to avoid any possibility of dehydration buildup. An example of the water schedule that I developed with the ARP veterinarian with very good results is given below:

Mon/Tue/Wed/Thu/Fri: water/juice in the lab while performing the tasks

At the end of the Fri recording session the animal will receive in the cage free water access until Saturday evening, when the full bottle is replaced with a bottle containing the average amount of water/juice the animal received in the lab during the 5 recording sessions, which will be its Sunday intake until the Monday recording session.

During the sessions the animal is always allowed to work to satiety (i.e., until the animal loose any interest in the task). Water access is free all the time when not in training or no experiments are planned for that week. Access to dry food and dry treats is free all the time.

It may be possible that experimental needs or magnet availability require recording/imaging sessions during the weekend. In this case the period of free water will be moved inside the week but the schedule will be modified in such a way to preserve the 24hrs/7 days free water schedule.

Special experimental conditions may require uninterrupted recordings for more than 5 days for a limited period of time. These periods, quite rare, will be of limited duration (max 2 weeks). In such a case we will work with the veterinarian to have the animal under direct veterinarian supervision during that period and will be put on an extended period of free water afterwards.

If, during the experimental planning, the period is estimated to last for more than 2 weeks, which is very unlikely it will ever be needed, I will apply for a protocol addendum specifying the reason why this is of critical importance at that stage of the project and for how long the uninterrupted sequence of recordings is planned to last. In addition to the IACUC approval (if approved) a written approval/direct monitoring log from the veterinarian will be added to the weight/water intake record to be placed together in the animals USDA record.

ANESTHESIA
SECTION C

1. List procedure(s) requiring anesthesia by species

Species	Procedure
Macaca mulatta	Surgery

2. Describe the pre-anesthesia protocol, including any fasting or pre-anesthetic drugs:

Animals are fasted 12 hours prior to surgery. Heart rate, respiratory rate, O2 saturation, temperature, and blood pressure will be monitored during the surgical procedure.

3. Anesthetic agent(s). If more than one agent is to be used (e.g. for induction and maintenance), list all agents by species:

Species	Anesthetic Agent	Dosage	Route
Macaca mulatta	Ketalar (prior to intubation)	.1/kg	IM
Macaca mulatta	Halothane	1/2% - 1% (titrated)	Inhalation

4. Describe procedures to monitor the depth of anesthesia: (e.g. respiratory rate, toe-pinch reflex, palpebral reflex)

Anesthesia will be monitored by ARP veterinarian technicians. Monitoring procedures include the observations of any behavioral sign of discomfort or sensitivity (presence of toe-pinch reflex, palpebral reflex or body movements) and continuous instrumental reading of respiratory rate, O2 solution level, heart rate, blood pressure, pulse oximetry, and body temperature.

5. Will a paralytic agent be used? Yes _____ No ✓

If yes, please specify.
Note: paralytic agents can only be administered to anesthetized animals, and animals must be monitored appropriately (i.e., blood pressure, ECG, etc.) to assure adequate anesthesia

Species	Agent	Dose	Route

SURGICAL PROCEDURES
SECTION D

1. Indicate where surgery will be performed, the person performing the surgery, and the qualifications and experience of that individual to perform the techniques involved: (Note: Major survival surgery on non-rodent mammalian species must be performed in a facility approved by the IACUC.)

   Bldg. _____ Room _____

   Name of Surgeon(s) _____

   Experience _____

2. Non-Survival Surgery (Animal will not recover from anesthesia) NA

   Survival Surgery (Animal will recover from anesthesia) Yes (fill out Section 4)

3. Describe in detail the surgical procedure including the surgical approach, closure, support care, and monitoring during the procedure. If necessary, attach a separate sheet.

Rhesus monkeys (macaca mulatta) will undergo a sequence of aseptic surgical procedures under inhalation anesthesia. The first surgery is for the implantation of the head strips. Animals will be given analgesics (Buprenex) to minimize post-surgical discomfort. Under general anesthesia, the skin will be incised down the midline over the center of the skull and reflected back. Two biocompatible and magnet compatible PEEK strips will be bolted to the skull using surgical-grade ceramic bone screws. Each of these two strips carries two attachments for the external head post, which will be added later. The skin is stitched back and the implant, fully covered by the skin, is left untouched for at least 6-8 weeks to settle. In a second surgery the two small attachments are exposed by small incisions in the skin and the head holder, made with PEEK and dental acrylic, is installed. The eye movement recordings in the magnet and in the training room are made by using human-compatible infrared eye tracking systems, which track the pupil position by a TV camera and therefore no further surgeries are done on the animal during the training in the lab, near the magnet bore or inside the bore and during the first complete set of fMRI images, which can take one year or more. In the second stage of the experimental protocol, one or, at most, two 15 mm diameter holes are trephined over the appropriate areas as determined by stereotaxic coordinates and previous functional imaging, and magnet-compatible recording chambers are placed over them to allow single unit recordings in the area of interest. If possible, the behavior of the animal will still be monitored by using infrared eye tracking systems for their perfect magnetic compatibility. On some animals, prior to the implantation of the recording chambers, a coil of Teflon-coated stainless-steel wire will be implanted under Tenon's capsule of the eyes, which allow eye position to be continuously monitored via the magnetic field/ search coil technique (Judge et al. 1980; Robinson 1963). This technique is far superior in terms of signal-to-noise ratio and signal stability to the infrared technique, but can be used only in recordings outside the magnet. The two coils are implanted in separate surgeries to allow full recovery of one eye before implanting the second eye to allow the animal to maintain full vision in one eye during the recovery. Inquires with other laboratories regarding the presence of eye coils in animals used inside high field magnets (left open during the imaging and checked for electrical insulation to avoid the generation of current loops) suggest that there are no adverse effects. If the quality of the infrared eye tracking is sufficient to finely quantify the behavior during the electrophysiological recordings, no eye coils will be implanted, but this depends largely on the type of behavior under analysis. Single unit recording/electrical stimulation and tracer or chemical micro-injections can be performed inside the magnet (Logothetis 2002 for review) if needed, but the vast majority of the electrophysiological sessions will be outside the magnet in a separate lab. The PI has many years of experience with these procedures, which can be performed rapidly and with a very low incidence of complications.

4. Fill out this section if survival surgery is to be performed.

A. Describe post-operative care, including supportive care, post-operative monitoring, analgesia, antibiotic therapy, arrangements for after hours, weekend, and holiday care. If necessary, attach a separate sheet.

Post-operative monitoring is done in the so that animals can be returned to their home cage as soon as they recover from the gas anesthesia. Ketamine is given for the transport. When animals begin to move about and can right themselves, they are given an analgesic (see below), and monitored until they are fully alert and sitting upright. Surgeries are scheduled in the AM (Mon - Thurs) to avoid recovering during nights and weekends. Lab staff are available at these times and off hours if needed. Antibiotics are given in consultation by the UAB veterinarians and sutures will be removed 10-14 days after surgery.

Rinsing/cleaning of the recording cylinders and gentle cleaning of the head implant are painless procedures that are done with the animal sitting in the monkey chair with its head blocked. They are done before and after every session and in any case every two/three days or more often, if needed. The PI will be responsible to organize weekend/ holiday care and water access changes.

At least twice a month (more often if necessary) the animal is anesthetized with Ketamine (0.1 mg/Kg) and brought to the lab for a deeper cleaning of the head implant. The head post is attached to the under-the-skin head strips by external plastic screws and it can be removed without touching the skin incisions. The upper head is shaved and cleaned with Betadine soap and the incisions on the skin around the strip attachments are cleaned and inspected for signs of infection. A general check of the animal, of the collar, and of the animal's temperature is also performed. At the end the animal, still anesthetized, is returned to its home cage and visually checked until sitting upright.

B. Comments regarding potential post-operative complications and / or pain:

Buprenex at a dose of 0.01 mg/kg Im will be given every 12 hours as needed for post-operative analgesia

C. Postoperative analgesic therapy:

Species	Agent	Dosage	Route	Frequency
Macaca mulatta	Buprenex	0.01 mg/kg	Im	12 hr.

D. Anticipated post-operative survival time: 6 months to 2 years

E. Will multiple survival surgeries be performed on a single animal? Yes ✔ No _____

If yes, explain and justify why this cannot be avoided. Attach a separate sheet if necessary.

Implantation of the skull plates, head post, eye coils (if needed), and recording wells must be done in stages according to the behavioral training paradigm. Implantation of the head strips is done first and, after 6-8 weeks, the head post is added. This allows the setting of the ceramic screws in the bone and a much better holding at the time the head post is installed. Using infrared eye tracking, all training phases and a first set of fMRI images can be done without eye coil surgeries. Eye coils are subject to mechanical stress each time the eyes are moved and their implant at the time of the head post not only is not necessary, but would also raise the possibility of having to replace them later on, with unneeded additional surgeries. As said earlier, such coils may not be needed on all animals. The two coils are implanted in separate surgeries to allow full recovery of one eye before implanting the second eye to allow the animal to maintain full vision in one eye during the recovery, usually at least one month apart. The implantation of the recording wells is done only after all the training and preliminary behavioral and imaging sessions are completed. There are two main reasons for this delayed installation. The first is that even with the best care, the exposure of the (intact) dura significantly increases the probability of infections inside or near the recording wells. The second is that the skull tends to grow back and starts to close the holes, with the need of additional surgeries for the removal of the new bone. It should also be noted that installation of the head post, eye coils, and recording wells, although done in a UAB surgical facility with full anesthesia, probably does not constitute "major survival surgery" since they do not involve the opening of a body cavity and there is no potential for permanent functional impairment. Opening of the conjunctiva and of small areas of the skull have been done in humans under local anesthesia.
Depending on the installation of eye coils or not, each animal will go through 3 (5 with the two eye coil surgeries) surgical procedures.
1. Head strips 2. Head post 3. First eye coil 4. Second eye coil 5. Recording wells
While unlikely, additional coil replacements may be needed.

UAB ANIMAL USE SAFETY INFORMATION

This project must be registered and authorized by UAB OH&S if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal or animal facility.

OH&S Administrative Use Only
Project #
Authorization Date

PI INFO

Name _____ Phone _____ Emergency # _____

Department: _____ Alternate Contact _____ Alt. Phone _____

PROJECT

Project Title _____ Species Macaca mulatta

FMR Imaging of Eye Stabilization Processes (APN 030405620;

IACUC Administrative Use Only

Funding Source NIH/NEI APN:

POTENTIALLY HAZARDOUS MATERIALS (Excluding Anesthetics)

Agent/Material is potentially hazardous for:

☐ Humans

☐ Animals (Species _____)

Agent(s)	Route of Administration	Excretion (e.g., urine/feces)	Human Health Risks or Other Concerns
NA			Standard protocols for handling and care of
			alert non-human primates.

SPECIAL PRECAUTIONS/INSTRUCTIONS (check all that apply)

☐ The PI or his/her technicians are responsible for the feeding and care of these animals (must receive IACUC approval)

☐ The following may be contaminated with potentially hazardous material and must be handled only by authorized personnel:

☐ Cage ☐ Pen ☐ Cage/pen accessories ☐ Water Bottle ☐ Animal Carcasses ☐ Bedding ☐ Other _____

☐ Cages/Pen must be decontaminated before cagewash (Method _____)

☐ Cages/Pen/Bedding must be autoclaved before cleaning or disposal

☐ Animal carcasses must be disposed of as follows:

☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures)

☐ Chem. And/or Bio. Contaminated (Red barrel incineration) ☐ Other _____

☐ All contaminated waste (soiled bedding and other animal waste) must be properly labeled and disposed of as follows:

☐ Chem. Contaminated (Yellow barrel incinerate) ☐ Bio. Contaminated (Autoclave/ Red barrel)

☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures) ☐ Other _____

☐ Other (incl. special tests or immunizations) _____

REQUIRED PERSONAL PROTECTIVE EQUIPMENT (PPE) (check all that apply)

☐ The following Personal Protective Equipment (PPE) must be worn/used in the room:

☐ Lab Coat ☐ Disposable Gloves ☐ Face Shield ☐ Safety Glasses ☐ Goggles

☐ NIOSH Certified Dust Mask ☐ Head/hair (beard) Cover ☐ Closed front gown with long sleeves and elastic cuffs

☐ Shoe Covers/Booties ☐ Disinfectant Foot Spray ☐ H_2O Repellant Coveralls/Jumpsuit

☐ Biosafety Cabinet req. ☐ N-95 or Equivalent Fitted Respirator

☐ Other _____

☐ PPE must be removed before leaving the room.

☐ PPE must be discarded or decontaminated after each use.

☐ Other _____

☐ Check here if additional information is attached.

Form Must Be Posted On Animal Room Door Where Animal is Used or Housed

IACUC Revised 11/10/00

OFFICE USE ONLY (revised 03/14/02)
IACUC Approval Number: 6580
Funded Date:
Not Funded/Delete Date:
Category

ONLINE, TYPE ONLY IN THE VISIBLE AREA

UAB Animal Use Review Form For New Proposals

General Information

Section A

Principal Investigator _____ Email _____

Department/Division _____ Extension _____ _____

Affiliation:

Graduate School		Medicine	✔
Med. Center Adm.		Nursing	
Med. Center Joint Dept.		Optometry	
S.H.R.P.		Public Health	
Dentistry		Academic Affairs	

Campus Address (Room, Bldg.) _____

Project Title A new approach to xenotransplantation in primates by combining tolerance induction and ~~cytoprotection of porcine pancreatic islets.~~

RECEIVED
SEP 26 2002
IACUC

Program Project or SCORE Title (If Applicable) _____

Project Period 04-01-2003 To 03-30-2006

External Supporting Agency NIH Application Deadline 10-01-02

Internal Supporting Agency _____ Application Deadline _____

Are all individuals having contact with the animals in this project participating in the ARP Personnel Health Program?

Yes ✔ No _____

	Species	Number Used Per Year	Approx. No. Days Housed Per Year	Approx. Daily Census Avg. / High	Animal Source Vendor	Housing Site*
1.	Pigs	6	1	2 /3		
2.	Rhesus	6	30	60 /90	Covance	
3.				/		

* If animals will be housed or undergo procedures outside of ARP animal facilities (in laboratories or other study areas) for more than 12 hours, please indicate the building and room number here: _____

I certify that the information provided on this form is accurate to the best of my knowledge and includes all animal procedures proposed in the corresponding grant or experimental plan.

_____ _____

Investigator Signature Date

Investigators responsible for experimental animal procedures other than the principal investigator:

Name(s) _____

Technical staff involved in the experimental procedures (please indicate training and experience):

Name	Hire Date	Length of Experience	Nature of Experience

1. Briefly explain the scientific merit of your proposal to justify the use of animals to any reasonable and well-informed lay person. This explanation should include your project's relevance to human or animal health and/or the advancement of knowledge. Include the rationale for the choice of species. If necessary, attach a separate sheet.

Pancreatic islet transplantation is an attractive treatment for patients with type I diabetes. However, there are major obstacles to overcome before islet transplantation can become a routine therapeutic procedure. One is the need for chronic immunosuppression and the other is the shortage of cadaveric organs for transplantation. In respect of the former, we have demonstrated islet allografts tolerance without maintenance immunosuppressive drug therapy in unrelated diabetic primates. With respect to the shortage of human pancreatic tissue, pigs are an attractive source of islets because they breed rapidly, a long history of porcine insulin in humans, and the potential for genetic engineering. Unfortunately, exposure of porcine islets to fresh human or primate serum or blood, resulted in acute islet damage mainly mediated by xenoreactive natural antibodies (XNA) and complement (C). Under certain circumstances, when XNA and C-mediated immune responses are inhibited for a few days, grafts can survive indefinitely despite the return of anti-donor antibodies and complement, a phenomenon referred as "accommodation" . Expression in the graft of anti-apoptotic or "protective genes", such as Bcl-2, A20 Bcl-xL and heme oxygenase-1 (HO-1), make the graft resistant to XNA and C-mediated rejection. The protein encoded by the Bcl-2 gene has been implicated in the prolongation of cell survival by blocking the early changes associated with apoptosis and necrosis. We have demonstrated that overexpression of Bcl-2 in pancreatic islets prevents loss of functional islet mass after transplantation and significantly reduces the number of islets required to achieve euglycemia. Within this context, we will (1) analyze the survival of genetically modifeid porcine islets to overexpress Bcl-2 and 2) we will asses the metabolic function after transplantation. The reasearch proposed in this grant will provide significant information to the field of xenotransplantation and the potential use of porcine islets as an alternative to human tissue for IDDM treatment.

2. Briefly justify the number of animals requested for the control and experimental group size and the statistical analysis planned. If necessary, attach a separate sheet.

See experimental plan.
18 pathogen-free pigs, 18 rhesus recipients

3. Please categorize the level of pain or discomfort associated with all procedures. Provide numbers of animals for each species and category (A, B, or C) as needed. (Please see instructions for explanation of categories.) If groups of animals fall into more than one category, fill out a line for each category. The total number for each species should be equal to the total number on page 1.

Category (A, B, or C)	Species	Number Per Year
B	rhesus	6

If any animals fall into Category C, justify the need to perform painful experiments without the use of anesthetics or analgesics. If necessary, attach a separate sheet.

4. The Animal Welfare Act (P.L. 99/158) requires that the principal investigator provide the following information:

a. Please provide assurance that alternatives to the use of animals were considered in planning these research activities:

There are no available valid in vitro or computer simulated model to replace animal studies. The research plan has been developed to address questions which have immediate potential applicability in human islet transplantation, and thus justify the use of non-human primate recipients. Advances in this model have potential to affect the lives of millions of diabetics. These studies will also elucidate immunological mechanisms and strategies to induce immunological unresponsiveness to xenografts in the absence of chronic immunosuppression.

b. Please provide assurance that these research activities do not *unnecessarily* duplicate previous experiments:

No duplication of previous experiments will be performed

c. Please describe the methods and sources used to determine that alternatives are not available and that unnecessary duplication of experiments will not occur. **If the sources include a database search, please include the databases searched, the date of the search, the years covered by the search, and the key words and/or search strategy used. If other sources are consulted, please include appropriate documentation.**

Check all that apply:

- ✓ Index Medicus (Medline, etc.)
- ____ Biological Abstracts
- ____ Current Research Information Service
- ____ National Agricultural Library
- ____ Other (describe)

SPECIFICS OF PROCEDURES INVOLVING ANIMALS
SECTION B

1. Euthanasia Methods: **(This question must be completed for all protocols)**:

   A. Inhalant agents (ether, halothane, methoxyflurane, CO_2)

   Species _____ Drug / Gas _____

   Method of Administration _____

   B. Injectable Agents (Barbiturates, KCl*. . .)

   Species Pigs Drug Sodidum pentob Dose 2 ml/kg Route i.v.

   C. Physical Methods

   Cervical dislocation (poultry, mice, rats <200g, rabbits <1kg)

   Species _____

   Decapitation with guillotine – Species _____

   Will the animal be sedated / anesthetized during cervical dislocation and/or decapitation?

   ( ) Yes – Fill out section C.

   ( ) No – Provide scientific justification for performing this procedure without sedation/anesthesia (e.g. interference with specific experimental parameters).

   D. Exsanguination* - Species _____

   E. Other Method (Describe) _____

   Species _____

   *Method to be used only in anesthetized animals – fill out Section C.

2. Immunizations / Antibody Production

Complete the following for *each species and immunogen.*

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses

B. Will adjuvants be used? Yes _____ No _____

Type of adjuvant:

Primary injection _____

Booster injection(s) _____

3. Other Injections:

If drugs or chemicals (other than anesthetics) are to be given, complete this section *for each species and agent.*

If anesthetics will be used, compete Section C.

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses
rhesus	streptozotocin	iv	3 ml	single	non	
rhesus	immunotoxin	iv	1.0 ml	2 doses	24 hour:	
rhesus	deoxyspergualin	iv	0.3 ml	15 doses	24 hour:	
rhesus	solumedrol	iv	1 ml	3 doses	24 hour:	

B. If toxic or other deleterious reactions may occur in animals, state the possible reaction(s) and procedures to deal with these reactions. If LD_{50} studies are planned, state the number of animals per dosage group (see instructions).

Streptozotocin. induces hypoglycemia and dehydration. Animals will be monitor closely after administration, lactated ringe's solution and Dextrose 5% will be used as nedded.
DSG and IT. induce dehydration and weight loss. Animals will be monitor closely after administration, lactated ringe's solution and Ensure will be used as nedded.

4. Are physical restraints used? Yes _____ No ✓
If yes, describe the restraint system and indicate the approximate time in the restraint for each experiment.

System _____

Time _____

5. Blood Samples (Note: Some blood collection methods such as intracardiac or retro-orbital techniques should be performed only in anesthetized animals – fill out Section C.

Route	saphenous	Amount	10-15 ml	Freq.	every 2 weeks max.
	tail vein		i drop		every day

6. Pain Threshold Tests

Type _____ Freq. _____

_____ _____

_____ _____

7. Special Diets/Food Deprivation

Type fasting overnight for surgery and metabolic studies Freq. every other week max.

_____ _____

_____ _____

8. Tumor inoculations / implantations (including hybridoma ascites tumors).

A.

Species	Tumor Type	Site

B. Describe procedures to monitor tumor size and ascitic fluid accumulation and frequency of tapping ascitic fluids. Also, describe criteria for euthanasia of animals if they become ill due to tumor growth:

9. Other procedures: (inhalation, infectious agents, inoculations, etc.).

Islet transplantation using gentically modified cells (see research plan)

USE FOR ADDITIONAL INFORMATION IF NECESSARY

Drugs :

rhesus	Doxycyclin	oral	1ml	140 dosis	every 24 hours
rhesus	Soluble complent	receptor 1	1 ml iv	2 dosis	every 24 hours
rhesus	buprenex	i.m.	0.3ml	8-12 dosis	every 12 hours

ANESTHESIA
SECTION C

1. List procedure(s) requiring anesthesia by species

Species	Procedure
Pigs	terminal donor-pancreas procurement
Rhesus	Handling, blood sampling, islet transplantation

2. Describe the pre-anesthesia protocol, including any fasting or pre-anesthetic drugs:

   Overnight fasting, no pre-anesthetic drugs

3. Anesthetic agent(s). If more than one agent is to be used (e.g. for induction and maintenance), list all agents by species:

Species	Anesthetic Agent	Dosage	Route
Pigs	Ketamine/isofluorane		inhaled
rhesus	ketamine/isofluorane		inhaled

4. Describe procedures to monitor the depth of anesthesia: (e.g. respiratory rate, toe-pinch reflex, palpebral reflex)

   Anesthesia will be monitored by respiratory rate, palpebral reflex, blood pressure and oxigen saturation in blood

5. Will a paralytic agent be used? Yes _____ No ✓

   If yes, please specify.
   Note: paralytic agents can only be administered to anesthetized animals, and animals must be monitored appropriately (i.e., blood pressure, ECG, etc.) to assure adequate anesthesia

Species	Agent	Dose	Route

SURGICAL PROCEDURES
SECTION D

1. Indicate where surgery will be performed, the person performing the surgery, and the qualifications and experience of that individual to perform the techniques involved: (Note: Major survival surgery on non-rodent mammalian species must be performed in a facility approved by the IACUC.)

   Bldg. _____ Room _____

   Name of Surgeon(s) _____

   Experience _____

2. Non-Survival Surgery (Animal will not recover from anesthesia) pigs

   Survival Surgery (Animal will recover from anesthesia) rhesus (fill out Section 4)

3. Describe in detail the surgical procedure including the surgical approach, closure, support care, and monitoring during the procedure. If necessary, attach a separate sheet.

Pigs.
Pancreas Procurement
Under the effects of anesthesia, the skin of the abdomen will be prepared with Betadine. A midline abdominal incision will be performed, the pancreas, duodenum and abdominal aorta will be exposed. Heparin (100 units/kg) will be given intravenously and 1 minute will be allowed for recirculation. The distal end of the abdominal aorta will be ligated and an 8 F cannula will be placed. Then, the aorta proximal to the celiac axis will be clamped and the preservation solution (UW, 4•C, 100 ml) will be infused by gravidity. Simultaneously, blood will be recovered directly from the inferior vena cava. The animal at this point will be euthanized and the pancreas removed. The dudenum will be clamped 2 cm proximal and 3 cm distal to the pancreatic duct and 20 cc of Betadine will be administered in the duodenal stump. After the harvest, the pancreatic duct is cannulated with a 24G angiocath . Strict sterile techniques will be enforced during the procedure including steril surgical instruments.

Rhesus.
Islet transplantation
Under the effects of anesthesia, the skin of the abdomen will be prepared. (hair removal and scrub with betadine). A midline abdominal incision will be performed, and the inferior mesenteric vein will be cannulated with an 7 F feeding tube. Islets will be infused by gravity. The abdomen will be closed in 3 layers, peritoneum/fascia with prolene 4-0, subcutaneosu tissue with Vycryl 4-0, skin with a staple gun. Strict sterile techniques will be enforced during the procedure including steril surgical instruments. The staples will be removed 15 days posttransplant.

Percutaneous liver biopsy:
The animal will be sedated with ketamine 10 mg/kg im. Cefazolin, 12.5 mg/kg, will be given as prophylactic antibiotic. Lidocaine, 1%, 1cc, will be used as local anesthetic (skin, muscles, peritoneum). A 2 mm incision will be performed under the last rib and a Tru-cut needle will be inserted and directed to the left lateral segment of the liver. Buprenex, 0.2 mL im will be used as post-procedural analgesic. Biopsies will take place as described in the experimental plan. Strict sterile techniques will be enforced during the procedure including steril surgical instruments.

Lymph node biopsy:
The animal will be sedated with ketamine 10 mg/kg im. Cefazolin, 12.5 mg/kg, will be given as prophylactic antibiotic. Lidocaine, 1%, 1cc, will be used as local anesthetic (skin, muscles, peritoneum). A 5 mm incision will be performed in the axillary or inguinal region. One lymph node will identified and excised. The incusion is closed with a single stitch of 3.0 silk suture. Buprenex, 0.2 mL im will be used as post-procedural analgesic. Biopsies will take place at days 7, 14 and 30 days post-transplant. Strict sterile techniques will be enforced during the procedure including steril surgical instruments.

4. Fill out this section if survival surgery is to be performed.

A. Describe post-operative care, including supportive care, post-operative monitoring, analgesia, antibiotic therapy, arrangements for after hours, weekend, and holiday care. If necessary, attach a separate sheet.

For all routine handling and bleeding, the monkeys are anesthetized with ketamine (10 mg/kg). For surgery, the animals are sedated pre-operatively with Ketamine (20 mg/kg IM) and Fentanyl (15ug/kg IM). After transplantation, a 24 hr. constant care period for recipients and donors is maintained with the continuous presence of a qualified staff member to administer fluids, antibiotics, and adequate Buprenex (0.02 ml IM) analgesia every 12 hours to minimize surgical stress and pain. To reduce fever and discomfort from •-CD3-IT, 81 mg aspirin is administered postoperatively on days 0 and +1 posttransplant. The combination of aspirin, Methylprednisolone and Buprenex will minimize fever and inflammatory reactions and provide analgesia. Thereafter, donors and recipients are monitored daily by the PI and staff and 3-5 times weekly by the veterinarians. Weekend care is always provided. Ketamine is given whenever possible to minimize discomfort and stress in handling or phlebotomizing and to protect the staff from biohazards.
Post-transplant care and islet transplant evaluation. All recipients will receive analgesics for 3 days (Buprenex, 0.15 mg/kg/i.m.), fluids as needed and enteral nutritional support (Ensure, 250 ml/day) for 3 days after the surgical procedure.

B. Comments regarding potential post-operative complications and / or pain:
See post-operative care

C. Postoperative analgesic therapy:

Species	Agent	Dosage	Route	Frequency
rhesus	buprenex	0.15 mg/kg/ir	im	12 hours

D. Anticipated post-operative survival time: indefinite

E. Will multiple survival surgeries be performed on a single animal? Yes _____ No ✔
If yes, explain and justify why this cannot be avoided. Attach a separate sheet if necessary.

UAB ANIMAL USE SAFETY INFORMATION

This project must be registered and authorized by UAB OH&S if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal or animal facility.

OH&S Administrative Use Only
Project #
Authorization Date

PI INFO

Name _____ Phone _____ Emergency # _____

Department _____ Alternate Contact _____ Alt. Phone _____

PROJECT

Species _____

Project Title _____

A new approach to xenotransplantation in primates by combining tolerance induction and Bcl-2 cytoprotection of porcine pancreatic islets.

IACUC Administrative Use Only
APN:

Funding Source _____

POTENTIALLY HAZARDOUS MATERIALS (Excluding Anesthetics)

OH&S Use Only | OH&S Use Only | OH&S Use Only

Agent/Material is potentially hazardous for:

☐ Humans
☐ Animals (Species _____)

Agent(s)	Route of Administration	Excretion (e.g., urine/feces)	Human Health Risks or Other Concerns
Streptozotocin	iv	urine	carcinogenic, diabetogenic

SPECIAL PRECAUTIONS/INSTRUCTIONS (check all that apply)

☐ The PI or his/her technicians are responsible for the feeding and care of these animals (must receive IACUC approval)

☑ The following may be contaminated with potentially hazardous material and must be handled only by authorized personnel:

☐ Cage ☐ Pen ☐ Cage/pen accessories ☐ Water Bottle ☑ Animal Carcasses ☐ Bedding ☐ Other _____)

☐ Cages/Pen must be decontaminated before cagewash (Method _____)

☐ Cages/Pen/Bedding must be autoclaved before cleaning or disposal

☑ Animal carcasses must be disposed of as follows:

☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures)

☑ Chem. And/or Bio. Contaminated (Red barrel incineration) ☐ Other _____

☑ All contaminated waste (soiled bedding and other animal waste) must be properly labeled and disposed of as follows:

☑ Chem. Contaminated (Yellow barrel incinerate) ☐ Bio. Contaminated (Autoclave/ Red barrel)

☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures) ☐ Other _____

☐ Other (incl. special tests or immunizations) _____

REQUIRED PERSONAL PROTECTIVE EQUIPMENT (PPE) (check all that apply)

☑ The following Personal Protective Equipment (PPE) must be worn/used in the room:

☑ Lab Coat ☑ Disposable Gloves ☐ Face Shield ☑ Safety Glasses ☐ Goggles

☑ NIOSH Certified Dust Mask ☑ Head/hair (beard) Cover ☐ Closed front gown with long sleeves and elastic cuffs

☑ Shoe Covers/Booties ☐ Disinfectant Foot Spray ☐ H_2O Repellant Coveralls/Jumpsuit

☐ Biosafety Cabinet req. ☐ N-95 or Equivalent Fitted Respirator

☐ Other _____

☑ PPE must be removed before leaving the room.

☐ PPE must be discarded or decontaminated after each use.

☐ Other _____

Form Must Be Posted On Animal Room Door Where Animal is Used or Housed

IACUC Revised 11/10/00

☐ Check here if additional information is attached.

OFFICE USE ONLY (revised 03/14/02)
IACUC Approval Number: 7023
Funded Date:
Not Funded/Delete Date:
Category

ONLINE, TYPE ONLY IN THE VISIBLE AREA

UAB Animal Use Review Form For New Proposals
General Information
Section A

Principal Investigator _____ Email _____

Department/Division _____ Extension _____

Affiliation:

Graduate School		Medicine	✔
Med. Center Adm.		Nursing	
Med. Center Joint Dept.		Optometry	
S.H.R.P.		Public Health	
Dentistry		Academic Affairs	

Campus Address (Room, Bldg.) _____

Project Title Evaluation of the effects of brain-death on islet recovery and functionality in primates

Program Project or SCORE Title (If Applicable) _____

Project Period 11-01-03 To 10-31-04

External Supporting Agency _____ Application Deadline _____

Internal Supporting Agency Division or Transplantation Application Deadline N/A

Are all individuals having contact with the animals in this project participating in the ARP Personnel Health Program?

Yes ✔ No _____

	Species	Number Used Per Year	Approx. No. Days Housed Per Year	Approx. Daily Census Avg. / High	Animal Source Vendor	Housing Site*
1.	Monkeys	14	1-2	1 / 2	No preference	
2.	SCID Mice	230	30	30 / 45	Jackson	
3.				/		

* If animals will be housed or undergo procedures outside of ARP animal facilities (in laboratories or other study areas) for more than 12 hours, please indicate the building and room number here: _____

I certify that the information provided on this form is accurate to the best of my knowledge and includes all animal procedures proposed in the corresponding grant or experimental plan.

Investigator Signature _____ Date OCT - 6

Investigators responsible for experimental animal procedures other than the principal investigator:

Name(s) _____

Technical staff involved in the experimental procedures (please indicate training and experience):

Name	Hire Date	Length of Experience	Nature of Experience

1. Briefly explain the scientific merit of your proposal to justify the use of animals to any reasonable and well-informed lay person. This explanation should include your project's relevance to human or animal health and/or the advancement of knowledge. Include the rationale for the choice of species. If necessary, attach a separate sheet.

See attached

2. Briefly justify the number of animals requested for the control and experimental group size and the statistical analysis planned. If necessary, attach a separate sheet.

14 primates animals will be requested for the entire project. 7 will be used as control donors (no brain death), and 7 will be subjected to brain-death before pancreas procurement. Islet yield and functionality will be compared statistically between groups using a student t test. 230 SCID mice will be required for the entire project. 15 animals will be used per islet isolation (total 14 isolations) for quality control. 5 animals will receive an optimal islet dose (2000 Islet equivalents (IEQ)/mouse), 5 will receive 1000 IEQ/mouse and 5 will get 500 IEQ. 20 extra animals are requested to replace animals not included in the study for technical problems.

1. Briefly explain the scientific merit of your proposal to justify the use of animals to any reasonable and well-informed lay person. This explanation should include your project's relevance to human or animal health and/or the advancement of knowledge. Include the rationale for the choice of species. If necessary, attach a separate sheet.

Application of a new steroid-free immunosuppressive protocol has markedly improved outcomes in pancreatic islet transplantation (PIT). However, large numbers of islets are required to achieve insulin-independence. While islet re-transplantation is effective, it lacks cost-effectiveness and is constrained by the shortfall of donor pancreatic tissue. Although there are approximately 1 million islets in the adult human pancreas, the current pancreas preservation and islet isolation techniques recover fewer than 50% of the islets. Furthermore, significant loss of functional islet mass (FIM) occurs in the peritransplant period. Thus, the disparity between human islet supply and potential demands of millions of diabetic patients mandates that improved methods for islet recovery and engraftment are needed.

Organ transplantation outcomes are influenced by antigen-dependent and independent events. Despite progressive improvements in immunosuppressive agents, the success rate of organs obtained from cadaveric donors, both over the short- and long-term, remains significantly inferior to those from living donors regardless of their genetic relationship with the recipient. The major difference between cadaveric and living donors is brain-death (BD). Acceptance of this well-defined clinical diagnosis enables removal of appropriate functioning organs while the circulation is still sustained. Such tissues/organs, however, experience profound physiological derangements that may be initiated by the central catastrophe, in addition to injury potentially mediated by subsequent effects of storage and reperfusion. ***Presently, cadaveric donors remain the sole source of pancreatic tissue for PIT.*** Several studies have demonstrated the deterioration of organs following BD by multiple interrelated events, including the effects of massive acute cerebral injury, hypotension, and the release of pro-inflammatory cytokines (PIC), such as TNF-α, IL-1β, IL-6 and IFN-γ from multiple cell types. Increased mRNA expression of these factors has also demonstrated in peripheral tissues. Although PIC have a profound impact on pancreatic β-cell function and death during type I diabetes, unfortunately ***no studies have been conducted to date to determine the effects of BD on islet isolation, culture, and transplantation.*** Furthermore, virtually all experimental studies in islet transplantation use young, healthy living animals as donors. In this regard, our preliminary studies in small animals have suggested that BD in rats is associated with a significant reduction in the yield and viability of isolated pancreatic islets and their functionality *in vivo* after transplantation. It could therefore be postulated that prevention/reduction of the deleterious effects of BD would mitigate islet loss and improve islet functionality and survival after transplantation. Moreover, development of such strategies could improve the quality of organs from "marginal" donors, thus broadening the criteria for donor acceptance for islet isolation and transplantation.

Islet yields and functionality after transplantation are different between species, especially in small animals versus large animals. These differences could be related to different susceptibilities of pancreatic islets to cell death induced by proinflammatory cytokines, oxidative stress, hyperglycemia, etc. In this regard, in order to develop therapeutic strategies, the evaluation of the effects of brain death on pancreatic islets needs to be performed in preclinical models, especially primates. In this regard, primate islet isolation and islet physiology are the closest to the human. Therefore, the proposed studies will be performed using primates as brain-death donors.

Islet functionality can be evaluated in vitro by glucose-stimulated insulin release. However, no correlation exist between in vitro analysis and functionality after the transplant. Therefore, the gold standard quality control test after islet isolation is transplantation into diabetic SCID-mice. This protocol request mice to evaluate in vivo functionality of islets obtained from brain-death donors.

3. Please categorize the level of pain or discomfort associated with all procedures. Provide numbers of animals for each species and category (A, B, or C) as needed. (Please see instructions for explanation of categories.) If groups of animals fall into more than one category, fill out a line for each category. The total number for each species should be equal to the total number on page 1.

Category (A, B, or C)	Species	Number Per Year
B	NHP	14
B	Mice	230

If any animals fall into Category C, justify the need to perform painful experiments without the use of anesthetics or analgesics. If necessary, attach a separate sheet.

4. The Animal Welfare Act (P.L. 99/158) requires that the principal investigator provide the following information:

a. Please provide assurance that alternatives to the use of animals were considered in planning these research activities:

There are no available valid in vitro or computer simulated model to replace animal studies. The research plan has been developed to address questions which have immediate potential applicability in human islet transplantation. Advances in this model have potential to affect the lives of millions of diabetics.

b. Please provide assurance that these research activities do not *unnecessarily* duplicate previous experiments:

No duplication of previous experiments will be performed

c. Please describe the methods and sources used to determine that alternatives are not available and that unnecessary duplication of experiments will not occur. **If the sources include a database search, please include the databases searched, the date of the search, the years covered by the search, and the key words and/or search strategy used. If other sources are consulted, please include appropriate documentation.**

brain-death / islets, 1966 to present

Check all that apply:

- ✓ Index Medicus (Medline, etc.)
- ✓ Biological Abstracts
- ___ Current Research Information Service
- ___ National Agricultural Library
- ___ Other (describe)

SPECIFICS OF PROCEDURES INVOLVING ANIMALS
SECTION B

1. Euthanasia Methods: **(This question must be completed for all protocols)**:

A. Inhalant agents (ether, halothane, methoxyflurane, CO_2)

Species Mice Drug / Gas CO2

Method of Administration inhalation

B. Injectable Agents (Barbiturates, KCl*. . .)

Species NHP Drug pentobarbital Dose 100 mg/kg Route i.v.

C. Physical Methods

Cervical dislocation (poultry, mice, rats <200g, rabbits <1kg)

Species _____

Decapitation with guillotine – Species _____

Will the animal be sedated / anesthetized during cervical dislocation and/or decapitation?

( ) Yes – Fill out section C.

( ) No – Provide scientific justification for performing this procedure without sedation/anesthesia (e.g. interference with specific experimental parameters).

D. Exsanguination* - Species _____

E. Other Method (Describe) _____

Species _____

*Method to be used only in anesthetized animals – fill out Section C.

2. Immunizations / Antibody Production

Complete the following for *each species and immunogen.*

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses

B. Will adjuvants be used? Yes _____ No _____

Type of adjuvant:

Primary injection _____

Booster injection(s) _____

3. Other Injections:

If drugs or chemicals (other than anesthetics) are to be given, complete this section *for each species and agent.*

If anesthetics will be used, compete Section C.

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses
Mice	Streptozotocin	ip		200 mg/kg	1	N/A

B. If toxic or other deleterious reactions may occur in animals, state the possible reaction(s) and procedures to deal with these reactions. If LD_{50} studies are planned, state the number of animals per dosage group (see instructions).

Streptozotocin induces hypoglycemia and dehydration on days 1-3 post-administration. Then, STZ induces hyperglycemia. Animals will be monitored closely. lactated Ringer's solution and dextrose 5% will be infused as needed.

4. Are physical restraints used? Yes _____ No ✓
If yes, describe the restraint system and indicate the approximate time in the restraint for each experiment.

System _____

Time _____

5. Blood Samples (Note: Some blood collection methods such as intracardiac or retro-orbital techniques should be performed only in anesthetized animals – fill out Section C.

Route	Amount	Freq.
tail vein	1 drop	daily for 1 week, 3/week
		thereafter (total 4 weeks)

6. Pain Threshold Tests

Type	Freq.

7. Special Diets/Food Deprivation

Type	Freq.
Fasting overnight for surgery (NHP)	days 7 and 30 (mice)
Fasting overnight for surgery and metabolic	

8. Tumor inoculations / implantations (including hybridoma ascites tumors).

A.

Species	Tumor Type	Site

B. Describe procedures to monitor tumor size and ascitic fluid accumulation and frequency of tapping ascitic fluids. Also, describe criteria for euthanasia of animals if they become ill due to tumor growth:

9. Other procedures: (inhalation, infectious agents, inoculations, etc.).

islet transplantation (mice), brain death induction (NHP)

ANESTHESIA
SECTION C

1. List procedure(s) requiring anesthesia by species

Species	Procedure
NHP	Routine handling, brain-death induction
mice	islet transplantation, blood sampling,

2. Describe the pre-anesthesia protocol, including any fasting or pre-anesthetic drugs:

Overnight fasting before surgical procedures, no pre-anesthetic drugs

3. Anesthetic agent(s). If more than one agent is to be used (e.g. for induction and maintenance), list all agents by species:

Species	Anesthetic Agent	Dosage	Route
NHP	ketamine	100 mg/kg	i.m.
NHP	isofluorane	variable	inhalation
Mice	Ketamine/xylazine	100 mg/kg /10 mg/kg	i.p.

4. Describe procedures to monitor the depth of anesthesia: (e.g. respiratory rate, toe-pinch reflex, palpebral reflex)

Anesthesia will be monitored by respiratory rate, palpebral reflex, and assesment of blood pressure / oxygen saturation.

5. Will a paralytic agent be used? Yes _____ No ✓

If yes, please specify.
Note: paralytic agents can only be administered to anesthetized animals, and animals must be monitored appropriately (i.e., blood pressure, ECG, etc.) to assure adequate anesthesia

Species	Agent	Dose	Route

SURGICAL PROCEDURES
SECTION D

1. Indicate where surgery will be performed, the person performing the surgery, and the qualifications and experience of that individual to perform the techniques involved: (Note: Major survival surgery on non-rodent mammalian species must be performed in a facility approved by the IACUC.)

   Bldg. _____ Room _____

   Name of Surgeon(s) _____

   Experience _____

2. Non-Survival Surgery (Animal will not recover from anesthesia) NHP

   Survival Surgery (Animal will recover from anesthesia) Mice (fill out Section 4)

3. Describe in detail the surgical procedure including the surgical approach, closure, support care, and monitoring during the procedure. If necessary, attach a separate sheet.

   See attached

Brain-death induction (NHP)

Normal male or female, 3-10 kg rhesus monkeys will be used. Animlas will be fast overnight the day before the procedure. Ketamine (100 mg/kg/i.m.) will be used to sedate the animal before endotraqueal intubation. Cefazolin, 12.5 mg/kg/single dose will be given i.v.Then, general anesthesia with isofluorane will be initiated. A peripheral vein (saphenous) will be cannulated with a 18-20 G angiocath and ½ ND/Dextrose 5% will be infused at 150 ml/hour. Temperature will be monitored with a rectal probe and urine output with a Foley catheter (5F). A 24G angiogath will be placed on the right radial artery to monitor arterial blood pressure using a standard HP monitor. A central line will be placed via external jugular vein (Hickman, Double or single lumen 5 F) to monitor central venous pressure, fluid infusion and for blood samples. Blood oxygen saturation will be monitor following standard techniques. Then, the animal will be cover with plastic drapes to maintain corporal temperature at 36-38 °C. Pre-warmed sheets will be used in case of hypothermia. Next, the occipital region of the head will be shaved, and prepared with betadine. Surgical drapes will be placed and a 5 mm hole will be drilled on the occipital bone, 1 cm aside from midline. A 4 or 5 F Foley catheter will be introduced into the extradural space with the tip pointing caudally. The Foley catheter will be fixed with 3-0 silk sutures. Inflating the balloon progressively with sterile saline (50 mL) will increase the intracranial pressure, thereby inducing rapidly progressive brain injury and brain-death. Mean arterial blood pressure will be maintained at > 70 mmHg. Urine will be replaced cc by cc each hour with fluids (see above). Brain-death will be defined by sharp rise and then subsequent drop of blood pressure and heart rate and will be confirmed by the absence of corneal reflexes, apnea, and "flat" electroencephalogram.
The animal will be maintained with mechanical ventilation, fluid therapy and homodynamic monitoring for 6 hours. Then, the skin of the abdomen will be shaved, and prepared with betadine. Following standard surgical techniques, a midline incision will be made. The tail of the pancreas will be dissected free. Then, the first and fourth segments of the duodenum will be ligated and separated from the rest of the small intestine using umbilical tape. The animal will receive heparin (100 U/kg/i.v.) and the aorta will be cannulated with an I.V. line connected to the preservation solution (University of Wisconsin Solution, 4°C). The vena cava will be cannulated with an extension I.v.tubing and 60 mL of blood will be obtained. Next, the intrathoracic aorta and superior vena cava will be occluded with a vascular clamp. The animal will be euthanized with pentobarbital, 100 mg/kg/iv. Death will be confirmed by direct thoracic observation. Next, the preservations solution will be infused and the intra-abdominal organs will be "cooled" with sterile ice. The pancreas will be removed and cleaned ex vivo. The pancreatic duct will be cannulated with a 24 G angiocath. Then, the pancreas will be transported to the lab for islet isolation.
Control, non brain-death animals will be subjected to the same procedures except inflation f the Foley catheter.

Renal/liver profiles will be obtained each hour to direct the electrolyte therapy. 1 cc heparinized blood will be obtained also each hour for cytokine analysis.

Islet transplantation (mice):

Mice (SCID, males, 30-40 grams) will be anesthetized (ketamine and xylazine, 100 mg/kg-10 mg/kg, i.p.), then the abdomen shaved and prepped with betadine. A midline incision is made and the portal vein is identified. Islets are infused into the portal vein using a 30G needle. Bleeding is controlled by local pressure and Surgicel. The abdomen is closed in two layers with Vycryl 6-0 (peritoneum and abdominal muscles) and the skin with Silk 6-0.

Intraperitoneal glucose tolerance test (days 7 and 30 post-transplant)

After overnight fast, rats are anesthetized with isofluorane, then the abdomen shaved and prepped with betadine. Dextrose (1 gram/kg body wt.) is injected i.p. Glucose determinations using a glucometer are performed from the tail vein (1 drop of blood) before dextrose infusion and at 2,5,10,15, 30 and 60 minutes.

4. Fill out this section if survival surgery is to be performed.

A. Describe post-operative care, including supportive care, post-operative monitoring, analgesia, antibiotic therapy, arrangements for after hours, weekend, and holiday care. If necessary, attach a separate sheet.

For handling and bleeding, mice will be anesthetized with ketamine/xylazine. After the transplant, continuous monitoring by qualified staff will be provided. Fluids will be given as needed. Buprenex (0.15 mg/kg/ i.p.) will be given q12 hours for 3 days. Weekend care will be always provided.
In case of dehydration, lactated ringer's will be used. Glucose determinations will be obtained daily for 7 days. Failure to achieve euglycemia (nonfasting glucose >200 mg/dL) for 3 consecutive days will be considered the end of the experiment and the animal will be euthanized. Euglycemic animals will be euthanized at the end of the study (30 days).

B. Comments regarding potential post-operative complications and / or pain:
See above

C. Postoperative analgesic therapy:

Species	Agent	Dosage	Route	Frequency
mice	buprenex	0.15 mg/kg	i.m.	q 12 hours

D. Anticipated post-operative survival time: 30 days

E. Will multiple survival surgeries be performed on a single animal? Yes _____ No ✓
If yes, explain and justify why this cannot be avoided. Attach a separate sheet if necessary.

UAB ANIMAL USE SAFETY INFORMATION

This project must be registered and authorized by UAB OH&S if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal or animal facility.

OH&S Administrative Use Only
Project #
Authorization Date

PI INFO

Name _____ Phone _____ Emergency # _____

Department _____ Alternate Contact _____ Alt. Phone _____

PROJECT

Project Title _____ Species Primates, mice

Evaluation of the effects of brain-death on islet recovery and functionality in primates

Funding Source Internal

IACUC Administrative Use Only
APN: 7023

POTENTIALLY HAZARDOUS MATERIALS (Excluding Anesthetics)

Agent/Material is potentially hazardous for:
- ☑ Humans
- ☑ Animals (Species Mice)

Agent(s)	Route of Administration	Excretion (e.g., urine/feces)	Human Health Risks or Other Concerns
streptozotocin (mice	i.v.	urine (inactive)	carcinogenic, diabetogenic
only)			

SPECIAL PRECAUTIONS/INSTRUCTIONS (check all that apply)

- ☐ The PI or his/her technicians are responsible for the feeding and care of these animals (must receive IACUC approval)
- ☑ The following may be contaminated with potentially hazardous material and must be handled only by authorized personnel:
  - ☐ Cage ☐ Pen ☐ Cage/pen accessories ☐ Water Bottle ☑ Animal Carcasses ☑ Bedding ☐ Other _____
- ☐ Cages/Pen must be decontaminated before cagewash (Method _____)
- ☐ Cages/Pen/Bedding must be autoclaved before cleaning or disposal
- ☑ Animal carcasses must be disposed of as follows:
  - ☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures)
  - ☑ Chem. And/or Bio. Contaminated (Red barrel incineration) ☐ Other _____
- ☑ All contaminated waste (soiled bedding and other animal waste) must be properly labeled and disposed of as follows:
  - ☑ Chem. Contaminated (Yellow barrel incinerate) ☐ Bio. Contaminated (Autoclave/ Red barrel)
  - ☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures) ☐ Other _____
- ☐ Other (incl. special tests or immunizations) _____

REQUIRED PERSONAL PROTECTIVE EQUIPMENT (PPE) (check all that apply)

- ☑ The following Personal Protective Equipment (PPE) must be worn/used in the room:
  - ☑ Lab Coat ☑ Disposable Gloves ☐ Face Shield ☑ Safety Glasses ☐ Goggles
  - ☑ NIOSH Certified Dust Mask ☑ Head/hair (beard) Cover ☐ Closed front gown with long sleeves and elastic cuffs
  - ☑ Shoe Covers/Booties ☐ Disinfectant Foot Spray ☐ H_2O Repellant Coveralls/Jumpsuit
  - ☐ Biosafety Cabinet req. ☐ N-95 or Equivalent Fitted Respirator
  - ☑ Other safety glasses worn only when animals are injected and cages containing hazard are dumped
- ☑ PPE must be removed before leaving the room.
- ☐ PPE must be discarded or decontaminated after each use.
- ☐ Other _____

Form Must Be Posted On Animal Room Door Where Animal is Used or Housed

IACUC Revised 11/10/00

☐ Check here if additional information is attached.

OFFICE USE ONLY (revised 03/14/02)
IACUC Approval Number: 7036
Funded Date:
Not Funded/Delete Date:
Category

ONLINE, TYPE ONLY IN THE VISIBLE AREA

UAB Animal Use Review Form For New Proposals
General Information
Section A

Principal Investigator _____ Email _____

Department/Division _____ Extension _____

Affiliation:

Graduate School		Medicine	✔
Med. Center Adm.		Nursing	
Med. Center Joint Dept.		Optometry	
S.H.R.P.		Public Health	
Dentistry		Academic Affairs	

Campus Address (Room, Bldg.) _____

Project Title Preclinical Studies to Optimize Islet Transplant Tolerance Induction in nonhuman primates (NHP)

Program Project or SCORE Title (If Applicable) JDRF Developmental Center for Immune Tolerance at UAB

Project Period 01/01/04 To 12/31/07

External Supporting Agency JDRF Application Deadline 10/22/03

Internal Supporting Agency _____ Application Deadline _____

Are all individuals having contact with the animals in this project participating in the ARP Personnel Health Program?

Yes ✔ No _____

	Species	Number Used Per Year	Approx. No. Days Housed Per Year	Approx. Daily Census Avg. / High	Animal Source Vendor	Housing Site*
1.	Rhesus monkeys	23	365	80 / 300	Labs	
2.				/		
3.				/		

OCT 22

* If animals will be housed or undergo procedures outside of ARP animal facilities (in laboratories or other study areas) for more than 12 hours, please indicate the building and room number here: _____

I certify that the information provided on this form is accurate to the best of my knowledge and includes all animal procedures proposed in the corresponding grant or experimental plan.

Investigator Signature Date

Investigators responsible for experimental animal procedures other than the principal investigator:

Name(s) _____

Technical staff involved in the experimental procedures (please indicate training and experience):

Name	Hire Date	Length of Experience	Nature of Experience

1. Briefly explain the scientific merit of your proposal to justify the use of animals to any reasonable and well-informed lay person. This explanation should include your project's relevance to human or animal health and/or the advancement of knowledge. Include the rationale for the choice of species. If necessary, attach a separate sheet.

Nowhere is the need for transplantation tolerance more urgent than in pancreas isolated islet transplantation to treat Type I insulin-dependent diabetes. The ranks of new diabetics continue to swell, with more than a million Type-I diabetics in the United States. Conservative management with exogenous insulin does not avert the life threatening complications of diabetes. Whole organ pancreas transplantation can replace islet function, but the attendant morbidity of the procedure limits applicability to patients with end-stage disease. In contrast, transplantation of isolated islets by infusion is a low morbidity surgical approach that has had a surge of recent clinical success. In the footsteps of major recent advances in clinical islet transplantation, the induction of durable immune tolerance emerges as the prevailing immunological challenge. Only tolerance induction can enable indefinite allograft acceptance, while eliminating the harmful side effects and economic burden of lifelong immunosuppression. Clearly, a greater understanding of the immune mechanisms, strategies, and genetics that favor nonhuman primate islet allograft tolerance is needed to bridge the chasm between rodent tolerance and clinical application. In this context, the importance of nonhuman primate tolerance studies cannot be overestimated.

We propose to examine modifications to a clinically promising islet transplant tolerance strategy, in nonhuman primates, with the goal of enhancing the safety and the consistency of tolerance outcome prior to human application. This strategy, that uses a concise peritransplant treatment protocol of two weeks duration without chronic immunosuppression, has led to robust, long-term tolerance in rhesus monkey allograft recipients, with donor-recipient MHC incompatibilities. Here, we will examine approaches to refine this model to optimize tolerance outcome.

2. Briefly justify the number of animals requested for the control and experimental group size and the statistical analysis planned. If necessary, attach a separate sheet.

Aim 1 of this Grant needs 22 monkeys as islet transplant recipients. 15 monkeys will be needed to serve as pancreas donors, giving a total for this phase of 37 monkeys.
Aim 2 of this Grant needs 18 monkeys as islet transplant recipients. 12 monkeys will be needed to serve as pancreas donors, giving a total for this phase of 30 monkeys.

Therefore the total animals required over four years is 67 monkeys minimum. Wherever possible other monkeys, required for associated projects in our other projects, will be used as control groups or as terminal pancreas donors.

Statistical analyses will be performed using non-parametric methods (e.g. Mann-Whitney).

3. Please categorize the level of pain or discomfort associated with all procedures. Provide numbers of animals for each species and category (A, B, or C) as needed. (Please see instructions for explanation of categories.) If groups of animals fall into more than one category, fill out a line for each category. The total number for each species should be equal to the total number on page 1.

Category (A, B, or C)	Species	Number Per Year
B	Rhesus macaques	23

If any animals fall into Category C, justify the need to perform painful experiments without the use of anesthetics or analgesics. If necessary, attach a separate sheet.

4. The Animal Welfare Act (P.L. 99/158) requires that the principal investigator provide the following information:

a. Please provide assurance that alternatives to the use of animals were considered in planning these research activities:

There are no validated in vitro or computer simulation models available to replace the complex immunological processes of islet transplantation and immune tolerance to be evaluated by the proposed studies. In vitro analyses are employed in many experiments of the proposal when they are appropriate. The research plan has been developed to address, in a nonhuman primate model, questions that have express clinical relevance to induction of immune tolerance in humans with autoimmune disease or islet allografts.

b. Please provide assurance that these research activities do not *unnecessarily* duplicate previous experiments:

No other research activities related to these experiments have been published. The research in this proposal is unique.

c. Please describe the methods and sources used to determine that alternatives are not available and that unnecessary duplication of experiments will not occur. **If the sources include a database search, please include the databases searched, the date of the search, the years covered by the search, and the key words and/or search strategy used. If other sources are consulted, please include appropriate documentation.**

Search performed 9/22/03
"Primates" AND "Immune Tolerance" AND "Islets" AND "Immunotoxin" (1966 - 2003)

Check all that apply:

- ✓ Index Medicus (Medline, etc.)
- ✓ Biological Abstracts
- ✓ Current Research Information Service
- ____ National Agricultural Library
- ____ Other (describe)

SPECIFICS OF PROCEDURES INVOLVING ANIMALS
SECTION B

1. Euthanasia Methods: **(This question must be completed for all protocols)**:

   A. Inhalant agents (ether, halothane, methoxyflurane, CO_2)

   Species _____ Drug / Gas _____

   Method of Administration _____

   B. Injectable Agents (Barbiturates, KCl*. . .)

   Species Rhesus Drug Pentobarbitol Dose 100 mg/kg Route i.v.

   C. Physical Methods

   Cervical dislocation (poultry, mice, rats <200g, rabbits <1kg)

   Species _____

   Decapitation with guillotine – Species _____

   Will the animal be sedated / anesthetized during cervical dislocation and/or decapitation?

   ( ) Yes – Fill out section C.

   ( ) No – Provide scientific justification for performing this procedure without sedation/anesthesia (e.g. interference with specific experimental parameters).

   D. Exsanguination* - Species _____

   E. Other Method (Describe) _____

   Species _____

   *Method to be used only in anesthetized animals – fill out Section C.

2. Immunizations / Antibody Production

Complete the following for *each species and immunogen.*

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses

B. Will adjuvants be used? Yes _____ No _____

Type of adjuvant:

Primary injection _____

Booster injection(s) _____

3. Other Injections:

If drugs or chemicals (other than anesthetics) are to be given, complete this section *for each species and agent.*

If anesthetics will be used, compete Section C.

A. Injection Protocol

Species	Agent	Route	Site	Volume	Number of Doses	Interval Between Doses
Rhesus	Immunotoxin	i.v.	Cephalic	1 - 2 ml	2	2 days
Rhesus	DSG	i.v.	Cephalic	1 - 2 ml	14	24 hours
Rhesus	STZ	i.v.	Cephalic	1 - 2 ml	1	n/a
Rhesus	Solumedrol	i.v.	Cephalic	< 1 ml	3	24 hours

continued on page 7

B. If toxic or other deleterious reactions may occur in animals, state the possible reaction(s) and procedures to deal with these reactions. If LD_{50} studies are planned, state the number of animals per dosage group (see instructions).

All the medications have a potential for side effects. All animals will be monitored DAILY for signs of illness, including lack of normal grooming, avoidance behavior, food intake etc. Body weight will be monitored at least weekly. Any complications will be treated with more frequent observation and blood tests. Appropriate medications will be administered to treat complications, including analgesia.

4. Are physical restraints used? Yes _____ No ✓
If yes, describe the restraint system and indicate the approximate time in the restraint for each experiment.

System _____

Time _____

5. Blood Samples (Note: Some blood collection methods such as intracardiac or retro-orbital techniques should be performed only in anesthetized animals – fill out Section C.

Route	Amount	Freq.
Cephalic/saphenous	<1% body wt / 2 weeks	Initally daily, then weekly

6. Pain Threshold Tests

Type	Freq.

7. Special Diets/Food Deprivation

Type	Freq.
Food withheld overnight prior to surgery	Maximum of 3 times
Enteral nutritional support (feeding tube)	3 - 6 times

8. Tumor inoculations / implantations (including hybridoma ascites tumors).

A. Species	Tumor Type	Site

B. Describe procedures to monitor tumor size and ascitic fluid accumulation and frequency of tapping ascitic fluids. Also, describe criteria for euthanasia of animals if they become ill due to tumor growth:

9. Other procedures: (inhalation, infectious agents, inoculations, etc.).

Ultrasound, X-ray and CT scans may be performed under ketamine sedation, as required in cases of sepsis or gastrointestinal complications.

USE FOR ADDITIONAL INFORMATION IF NECESSARY

3. A. Injection protocols (Continued)

Species	Agent	Route	Volume	Number of Doses	Interval Between Doses
Rhesus	Etanercept	s.c.	< 1 ml	1 – 3	72 – 96 h
Rhesus	Interleukin-10	s.c.	< 1 ml	14 – 42	8 – 24 h

ANESTHESIA
SECTION C

1. List procedure(s) requiring anesthesia by species

Species	Procedure
Rhesus	Routine handling (medications, blood samples, lymph node biopsies, enteric feeding)
Rhesus	Surgical procedures (organ procurement & transplantation)

2. Describe the pre-anesthesia protocol, including any fasting or pre-anesthetic drugs:

Food withheld overnight before surgery

3. Anesthetic agent(s). If more than one agent is to be used (e.g. for induction and maintenance), list all agents by species:

Species	Anesthetic Agent		Dosage	Route
Rhesus (routine)	Ketamine (10 mg/kg)	plus	Acepromazine (0.002 mg/kg)	i.m.
Rhesus (surgical)	Ketamine (10 mg/kg)	plus	Acepromazine (0.002 mg/kg)	i.m.
"	Fentanyl		0.33 ml/kg	i.v.
"	Isofluorane		1 - 4 %	inhalation

4. Describe procedures to monitor the depth of anesthesia: (e.g. respiratory rate, toe-pinch reflex, palpebral reflex)

Heart rate, respiration rate, toe pinch, palpebral reflex

5. Will a paralytic agent be used? Yes _____ No ✔

If yes, please specify.

Note: paralytic agents can only be administered to anesthetized animals, and animals must be monitored appropriately (i.e., blood pressure, ECG, etc.) to assure adequate anesthesia

Species	Agent	Dose	Route

SURGICAL PROCEDURES
SECTION D

1. Indicate where surgery will be performed, the person performing the surgery, and the qualifications and experience of that individual to perform the techniques involved: (Note: Major survival surgery on non-rodent mammalian species must be performed in a facility approved by the IACUC.)

   Bldg. _____ Room _____

   Name of Surgeon(s) _____

   Experience _____

2. Non-Survival Surgery (Animal will not recover from anesthesia) Yes

   Survival Surgery (Animal will recover from anesthesia) Yes (fill out Section 4)

3. Describe in detail the surgical procedure including the surgical approach, closure, support care, and monitoring during the procedure. If necessary, attach a separate sheet.

   Partial pancreatectomy:
   The animal is placed in the supine position with a grounding pad placed in contact with the back. The abdomen is prepared with Betadine. A midline laparotomy incision is performed and the intestines reflected upward. Dissection is begun at the pancreatic tail taking care not to injure the pancreas. After separation of the pancreas from the spleen, the pancreas is mobilized from the retroperitoneum with ligation of small retroperitoneal vein branches. The pancreas is mobilized to the right of the superior mesenteric artery. The splenic artery and vein are ligated and divided. The distal pancreas is removed following suture ligation of the proximal pancreatic duct. Parenchymal bleeding from the pancreatic remnant is electrocoagulated. After achieving hemostasis, the midline incision is closed into layers using a running proline 3-0 suture for the abdominal wall and a 3 -0 vicryl suture is subcutaneously to close the skin. The skin is stapled and the wound is covered with adhesive collodion. Prophylactic antibiotic therapy is Cephazolin (25 mg/kg/day i.m on days 0 -5). Analgesia is Buprenex at 0.1 mg/kg q 12 hours for the first 2 days, or additionally as needed. Postoperative care with fluids, antibiotics, and analgesia is routine, since the animals show only mild, transient hyperglycemia.

   Recipient Islet Infusion:
   The animal is placed in the supine position with a grounding pad placed in contact with the back. The abdomen is prepared and dressed following standard surgical procedures. The abdomen is opened with a 10 cm midline incision and the inferior mesenteric vein exposed. A short segment of the inferior mesenteric vein (1-2 cm) is dissected free and an 8 F feeding tube inserted between two silk ties. The tip of the tube is directed and placed in the portal vein. A tree-line stop connector is attached to the feeding tube and ½ NS is infused (30 ml/hour) to maintain the tube without blood clots. A 60 ml syringe is attached to the 3-line connector, the ½ NS flush is discontinued and 15000-25 000 Islet equivalents / kg are infused by gravity. After removal of the feeding tube, and if vascular congestion is observed in the colon, reconstruction of the inferior mesenteric vein is performed with 8-0 Prolene. The abdomen is closed with a single running 2-0 Prolene suture; the subcutaneous tissue with Vycril 3-0 and the skin with a stapler gun.

   Lymph node biopsy:
   This occurs at day 5 and day 28 post-transplant. The animal is sedated with ketamine. Lidocaine, 1ml is used as a local anesthetic (skin, muscles, peritoneum). A 5mm incision is made in the axillary or inguinal region. One lymph node is identified and excised. The incision is closed with a single stitch of 3.0 silk suture.

   Skin transplant:
   The animal is sedated with ketamine. Lidocaine, 1ml is used as a local anesthetic (skin, muscles, peritoneum). Full thickness skin from the original kidney donor is sutured onto an excised area on the lateral thorax of the recipient. Animals are placed in jackets to protect the graft.

4. Fill out this section if survival surgery is to be performed.

A. Describe post-operative care, including supportive care, post-operative monitoring, analgesia, antibiotic therapy, arrangements for after hours, weekend, and holiday care. If necessary, attach a separate sheet.

After transplantation surgery, trained and experienced staff monitor the primates, with emergency on-call cover from a member of the surgical team plus ARP veterinarian staff.
Following surgery, primates are monitored until fully alert & responsive, then returned to the cage. Blood glucose is monitored every few hours, and hypoglycemia is treated by infusion of 5% dextrose as required. Blood samples are sent for routine analysis (renal/liver panel, including sodium, potassium, creatinine, hematocrit etc) daily, and analgesia is acheived with buprenex (0.1 mg/kg i.m.).
Post-operative antibiotic care consists of Cefazolin (12.5 mg/kg i.m.) twice daily for 5 days.
Supplemental nutrition is administered by oral gavage in cases of anorexia.

B. Comments regarding potential post-operative complications and / or pain:
Buprenex (0.02 ml i.m.) is administered during the first 3-4 days for post-operative analgesia. Enteric nutritional support (high protein Ensure) is provided as required.

C. Postoperative analgesic therapy:

Species	Agent	Dosage	Route	Frequency
Rhesus	Buprenex	0.1 mg/kg	i.m.	every 12 hours

D. Anticipated post-operative survival time: > 2000 days for transplant recipients

E. Will multiple survival surgeries be performed on a single animal? Yes ✔ No ____
If yes, explain and justify why this cannot be avoided. Attach a separate sheet if necessary.

1. MAJOR: Intrahepatic infusion of donor islets
2. MINOR: Lymph node biopsy at days 4 & 30 for tolerance status monitoring.
3. MINOR: Skin transplant to challenge primary graft & prove durable tolerance.
4. MAJOR: Second intrahepatic infusion of donor islets if the primary infusion was insufficient, due to reduced functional islet mass, in the absence of evidence of rejection.

UAB ANIMAL USE SAFETY INFORMATION

This project must be registered and authorized by UAB OH&S if you will be using biohazards, radioisotopes, carcinogens, or toxic chemicals in the animal or animal facility.

OH&S Administrative Use Only
Project #
Authorization Date

PI INFO

Name _____ Phone _____ Emergency # _____

Department _____ Alternate Contact _____ Alt. Phone _____

PROJECT

Species Rhesus Macaque

Project Title ~~Preclinical Studies to Optimize Islet Transplant~~ Tolerance Induction in nonhuman primates (NHP)

Funding Source JDRF

IACUC Administrative Use Only
APN:

POTENTIALLY HAZARDOUS MATERIALS (Excluding Anesthetics)

Agent/Material is potentially hazardous for:
- ☐ Humans
- ☑ Animals (Species _____)

Agent(s)	Route of Administration	Excretion (e.g., urine/feces)	Human Health Risks or Other Concerns
Streptozotocin	i.p.	Urine	Potentially carcinogenic and islet toxic if direct skin contact or injection occurs

SPECIAL PRECAUTIONS/INSTRUCTIONS (check all that apply)

- ☐ The PI or his/her technicians are responsible for the feeding and care of these animals (must receive IACUC approval)
- ☑ The following may be contaminated with potentially hazardous material and must be handled only by authorized personnel:
  - ☑ Cage ☐ Pen ☐ Cage/pen accessories ☐ Water Bottle ☑ Animal Carcasses ☐ Bedding ☐ Other _____)
- ☐ Cages/Pen must be decontaminated before cagewash (Method _____)
- ☐ Cages/Pen/Bedding must be autoclaved before cleaning or disposal
- ☑ Animal carcasses must be disposed of as follows:
  - ☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures)
  - ☑ Chem. And/or Bio. Contaminated (Red barrel incineration) ☐ Other _____
- ☑ All contaminated waste (soiled bedding and other animal waste) must be properly labeled and disposed of as follows:
  - ☑ Chem. Contaminated (Yellow barrel incinerate) ☐ Bio. Contaminated (Autoclave/ Red barrel)
  - ☐ Rad. Contaminated (Package, Store, and Manifest as per Radiation Safety Procedures) ☐ Other _____
- ☐ Other (incl. special tests or immunizations) _____

REQUIRED PERSONAL PROTECTIVE EQUIPMENT (PPE) (check all that apply)

☑ The following Personal Protective Equipment (PPE) must be worn/used in the room:

- ☑ Lab Coat
- ☑ NIOSH Certified Dust Mask
- ☐ Shoe Covers/Booties
- ☐ Biosafety Cabinet req.
- ☐ Other
- ☑ Disposable Gloves
- ☑ Head/hair (beard) Cover
- ☐ Disinfectant Foot Spray
- ☐ N-95 or Equivalent Fitted Respirator
- ☑ Face Shield
- ☑ Closed front gown with long sleeves and elastic cuffs
- ☑ H_2O Repellant Coveralls/Jumpsuit
- ☐ Safety Glasses
- ☑ Goggles

- ☑ PPE must be removed before leaving the room.
- ☐ PPE must be discarded or decontaminated after each use.
- ☐ Other _____

Form Must Be Posted On Animal Room Door Where Animal is Used or Housed

IACUC Revised 11/10/0(

☐ Check here if additional information is attached.

REPORT SUMMARY

COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

20680
Accession No.

HS ☐ DX ☒ RES ☐
Necropsy ☒ Biopsy ☐

9/14/03
Date Received

Clinician

10/24/03
Final Report Date

INVESTIGATOR

Name: Dept: Account No.:

Phone: Contact: Contact Phone:

REASON SUBMITTED – REQUESTED SERVICE: Diagnostic Necropsy

SOURCE
Vendor:
Site: Date Obtained: 9/1/03 Building: Room: Cubicle: Isolator:

DESCRIPTION

Genus & Species: Macaca mulatta	A
Strain:	
Color	brown
Age (mo)	36
Sex:	M
Body Wt. (g)	2600
ID No.	3265
Physical Exam	Dead
Arrival Status:	Dead
If Euthanized, Method	
Gross Lesions:	Yes
Photographs:	No

NECROPSY
Date: 9/14/03 Time: Prosector:
Fixative: 10% Neutral Buffered Formalin Pathologist:

DIAGNOSES (Only Positive Findings Reported):
Kidney, cortical tubular degeneration/hypertrophy with presence of megalocytes and intranuclear eosionophilic inclusions, moderate; Omentum and abdominal surface of the diaphragm, serositis, fibrinopurulent, diffuse and multifocal, respectively; Lymph node, pericortical cells absent; Bone marrow (femoral), myeloid metaplasia, undifferentiated precursors, diffuse; Skeletal muscle (quadriceps), degeneration/necrosis, diffuse, slight to severe; Spleen, periareriolar sheaths, hypocellular and vasculitis, trabecular veins, lymphoplasmacytic; Liver, serositis, fibrinous, focal and necrosis, individual cell, multifocal; Lung, interstitial pneumonia with widening of alveolar spaces by poorly differentiated hematopoietic cells and histiocytic cells.

REMARKS: Incidental/age/strain associated findings are not reported. A pure culture of E. coli was recovered from the fibrin-covered abdominal visceral surfaces and abdominal fluid. There was clear evidence both grossly and histologically of this fibrinopurulent bacterial peritonitis. In addition, there was widespread cytomegaly of renal cortical tubular cells with tubular cell degeneration suggesting recrudescence of cytomegaloviral infection. Changes in the lungs most likely reflect a phase of ARDS or "shock" lung. White cells in the bone marrow were almost exclusively immature precursor cells suggesting activation of the proliferative phase of polymorphonuclear cell production and release of mature and maturing cells into peripheral circulation, most likely in response to the peritonitis. Immature (unsegmented)

cells were noted in the vasculature of most of the organs examined, most notably in alveolar capillaries. Another factor contributing to the presence of immature white cells in circulation is TNF, which was administered to this animal as part of the experimental protocol. TNF will increase the release of polymorphonuclear cells from the bone marrow. The phlebitis noted in the liver and the spleen lacked the endothelial necrosis and thrombosis of acute rejection vasculitis, as well as the fibroplasia and "foamy" macrophage accumulation of subacute rejection vasculitis. Thus, no specific interpretation of this change as related to transplantation effects can be made. The hypocellularity of the lymphoid tissues was thought to be the result of immunosuppression therapy associate with these transplantation experiments. Degeneration/necrosis in skeletal muscle was also thought to be the result of experimental treatments associated with this transplantation work. The weight loss noted was at least partly a result of this muscle loss. Persistently high CK values in the post transplant period also most likely reflect this muscle damage. Clinical chemistry values on the day of necropsy were those of an animal in extremis and were not helpful in determining a cause of death. A morphologic change to explain the persistently high values for hepatic enzymes and serum alkalin phosphatase in the post transplant period was not noted. The likeliest explanation for this animal's sudden death is gram negative endotoxemia.

Veterinary Pathologist

HISTORY: An islet cell transplant was done on 8/26/03. At that time he weighed about 4 kg. The monkey was judged to be doing OK and eating well yesterday 9/13. He was discovered ill this morning and died spontaneously between 7-8 am.

GROSS PATHOLOGY: Post mortem interval is about 3 hours. There is an 1.5 cm long sutured incision on the abdominal midline. A lymph node biopsy had been done in the left axilla. The subcuticular tissues and the skeletal muscle is tacky. The eyes are sunken in the orbits. Body condition is poor. Animal is thin with little adipose tissue anywhere and little muscular tissue on extremities. In the posterior part of the abdomen there are pale yellow patches and strands of elastic, loosely adherent material between viscera. There is an increased amount of pericardial fluid; the omentum is translucent rather than clear. The liver is light brown. There are patches of the same pale yellow, elastic, loosely adherent material noted above on the abdominal surface of the diaphragm, between the liver and the diaphragm, and between the liver and the stomach. Blood is collected for clinical chemistry determinations. A swab of the posterior abdominal surfaces is submitted for microbiologic culture and as requested per in antibiotic sensitivity.

Individual Animal Data
COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

UAB accession no. 20680

GROSS EXAM: Abnormal

ECTO/ENDOPARASITES: ☐

POLYMERASE CHAIN REACTION: ☐
Helicobacter bilis *Helicobacter hepaticus*

BACTERIAL CULTURES: ☒
Nasopharynx ☐

Liver ☐

Cecum ☐

Other ☒ Escherichia coli - peritoneal surface swab and fluid

MYCOPLASMA CULTURES: ☐
M. pulmonis

SEROLOGIES (See attached report for test results): Not Performed
(AGENT ABBREVIATIONS: CARB = cilia-associated respiratory bacillus, CMV = mouse or guinea pig cytomegalovirus, CPIL = *Clostridium piliforme*, ECUN = *Encephalitozoon cuniculi*, H-1 = Toolan H-1, KRV = Kilham rat virus, LCM = lymphocytic choriomeningitis, MAD = mouse adenovirus, MHV = mouse hepatitis virus, MRV = mouse rotavirus, MTV = mouse thymic virus, MVM = minute virus of mice, MPV = mouse parvoviruses, POX = mouse pox, PVM = pneumonia virus of mice, RCV = rabbit coronavirus, RRV = rabbit rotavirus, SDAV = sialodacryoadenitis virus, SV5 = simian virus 5, TCUN = *Treponema cuniculi*.)

BLOCKS:10

OTHER TESTS:

DIAGNOSES (ONLY POSITIVE FINDINGS REPORTED):
Kidney, cortical tubular degeneration/hypertrophy with presence of megalocytes and intranuclear eosionophilic inclusions, moderate; Omentum and abdominal surface of the diaphragm, serositis, fibrinopurulent, diffuse and multifocal, respectively; Lymph node, pericortical cells absent; Bone marrow (femoral), myeloid metaplasia, undifferentiated precursors, diffuse; Skeletal muscle (quadriceps), degeneration/necrosis, diffuse, slight to severe; Spleen, periareriolar sheaths, hypocellular and vasculitis, trabecular veins, lymphoplasmacytic; Liver, serositis, fibrinous, focal and necrosis, individual cell, multifocal; Lung, interstitial pneumonia with widening of alveolar spaces by poorly differentiated hematopoietic cells and histiocytic cells.

REMARKS: A pure culture of E. coli was recovered from the fibrin-covered abdominal visceral surfaces and abdominal fluid. There was clear evidence both grossly and histologically of this fibrinopurulent bacterial peritonitis. In addition, there was widespread cytomegaly of renal cortical tubular cells with tubular cell degeneration suggesting recrudescence of cytomegaloviral infection. Changes in the lungs most likely reflect a phase of ARDS or "shock" lung. White cells in the bone marrow were almost exclusively immature precursor cells suggesting activation of the proliferative phase of polymorphonuclear cell production and release of mature and maturing cells into peripheral circulation, most likely in response to the peritonitis. Immature (unsegmented) cells were noted in the vasculature of most of the organs examined, most notably in alveolar capillaries. Another factor contributing to the presence of immature white cells in circulation is TNF, which was administered to this animal as part of the experimental protocol. TNF will increase the release of polymorphonuclear cells from the bone marrow. The phlebitis noted in the liver and the spleen lacked the endothelial necrosis and thrombosis of acute rejection vasculitis, as well as the fibroplasia and "foamy" macrophage accumulation of subacute rejection vasculitis. Thus, no specific interpretation of this change as related to transplantation effects can be made. The hypocellularity of the lymphoid tissues was thought to be the result of immunosuppression therapy associate with these transplantation experiments. Degeneration/necrosis in skeletal muscle was also thought to be the result of experimental treatments associated with this transplantation work. The weight loss noted was at least partly a result of this muscle loss. Persistently high CK values in the post transplant period also most likely reflect this muscle damage. Clinical chemistry values on the day of necropsy were those of an animal in extremis and were not helpful in determining a cause of death. A morphologic change to explain the persistently high values for hepatic enzymes and serum alkaline phosphatase in the post transplant period was not noted. The likeliest explanation for this animal's sudden death is gram negative endotoxemia.

Veterinary Pathologist

REPORT SUMMARY

COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

20694
Accession No.

HS ☐ DX ☒ RES ☐
Necropsy ☒ Biopsy ☐

9/28/03
Date Received

Clinician

2/17/04
Final Report Date

INVESTIGATOR

Name: Dept: Account No.:

Phone: Contact: Contact Phone:

REASON SUBMITTED – REQUESTED SERVICE: Diagnostic Necropsy

SOURCE

Vendor:
Site: Date Obtained: 9/97 Building: Cubicle:
Room: Isolator:

DESCRIPTION

Genus & Species: Macaca mulatta	A
Strain:	
Color	
Age (mo)	96
Sex:	M
Body Wt. (kg)	11.05
ID No.	
Physical Exam	Dead
Arrival Status:	Dead
If Euthanized, Method	Sodium Pentathol
Gross Lesions:	No
Photographs:	No

NECROPSY

Date: 09/28/03 Time: 2pm Prosector:
Fixative: Buffered Formalin Pathologist:

DIAGNOSES (Only Positive Findings Reported):
Lung, bronchiolitis, necrotizing and alveolitis, diffuse, acute.

REMARKS: Incidental/age/strain associated findings are not reported.
In the lungs there is acute diffuse alveolitis with neutrophis in the septa and protein rich edema fluid in the alveoli. There is severe necrotizing bronchiolitis, diffuse in small airways and more patchy in the larger airways. Trachea and bronchi are unaffected. There is some hepatocellular individualization (dissociation from cords). This latter is a nonspecific change seen in hyperthermia among other conditions. Other tissues examined histologically include kidney, heart, spleen, stomach, large intestine, bone marrow, skeletal muscle (quadriceps), lymph node (axillary), thyroid and parathyroid glands, salivary gland, testis and urinary bladder. Changes noted in the lung are most suggestive of an acute adenoviral infection but no inclusions were seen and no viral particles were detected with EM. There are a number of other less likely differential diagnoses. We are consulting with colleagues about these and other possibilities.
ADDENDUM: We are no closer to a definitive diagnosis after consultation than we were before. It has been suggested that the changes in the lungs could be those of peracute endotoxic shock. While this is true, we have no history of any

illness or manipulation in this animal to suggest a source of endotoxin. It is unlikely that a definitive diagnosis can be made in this individual case.

Veterinary Pathologist

HISTORY: This 8-year-old macaque was received from in 9/97 as one of a group of 27. He tested negative for BV, STLV-1, SRV and SIV at that time. He has since had two at least two negative TB tests. He was vaccinated for tetanus in 3/00. He was Varicella virus negative by tests in 7/02 and 1/03. He was a self-mutilator who had an extended period of lesion healing and reinjury from 3/00 to 11/00. He was treated for a cough in 10/02. He was used as a kidney donor 7/98 and was used on a DSG testing protocol with thymoglobulin for 1 to 2 weeks. Blood work done on 9/18/03 was relatively normal. He was scheduled to be used as a pancreatic islet cell donor on 9/29. He had been in excellent health, active and eating well with a normal consistency but slightly decreased volume of feces. He was found very ill this morning, 9/28/03. The animal was sedated for physical exam and given supportive fluids. The clinical vet. ausculted very wet lung sounds. Temperature was subnormal. It was difficult to raise a vein for blood collection. Blood collected shortly before euthanasia indicated a high white cell count mostly neutrophils. There was no response to supportive therapy and the animal was euthanized *in extremis*. Slightly blood tinged fluid ran freely from the animal's nares after death.

GROSS PATHOLOGY: The body is in excellent condition with adequate adipose tissue stores. There are about 60 ml of clear, pale yellow fluid in the thoracic cavity. The lungs are heavy with some frothy fluid in the trachea. On section of the lungs, abundant, clear fluid seeps from the airways. The lobes vary from red-purple areas to areas of dull purple-grey to areas in which the parenchyma is mottled purple and pink in a pattern reminiscent of liver lobules. The GI tract is devoid of ingesta other than a few small (<1 cm in diameter), firm masses of fecal material in the diverticula of the descending colon.

Individual Animal Data
COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

UAB accession no. 20694

GROSS EXAM: Abnormal

ECTO/ENDOPARASITES: ☐

POLYMERASE CHAIN REACTION: ☐
Helicobacter bilis *Helicobacter hepaticus*

BACTERIAL CULTURES: ☐
Nasopharynx ☐

Liver ☐

Cecum ☐

Other ☐

MYCOPLASMA CULTURES: ☐
M. pulmonis

SEROLOGIES (See attached report for test results): Not performed
(AGENT ABBREVIATIONS: CARB = cilia-associated respiratory bacillus, CMV = mouse or guinea pig cytomegalovirus, CPIL = *Clostridium piliforme*, ECUN = *Encephalitozoon cuniculi*, H-1 = Toolan H-1, KRV = Kilham rat virus, LCM = lymphocytic choriomeningitis, MAD = mouse adenovirus, MHV = mouse hepatitis virus, MRV = mouse rotavirus, MTV = mouse thymic virus, MVM = minute virus of mice, MPV = mouse parvoviruses, POX = mouse pox, PVM = pneumonia virus of mice, RCV = rabbit coronavirus, RRV = rabbit rotavirus, SDAV = sialodacryoadenitis virus, SV5 = simian virus 5, TCUN = *Treponema cuniculi*.)

BLOCKS:13

OTHER TESTS: EM on lung

DIAGNOSES (ONLY POSITIVE FINDINGS REPORTED):
Lung, bronchiolitis, necrotizing and alveolitis, diffuse, acute

REMARKS: In the lungs there is acute diffuse alveolitis with neutrophis in the septa and protein rich edema fluid in the alveoli. There is severe necrotizing bronchiolitis, diffuse in small airways and more patchy in the larger airways. Trachea and bronchi are unaffected. There is some hepatocellular individualization (dissociation from cords). This latter is a nonspecific change seen in hyperthermia among other conditions. Other tissues examined histologically include kidney, heart, spleen, stomach, large intestine, bone marrow, skeletal muscle (quadriceps), lymph node (axillary), thyroid and parathyroid glands, salivary gland, testis and urinary bladder. Changes noted in the lung are most suggestive of an acute adenoviral infection but no inclusions were seen and no viral particles were detected with EM. There are a number of other less likely differential diagnoses. We are consulting with colleagues about these and other possibilities.

ADDENDUM: We are no closer to a definitive diagnosis after consultation than we were before. It has been suggested that the changes in the lungs could be those of peracute endotoxic shock. While this is true, we have no history of any illness or manipulation in this animal to suggest a source of endotoxin. It is unlikely that a definitive diagnosis can be made in this individual case.

Veterinary Pathologist

REPORT SUMMARY

COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

20754
Accession No.

HS ☐ DX ☒ RES ☐
Necropsy ☒ Biopsy ☐

2/9/04
Final Report Date

12/17/03
Date Received

Clinician

INVESTIGATOR

Name:
Dept:
Account No.:
Phone:
Contact:
Contact Phone:

REASON SUBMITTED – REQUESTED SERVICE: Diagnostic Necropsy

SOURCE
Vendor:
Site:
Date Obtained:
Building:
Room:
Cubicle:
Isolator:

DESCRIPTION

Genus & Species:	Macacca mulatta
Strain:	
Color	
Age (mo)	24
Sex:	F
Body Wt. (g)	1600
ID No.	
Physical Exam	Abnormal
Arrival Status:	Abnormal
If Euthanized, Method	
Gross Lesions:	Yes
Photographs:	No

NECROPSY
Date: 12/17/03 Time: 2 PM Prosector:
Fixative: 10% Neutral Buffered Formalin Pathologist:

DIAGNOSES (Only Positive Findings Reported):
Stomach, lymphoplasmacytic gastropathy with mucosal atrophy and lymphoid nodule formation; Small intestine, lymphoplasmacytic and eosinophilic enteropathy with glandular hyperplasia, villous blunting and fusion, marked , diffuse; Large intestine, lymphoplasmacytic and eosinophilic colonopathy with irregular surface epithelial cells (variation in cell and nuclear size and shape and nuclear placement in cell), glandular epithelial pseudostratification (piling up of cells), and mucosal hyperplasia; Spleen, hypoplasia of white pulp; Bone marrow, hypocellularity.

REMARKS: Incidental/age/strain associated findings are not reported. The changes in the gastrointestinal tract resemble those seen in the two previous accessions 20543 (ID 2130) and 20591 (ID 1950). Differences are that there is no spirochetosis and no debris- and lipofuschin-filled macrophages in the lamina propria of the gut. The absence of these changes may reflect the effect of treatment(s) given this monkey in efforts to treat her disease. Gut changes are those of chronic mild injury to the mucosa. In inflammatory bowel disease the changes usually are not limited to the mucosa and submucosa as they are in these three monkeys; also, the changes are not usually so diffuse. Other possiblilities are that the changes in the stomach may be the result of Helicobacter infection, a common problem in macaques but not easily confirmed histologically. The resulting atrophic gastric mucosa is not properly preparing ingesta for the small intestine leading to enteropathy. Another possibility is a hypersensitivity enteropathy, such as celiac

disease (gluten sensitive enteropathy). The changes in the small intestine are morphologically similar to those described for celiac disease. However, celiac disease reportedly does not affect colon. At this point an individual diagnosis explaining all of the GI tract changes is not possible. Changes in the splenic white pulp and the bone marrow reflect the overall poor health of the animal.

Veterinary Pathologist

HISTORY: Animal ID is CP7B. The animal is small for age. A skin graft was done in March 1003. She has not grown or gained weight as she should. Because of GI changes noted in two other young monkeys with similar signs, she has been treated for inflammatory bowel disease with no positve response. Treatment included steroids, sulfasalazine, and special food. Her top weight was 3 kg. in April, 2003. Eyes were collected for study by physiologic optics group.

GROSS PATHOLOGY: Animal in poor body condition. She is both too small and too thin. The spleen is smaller than expected. She has no detectable external or internal body fat. The intestines are more dilated than expected; the ingesta is normal color and consistency. A thymus is not detected.

Individual Animal Data
COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

UAB accession no. 20754

GROSS EXAM: Abnormal

ECTO/ENDOPARASITES: ☐

POLYMERASE CHAIN REACTION: ☐
Helicobacter bilis *Helicobacter hepaticus*

BACTERIAL CULTURES: ☐
Nasopharynx ☐

Liver ☐

Cecum ☐

Other ☐

MYCOPLASMA CULTURES: ☐
M. pulmonis

SEROLOGIES (See attached report for test results): Not Performed
(AGENT ABBREVIATIONS: CARB = cilia-associated respiratory bacillus, CMV = mouse or guinea pig cytomegalovirus, CPIL = *Clostridium piliforme*, ECUN = *Encephalitozoon cuniculi*, H-1 = Toolan H-1, KRV = Kilham rat virus, LCM = lymphocytic choriomeningitis, MAD = mouse adenovirus, MHV = mouse hepatitis virus, MRV = mouse rotavirus, MTV = mouse thymic virus, MVM = minute virus of mice, MPV = mouse parvoviruses, POX = mouse pox, PVM = pneumonia virus of mice, RCV = rabbit coronavirus, RRV = rabbit rotavirus, SDAV = sialodacryoadenitis virus, SV5 = simian virus 5, TCUN = *Treponema cuniculi*.)

BLOCKS:10

OTHER TESTS:

DIAGNOSES (ONLY POSITIVE FINDINGS REPORTED):
Stomach, lymphoplasmacytic gastropathy with mucosal atrophy and lymphoid nodule formation; Small intestine, lymphoplasmacytic and eosinophilic enteropathy with glandular hyperplasia, villous blunting and fusion, marked , diffuse; Large intestine, lymphoplasmacytic and eosinophilic colonopathy with irregular surface epithelial cells (variation in cell and nuclear size and shape and nuclear placement in cell), glandular epithelial pseudostratification (piling up of cells), and mucosal hyperplasia; Spleen, hypoplasia of white pulp; Bone marrow, hypocellularity.

REMARKS: The changes in the gastrointestinal tract resemble those seen in the two previous accessions 20543 (ID 2130) and 20591 (ID 1950). Differences are that there is no spirochetosis and no debris- and lipofuschin-filled macrophages in the lamina propria of the gut. The absence of these changes may reflect the effect of treatment(s) given this monkey in efforts to treat her disease. Gut changes are those of chronic mild injury to the mucosa. In inflammatory bowel disease the changes usually are not limited to the mucosa and submucosa as they are in these three monkeys; also, the changes are not usually so diffuse. Other possiblilities are that the changes in the stomach may be the result of Helicobacter infection, a common problem in macaques but not easily confirmed histologically. The resulting atrophic gastric mucosa is not properly preparing ingesta for the small intestine leading to enteropathy. Another possibility is a hypersensitivity enteropathy, such as celiac disease (gluten sensitive enteropathy). The changes in the small intestine are morphologically similar to those described for celiac disease. However, celiac disease reportedly does not affect colon. At this point an individual diagnosis explaining all of the GI tract changes is not possible. Changes in the splenic white pulp and the bone marrow reflect the overall poor health of the animal.

Veterinary Pathologist

REPORT SUMMARY

COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

20767
Accession No.

HS ☐ DX ☒ RES ☐
Necropsy ☒ Biopsy ☐

2/4/04
Final Report Date

1/9/04
Date Received

Clinician

INVESTIGATOR

Name:
Phone:
Dept:
Contact:
Account No.:
Contact Phone:

REASON SUBMITTED – REQUESTED SERVICE: Diagnostic Necropsy

SOURCE
Vendor: University of Miami
Site:
Date Obtained:
Building:
Room:
Cubicle:
Isolator:

DESCRIPTION

Genus & Species:	Aotus spp.
Strain:	owl monkey
Color	Tricolor
Age (mo)	114
Sex:	F
Body Wt. (g)	1000
ID No.	702
Physical Exam	Dead
Arrival Status:	Dead
If Euthanized, Method	
Gross Lesions:	Yes
Photographs:	

NECROPSY
Date: 1/9/04 Time: 2 PM Prosector:
Fixative: 10% Neutral Buffered Formalin Pathologist

DIAGNOSES (Only Positive Findings Reported):
Heart, cardiomyopathy with multifocal loss of myocytes and fibrosis; Lung, edema, diffuse, moderate with presence of hemosiderin-containing macrophages; Liver, bile duct, periportal fibrosis and biliary hyperplasia, very slight to slight and subcapsular nodular regeneration with fatty change; Kidney, glomerulonephritis, moderate with marked tubular loss/dilation with proteinic material, periglomerular and interstitial fibrosis, and marked interstitial accumulation of eosinphils and lymphoid cells; Kidney, arteriolitis, hyperplastic; Intestine, enterotyphlocolitis, eosinophilic, slight; Bone marrow, eosinophilic hyperplasia; Ovary, luteoma (luteinized thecoma).

REMARKS: Incidental/age/strain associated findings are not reported. Pulmonary edema in the presence of hemosiderocytes is not the result of the commonly noted terminal changes in alveolocapillary membranes occurring with spontaneous deaths but rather related to increased hydrostatic pressure; the hemosiderocytes are colloquially referred to as heart failure cells. Spontaneous unexplained cardiomyopathies are reported in Aotus. The hepatic changes are not those usually associated with right-sided heart failure and their relationship to the other lesions in this animal is uncertain. In one text the glomerulonephritis was described as including the interstitial nephritis; another text described them as separate diseases. In this animal the interstitial nephritis predominated. This is a recognized problem in Aotus.

The onion-skin pattern of renal arteriolitis noted in this animal is associated with hypertension in humans. The eosinophilic nature of the intestinal inflammation suggests hypersensitivity. The ovarian change is incidental. Vitamin E excess does not figure in the demise of this animal. She was likely in fragile health with sufficient renal and cardiac problems that the excitement and stress associated with normal sample collection caused her death. Cardiac decompensation with pulmonary edema is the likeliest proximal cause of death.

Veterinary Pathologist

HISTORY: On 07/29/97 the monkey was given an injection of Herpes simplex virus vector into the brain. Because of the sensitivity of the Aotus to this virus, this species is valuable in assessing the safety of these vectors intended for use against brain tumors in humans. She had 2-3 day episodes of soft stool/diarrhea of normal color 2 times in the last two months. The diarrhea resolved without treatment both times. No other clinical signs were noted. On 01/08/04 she vomited 3 times during fecal collection and administration of vitamin injection done without sedation. It was calculated that the IM injection of vitamin E given at that time was greater than recommended. Last blood work was done in October or November. She was found dead in her cage the morning of 01/09/04.

GROSS PATHOLOGY: There is clear froth in the trachea and bronchi. The liver is firmer than expected and has a diffusely bosselated capsular surface. The hepatic parenchyma is irregularly mottled purple-brown and tan. Both kidneys are firmer than expected on section. The right kidney is 2.5 cm from pole to pole and 1.0 cm wide at the hilus. The cortex is thinner than expected and of irregular width The cortical surface is diffusely, irregularly nodular. The left kidney is similar but the cortical surface is more severely nodular and pitted and the cortex is focally thinner. The cortices are pale tan. The left kidney is uncut to differentiate it from the right at trimming. The ovaries are yellow tan.

Individual Animal Data
COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

UAB accession no. 20767

GROSS EXAM: Normal

ECTO/ENDOPARASITES: ☐

POLYMERASE CHAIN REACTION: ☐
Helicobacter bilis *Helicobacter hepaticus*

BACTERIAL CULTURES: ☐
Nasopharynx ☐

Liver ☐

Cecum ☐

Other ☐

MYCOPLASMA CULTURES: ☐
M. pulmonis

SEROLOGIES (See attached report for test results): Not done
(AGENT ABBREVIATIONS: CARB = cilia-associated respiratory bacillus, CMV = mouse or guinea pig cytomegalovirus, CPIL = *Clostridium piliforme*, ECUN = *Encephalitozoon cuniculi*, H-1 = Toolan H-1, KRV = Kilham rat virus, LCM = lymphocytic choriomeningitis, MAD = mouse adenovirus, MHV = mouse hepatitis virus, MRV = mouse rotavirus, MTV = mouse thymic virus, MVM = minute virus of mice, MPV = mouse parvoviruses, POX = mouse pox, PVM = pneumonia virus of mice, RCV = rabbit coronavirus, RRV = rabbit rotavirus, SDAV = sialodacryoadenitis virus, SV5 = simian virus 5, TCUN = *Treponema cuniculi*.)

BLOCKS:12

OTHER TESTS:

DIAGNOSES (ONLY POSITIVE FINDINGS REPORTED):
Heart, cardiomyopathy with multifocal loss of myocytes and fibrosis; Lung, edema, diffuse, moderate with presence of hemosiderin-containing macrophages; Liver, bile duct, periportal fibrosis and biliary hyperplasia, very slight to slight and subcapsular nodular regeneration with fatty change; Kidney, glomerulonephritis, moderate with marked tubular loss/dilation with proteinic material, periglomerular and interstitial fibrosis, and marked interstitial accumulation of eosinphils and lymphoid cells; Kidney, arteriolitis, hyperplastic; Intestine, enterotyphlocolitis,

eosinophilic, slight; Bone marrow, eosinophilic hyperplasia; Ovary, luteoma (luteinized thecoma).

REMARKS: Pulmonary edema in the presence of hemosiderocytes is not the result of the commonly noted terminal changes in alveolocapillary membranes occurring with spontaneous deaths but rather related to increased hydrostatic pressure; the hemosiderocytes are colloquially referred to as heart failure cells. Spontaneous unexplained cardiomyopathies are reported in Aotus. The hepatic changes are not those usually associated with right-sided heart failure and their relationship to the other lesions in this animal is uncertain. In one text the glomerulonephritis was described as including the interstitial nephritis; another text described them as separate diseases. In this animal the interstitial nephritis predominated. This is a recognized problem in Aotus. The onion-skin pattern of renal arteriolitis noted in this animal is associated with hypertension in humans. The eosinophilic nature of the intestinal inflammation suggests hypersensitivity. The ovarian change is incidental. Vitamin E excess does not figure in the demise of this animal. She was likely in fragile health with sufficient renal and cardiac problems that the excitement and stress associated with normal sample collection caused her death. Cardiac decompensation with pulmonary edema is the likeliest proximal cause of death.

Veterinary Pathologist

REPORT SUMMARY

COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

20773
Accession No.

HS ☐ DX ☒ RES ☐
Necropsy ☒ Biopsy ☐

1/15/04
Date Received

Clinician

3/3/04
Final Report Date

INVESTIGATOR

Name: Dept: Account No.

Phone: Contact: Contact Phone:

REASON SUBMITTED – REQUESTED SERVICE: Diagnostic Necropsy

SOURCE
Vendor:
Site: Date Obtained: Building: Cubicle:
Room: Isolator:

DESCRIPTION

Genus & Species:	Macaca nemestrina
Strain:	
Color	
Age (mo)	66
Sex:	M
Body Wt. (g)	9000
ID No.	
Physical Exam	Abnormal
Arrival Status:	Abnormal
If Euthanized, Method	
Gross Lesions:	Yes
Photographs:	No

NECROPSY
Date: 1/15/04 Time: 4 PM Prosector:
Fixative: 10% Neutral Buffered Formalin Pathologist

DIAGNOSES (Only Positive Findings Reported):
Bone, femur and tibia, left, osteoporosis with lacunar osteoclasis (enlargement of Howship's lacunae), multifocal but most severe on endosteal surface of the bones; Bone, tarsus, left, osteomyelitis, focal with fracture of articular cartilage and fibrinopurulent arthritis and chronic synovitis and atrophy of tarsal bones (osteoporosis) characterized by loss of subchondral bone and loss of continuity and narrowing of trabeculae; Skeletal muscle, semimembranosus/semitendinosus and craniotibialis/gastrocnemius, atrophy with increased numbers of sarcolemmal nuclei/field, slight increase in fibrous connective tissue, very slight increase in adipose tissue and decreased myocyte width/diameter.

REMARKS: Incidental/age/strain associated findings are not reported. Muscle and bone atrophy (osteoporosis) in an individual limb is usually the result of disuse and lack of weight bearing whatever the cause (fracture, arthritis, sprain, immobilization). Tarsal arthritis and synovitis are the likely cause of the atrophy noted in the left hind limb of this animal. This was a problem of some duration as evidenced by the chronicity of the changes in the joint and the bone loss noted by histologic and radiologic examinations. It is not unusual to be unable to isolate organisms from joint cultures.

Veterinary Pathologist

HISTORY: This animal, 98P162, was SIV infected. He was noticed to be lame with a swollen left ankle. There was decreased density of the bones of the left hind leg as compared to the right on radiographic study. Diagnosis by a physician radiologist was osteopenia. The animal was euthanized for recovery of study pertinent tissues and took samples from the muscle, femur, tibia, tarsi and feet from both hind legs for histologic examination.

GROSS PATHOLOGY: There was marked decrease in muscle mass and bone diameter in left hind leg as compared to the right. The left tarsus was swollen. Swab of left tarsal joint cavity submitted for microbiologic culture.

Individual Animal Data
COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

UAB accession no. 20773

GROSS EXAM: Abnormal

ECTO/ENDOPARASITES: ☐

POLYMERASE CHAIN REACTION: ☐
Helicobacter bilis *Helicobacter hepaticus*

BACTERIAL CULTURES: ☐
Nasopharynx ☐

Liver ☐

Cecum ☐

Other ☒ No growth

MYCOPLASMA CULTURES: ☐
M. pulmonis

SEROLOGIES (See attached report for test results): Not Performed
(AGENT ABBREVIATIONS: CARB = cilia-associated respiratory bacillus, CMV = mouse or guinea pig cytomegalovirus, CPIL = *Clostridium piliforme*, ECUN = *Encephalitozoon cuniculi*, H-1 = Toolan H-1, KRV = Kilham rat virus, LCM = lymphocytic choriomeningitis, MAD = mouse adenovirus, MHV = mouse hepatitis virus, MRV = mouse rotavirus, MTV = mouse thymic virus, MVM = minute virus of mice, MPV = mouse parvoviruses, POX = mouse pox, PVM = pneumonia virus of mice, RCV = rabbit coronavirus, RRV = rabbit rotavirus, SDAV = sialodacryoadenitis virus, SV5 = simian virus 5, TCUN = *Treponema cuniculi*.)

BLOCKS:12

OTHER TESTS:

DIAGNOSES (ONLY POSITIVE FINDINGS REPORTED):
Bone, femur and tibia, left, osteoporosis with lacunar osteoclasis (enlargement of Howship's lacunae), multifocal but most severe on endosteal surface of the bones; Bone, tarsus, left, osteomyelitis, focal with fracture of articular cartilage and fibrinopurulent arthritis and chronic synovitis and atrophy of tarsal bones (osteoporosis) characterized by loss of subchondral bone and loss of continuity and narrowing of trabeculae; Skeletal muscle, semimembranosus/semitendinosus and craniotibialis/gastrocnemius, atrophy with increased numbers of sarcolemmal nuclei/field, slight increase in fibrous connective tissue, very slight increase in adipose tissue and decreased myocyte width/diameter.

REMARKS: Muscle and bone atrophy (osteoporosis) in an individual limb is usually the result of disuse and lack of weight bearing whatever the cause (fracture, arthritis, sprain, immobilization). Tarsal arthritis and synovitis are the likely cause of the atrophy noted in the left hind limb of this animal. This was a problem of some duration as

evidenced by the chronicity of the changes in the joint and the bone loss noted by histologic and radiologic examinations. It is not unusual to be unable to isolate organisms from joint cultures.

Veterinary Pathologist

REPORT SUMMARY

COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

20792
Accession No.

HS ☐ DX ☒ RES ☐
Necropsy ☒ Biopsy ☐

2/11/04
Date Received

Clinician

3/1/04
Final Report Date

INVESTIGATOR

Name: Dept: Surgery Account No.:

Phone: Contact: Contact Phone:

REASON SUBMITTED – REQUESTED SERVICE: Diagnostic necropsy

SOURCE

Vendor:
Site: Date Obtained: Building: Cubicle:
Room: Isolator:

DESCRIPTION

Genus & Species:	Macaca mulatta
Strain:	
Color	
Age (mo)	36
Sex:	M
Body Wt. (g)	2900
ID No.	282
Physical Exam	Abnormal
Arrival Status:	Abnormal
If Euthanized, Method	
Gross Lesions:	
Photographs:	

NECROPSY

Date: 1/11/04 Time: Prosector:
Fixative: 10% Neutral Buffered Formalin Pathologist:

DIAGNOSES (Only Positive Findings Reported):
Liver, hepatocellular vacuolation, diffuse, moderate; Lymph node, histiocytosis, medullary sinusoids, slight to moderate, and follicular hyalinosis (exhaustion), diffuse, slight, mesenteric, mesocolonic, lumbar and inguinal nodes; GI tract, gastroenterocolitis, lymphoplasmacytic and eosinophilic, diffuse with most severe change in the large intestine; Stomach, gastritis, diffuse, slight to moderate in section of fundus and pylorus examined; Small intestine, villous atrophy with glandular hyperplasia and debris- and lipofuschin-filled histiocytes near villous tips, very slight to slight with duodenum least affected; Large intestine, typhlocoloproctitis, diffuse with inflammatory cells among the surface epithelial cells, shortening of surface epithelial cells and debris- and lipofuschin-filled histiocytes in superficial lamina propria; Cecum, proliferative typhlitis, multifocal; Rectum, proliferative proctitis, multifocal with multifocal necrosis of surface epithelial cells, crypt abscess formation and neutrophils in luminal material, multifocal.

REMARKS: Incidental/age/strain associated findings are not reported. Liver changes are those of fasting in an animal with some adipose reserves. The changes in the lymph nodes are those of chronic immune stimulation. Changes in the stomach may be related to Helicobacter infection, which is common in macaques, or may be part of the overall GI inflammation. Changes in the large intestine are similar to those described in chronic colitis of young rhesus macaques. The changes in the entire GI tract are those of chronic injury/immune stimulation. The eosinophils suggest a

hypersensitivity component to the inflammation. No bacteria or fungi are noted with special stains (Gram, PAS, acid fast). Findings in the necropsies of several of these undersized young macaques with multiple bouts of diarrhea, weight loss and general failure to thrive have not been consistently diagnostic of a specific IBD, however, the superficial nature of the inflammation would tend to rule out Crohn's-like disease. Their blood work (low albumin, TP) is that of protein losing enteropathy - a nonspecific diagnosis encompassing a number of disease problems. In this animal there was no consistent abnormality in the hemograms done over a 3 year period. Clinical blood tests for celiac disease could help in determining the role, if any, of dietary sensitivity to gluten in these poor doing young macaques. I am uncertain whether the blood tests developed for humans would be diagnostic in macaques, but dietary manipulation and patience could also be tried. According to material I have consulted on the human disease, return of normal villous morphology (and normal absorptive capacity) may occur in as little as 2-4 months but has been known to take more than a year. Inflammatory bowel disease is often a diagnosis by exclusion requiring a rigorous and systematic clinical diagnostic approach. References that might be helpful: 1. Infectious Agent and Immune Response Characteristics of Chronic Enterocolitis in Captive Rhesus Macaques, Sestak, K, Merritt, CK, Borda, J, et al., Infection and Immunity, 71: 4079-4086, 2003. 2. Chronic Colitis, Juvenile Macaca mulatta, Adler, RR, Schmucker, DL, and Lowenstine, LJ, In "Monographs on Pathology of Laboratory Animals, Nonhuman Primates", eds. TC Jones, U Mohr and RD Hunt, Vol. 2; 81-87, 1993, Springer-Verlag, NY. Also, according to a noted expert in nonhuman primate disease, a similar problem with chronic diarrhea is seen in children in developing countries after the children have survived documented episodes of viral, bacterial, or parasitic diarrhea.

Veterinary Pathologist

HISTORY: There has been a history of weight fluctuation between about 2.0 to 3.0 kg for 2 years with multiple bouts of diarrhea. Serum albumin has been low consistently with values ranging from 1.9 to 3 at various time points dependent upon whether has diarrhea or not, but he has had some periods of health and weight gain. ID # - RQ2820

GROSS PATHOLOGY: The animal has minimal body fat. There is a midline laparotomy incision and the pancreas and duodenum are absent. Stomach is sutured closed at pylorus; omentum reddened. Eyes were removed by clinical vet. after euthanasia but before submission to pathologist for necropsy; the eyes were given to physiololgic optics. The animal was perfused with transplantation fluid during pancreatectomy. There is less than 5 cc of dark red, serous fluid in the gastric lumen. Large intestinal mucosa is thicker than expected. The liver and kidneys are medium brown.

Individual Animal Data
COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

UAB accession no. 20792

GROSS EXAM: Abnormal

ECTO/ENDOPARASITES: ☐

POLYMERASE CHAIN REACTION: ☐
Helicobacter bilis *Helicobacter hepaticus*

BACTERIAL CULTURES: ☐
Nasopharynx ☐

Liver ☐

Cecum ☐

Other ☐

MYCOPLASMA CULTURES: ☐
M. pulmonis

SEROLOGIES (See attached report for test results): Not Performed
(AGENT ABBREVIATIONS: CARB = cilia-associated respiratory bacillus, CMV = mouse or guinea pig cytomegalovirus, CPIL = *Clostridium piliforme*, ECUN = *Encephalitozoon cuniculi*, H-1 = Toolan H-1, KRV = Kilham rat virus, LCM = lymphocytic choriomeningitis, MAD = mouse adenovirus, MHV = mouse hepatitis virus, MRV = mouse rotavirus, MTV = mouse thymic virus, MVM = minute virus of mice, MPV = mouse parvoviruses, POX = mouse pox, PVM = pneumonia virus of mice, RCV = rabbit coronavirus, RRV = rabbit rotavirus, SDAV = sialodacryoadenitis virus, SV5 = simian virus 5, TCUN = *Treponema cuniculi*.)

BLOCKS:12 OTHER TESTS: Special stains

DIAGNOSES (ONLY POSITIVE FINDINGS REPORTED):
Liver, hepatocellular vacuolation, diffuse, moderate; Lymph node, histiocytosis, medullary sinusoids, slight to moderate, and follicular hyalinosis (exhaustion), diffuse, slight, mesenteric, mesocolonic, lumbar and inguinal nodes; GI tract, gastroenterocolitis, lymphoplasmacytic and eosinophilic, diffuse with most severe change in the large intestine; Stomach, gastritis, diffuse, slight to moderate in section of fundus and pylorus examined; Small intestine, villous atrophy with glandular hyperplasia and debris- and lipofuschin-filled histiocytes near villous tips, very slight to slight with duodenum least affected; Large intestine, typhlocoloproctitis, diffuse with inflammatory cells among the surface epithelial cells, shortening of surface epithelial cells and debris- and lipofuschin-filled histiocytes in superficial lamina propria; Cecum, proliferative typhlitis, multifocal; Rectum, proliferative proctitis, multifocal with multifocal necrosis of surface epithelial cells, crypt abscess formation and neutrophils in luminal material, multifocal.

REMARKS: Liver changes are those of fasting in an animal with some adipose reserves. The changes in the lymph nodes are those of chronic immune stimulation. Changes in the stomach may be related to Helicobacter infection, which is common in macaques, or may be part of the overall GI inflammation. Changes in the large intestine are similar to those described in chronic colitis of young rhesus macaques. The changes in the entire GI tract are those of chronic injury/immune stimulation. The eosinophils suggest a hypersensitivity component to the inflammation. No bacteria or fungi are noted with special stains (Gram, PAS, acid fast). Findings in the necropsies of several of these undersized young macaques with multiple bouts of diarrhea, weight loss and general failure to thrive have not been consistently diagnostic of a specific IBD, however, the superficial nature of the inflammation would tend to rule out Crohn's-like disease. Their blood work (low albumin, TP) is that of protein losing enteropathy - a nonspecific diagnosis encompassing a number of disease problems. In this animal there was no consistent abnormality in the hemograms done over a 3 year period. Clinical blood tests for celiac disease could help in determining the role, if any, of dietary sensitivity to gluten in these poor doing young macaques. I am uncertain whether the blood tests developed for humans would be diagnostic in macaques, but dietary manipulation and patience could also be tried. According to material I have consulted on the human disease, return of normal villous morphology (and normal absorptive capacity) may occur in as little as 2-4 months but has been known to take more than a year. Inflammatory bowel disease is often a diagnosis by exclusion requiring a rigorous and systematic clinical diagnostic approach.

Veterinary Pathologist

REPORT SUMMARY

COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

20817
Accession No.

HS ☐ DX ☒ RES ☐
Necropsy ☒ Biopsy ☐

2/26/04		3/15/04
Date Received	Clinician	Final Report Date

INVESTIGATOR

Name:	Dept:	Account No
Phone:	Contact:	Contact Phone:

REASON SUBMITTED – REQUESTED SERVICE: Diagnostic necropsy

SOURCE

Vendor:		Building:	Cubicle:
Site:	Date Obtained:	Room:	Isolator:

DESCRIPTION

Genus & Species:	Macaca nemestrina
Strain:	
Color	Agouti
Age (mo)	60
Sex:	M
Body Wt. (g)	8000
ID No.	
Physical Exam	Abnormal
Arrival Status:	Abnormal
If Euthanized, Method	
Gross Lesions:	Yes
Photographs:	Yes

NECROPSY

Date: 02/26/04	Time: 3 PM	Prosector:
Fixative: 10% Neutral Buffered Formalin		Pathologist:

DIAGNOSES (Only Positive Findings Reported):
Pharynx, pharyngitis, fibrinopurulent, extensive with multifocal vesicles, erosions, intracellular bacteria and extension of inflammation and necrosis deep into oral connective tissue (cellulitis), adjacent skeletal muscle and mucous glands; Larynx (epiglottis,ventricle and folds), laryngitis and epiglottitis, fibrinopurulent, multifocal with extension of inflammation (edema, necrosis, neutrophils) deep to elastic cartilage of the epiglottis and into adjacent skeletal muscle and connective tissue (cellulitis); Lungs, hyperinflation, peripheral, multifocal, extensive and congestion, marked.

REMARKS: Incidental/age/strain associated findings are not reported. Special stains to detect bacteria and fungi were done on pharyngeal and laryngeal tissue. Large, gram positive cocci arranged in clumps and both gram positive and gram negative rods were noted. There was no evidence of fungal elements nor were any cultured from the incubated broth. The large cocci are likely Staphylococcus. The rods morphologically are coryneforms and coliforms, respectively, as were cultured from this tissue. This animal had a pseudomembranous pharyngitis and laryngitis. The inflammation is confined largely to mucosa covered by nonkeratinized stratified squamous epithelium with a few small foci extending far enough into the larynx to affect its pseudostratified ciliated epithelium. The depth of the inflammation in affected tissues suggests a virulent etiologic agent. Capillaries in the lungs are filled with neutrophils. PMNs are also numerous in all

larger pulmonary vessels examined. The WBC supports this morphologic evidence of neutrophilia. There are also many multinucleated cells in the capillaries. These are likely megakaryocytes and their presence indicates active release of blood cells from the bone marrow storage pool as is common in acute inflammation. Blood vessels in pharyngeal tissue and lungs have hypertrophic endothelial cells. This is a nonspecific response to injury including that caused by inflammation. The mandibular lymph node has some medullary histiocytosis, another nonspecific response to inflammation. Tonsils are not found in the tissue submitted. No evidence of inclusions bodies or other evidence of viral infection is seen. The presence of vesicles is troublesome and an oral swab for viral culture might have been a helpful aid in diagnosis. However, bacterial agents such as Staph. aureus are capable of causing vesicles. An agent more commonly isolated in the past from this type of severe, rapidly progressive pharyngitis in children is Hemophilus influenzae. See Merck Manual, Sec. 19, Ch. 265, Childhood infections, "Acute Epiglottitis. The swab culture obtained from this animal is not of a quality suitable to detect this organism.

Veterinary Pathologist

HISTORY: This 5-year-old, male, pigtailed macaque arrived at UAB about 2 months ago and was in quarantine for 4 to 6 weeks. He was given a hind leg subcutaneous injection of a 1:10 dilution of SIVmac239 on 2/9/04 and he has just now tested seropositive for this virus. On 2/23/04 he was noted to be open-mouth breathing and drooling and had a clear nasal discharge. He was sedated for a physical exam. The uvula was judged to be swollen. He had difficulty swallowing. His body temperature was normal. By the end of Monday he had not improved and he was given an injection of penicillin and dexamethasone (for swelling). On Tuesday he was not improved and although the swelling in his uvula was decreased, a slightly raised area with a mucosa paler than that adjacent was noted. This focus was interpreted to be a vesicle. The open mouth breathing with stridor was still present. His vocal folds were swollen and he was refusing to eat or drink. He did not swallow fluid introduced into his mouth. He had lost about 2 kg. He was given subcutaneous fluids. Intratracheal intubation was done and a thoracic radiograph made. There were no radiographic abnormalities in the chest. His WBC had increased from a base line of about 5 X 1000 to 20 X 1000 with neutrophilia. There was prerenal azotemia. On Wednesday he had a brown, serous nasal discharge. Treatment with antibiotic and steroid was repeated. Difficult breathing continued but he could breathe through his nasal passages when his mouth was held closed On Thursday he was found unresponsive in his cage. His temperature was subnormal (95oF). His chest ausculted normal but open mouth breathing continued. The buccal pouches/subhyoid air sacs were bulging and judged to be air-filled. The uvula was again swollen as were the tonsils. There was an opaque, brown nasal discharge. The soft palate was pale tan with a red central focus. Foci interpreted as vesicles again seen in pharyngeal mucosa. The WBC was still high with neutrophilia. The animal died before he could be euthanized.

GROSS PATHOLOGY: Soft palate- vesicles? raw. light tan - raised hemorrhagic spot in center. Buccal pouches - rough, pale yellow mucosal surface. Samples collected: (1) necrotic soft palate. (2) tonsil. (3) LN- mandibular (4) vocal fold, epiglottis area (5) swab culture of necrotic area (6) piece of lung.

Individual Animal Data
COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

UAB accession no. 20817

GROSS EXAM: Abnormal

ECTO/ENDOPARASITES: ☐

POLYMERASE CHAIN REACTION: ☐
Helicobacter bilis *Helicobacter hepaticus*

BACTERIAL CULTURES: ☐
Nasopharynx ☐

Liver ☐

Cecum ☐

Other ☒ Escherichia coli Enterococcus sp. Staphylococcus epidermidis
Corynebacterium sp. (non-pathogenic) CULTURE FROM ORAL CAVITY

MYCOPLASMA CULTURES: ☐
M. pulmonis

SEROLOGIES (See attached report for test results): Not Performed

(AGENT ABBREVIATIONS: CARB = cilia-associated respiratory bacillus, CMV = mouse or guinea pig cytomegalovirus, CPIL = *Clostridium piliforme*, ECUN = *Encephalitozoon cuniculi*, H-1 = Toolan H-1, KRV = Kilham rat virus, LCM = lymphocytic choriomeningitis, MAD = mouse adenovirus, MHV = mouse hepatitis virus, MRV = mouse rotavirus, MTV = mouse thymic virus, MVM = minute virus of mice, MPV = mouse parvoviruses, POX = mouse pox, PVM = pneumonia virus of mice, RCV = rabbit coronavirus, RRV = rabbit rotavirus, SDAV = sialodacryoadenitis virus, SV5 = simian virus 5, TCUN = *Treponema cuniculi*.)

BLOCKS:10 OTHER TESTS: Fungal slant culture

DIAGNOSES (ONLY POSITIVE FINDINGS REPORTED):
Pharynx, pharyngitis, fibrinopurulent, extensive with multifocal vesicles, erosions, intracellular bacteria and extension of inflammation and necrosis deep into oral connective tissue (cellulitis), adjacent skeletal muscle and mucous glands; Larynx (epiglottis,ventricle and folds), laryngitis and epiglottitis, fibrinopurulent, multifocal with extension of inflammation (edema, necrosis, neutrophils) deep to elastic cartilage of the epiglottis and into adjacent skeletal muscle and connective tissue (cellulitis); Lungs, hyperinflation, peripheral, multifocal, extensive and congestion, marked.

REMARKS: Special stains to detect bacteria and fungi were done on pharyngeal and laryngeal tissue. Large, gram positive cocci arranged in clumps and both gram positive and gram negative rods were noted. There was no evidence of fungal elements nor were any cultured from the incubated broth. The large cocci are likely Staphylococcus. The rods morphologically are coryneforms and coliforms, respectively, as were cultured from this tissue. This animal had a pseudomembranous pharyngitis and laryngitis. The inflammation is confined largely to mucosa covered by nonkeratinized stratified squamous epithelium with a few small foci extending far enough into the larynx to affect its pseudostratified ciliated epithelium. The depth of the inflammation in affected tissues suggests a virulent etiologic agent. Capillaries in the lungs are filled with neutrophils. PMNs are also numerous in all larger pulmonary vessels examined. The WBC supports this morphologic evidence of neutrophilia. There are also many multinucleated cells in the capillaries. These are likely megakaryocytes and their presence indicates active release of blood cells from the bone marrow storage pool as is common in acute inflammation. Blood vessels in pharyngeal tissue and lungs have hypertrophic endothelial cells. This is a nonspecific response to injury including that caused by inflammation. The mandibular lymph node has some medullary histiocytosis, another nonspecific response to inflammation. Tonsils are not found in the tissue submitted. No evidence of inclusions bodies or other evidence of viral infection is seen. The presence of vesicles is troublesome and an oral swab for viral culture might have been a helpful aid in diagnosis. However, bacterial agents such as Staph. aureus are capable of causing vesicles. An agent more commonly isolated in the past from this type of severe, rapidly progressive pharyngitis in children is Hemophilus influenzae. See Merck Manual, Sec. 19, Ch. 265, Childhood infections, "Acute Epiglottitis. The swab culture obtained from this animal is not of a quality suitable to detect this organism.

Veterinary Pathologist

REPORT SUMMARY

COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

20867
Accession No.

HS ☐ DX ☒ RES ☐
Necropsy ☒ Biopsy ☐

3/25/04
Date Received

Clinician

4/12/04
Final Report Date

INVESTIGATOR

Name: Dept: Account No.:

Phone: Contact: Contact Phone:

REASON SUBMITTED – REQUESTED SERVICE: Diagnostic necropsy

SOURCE

Vendor: Building: Cubicle:
Site: Date Obtained: Room: Isolator:

DESCRIPTION

Genus & Species: Macaca mulatta
Strain:
Color: brown
Age (mo):
Sex: F
Body Wt. (g): 4000
ID No.:

Physical Exam
Arrival Status: Dead
If Euthanized, Method:
Gross Lesions:
Photographs: No

NECROPSY

Date: 3/25/04 Time: 11 AM Prosector:
Fixative: 10% Neutral Buffered Formalin Pathologist:

DIAGNOSES (Only Positive Findings Reported):
Lungs, interstitial edema, moderate with airway macrophages some having small numbers of brown, cytoplasmic granules; Liver, hepatocellular vacuolation, moderate and cells resembling islet cells in sinusoids; Spleen and lymph nodes (abdominal), follicular hyalinosis and hypocellularity of follicular centers; Kidney, interstitial nephritis, multifocal, slight with interstial fibrosis, tubular atrophy and thickening of glomerular (Bowman's) capsules and proliferation of glomerular parietal epithelium in affected foci.

REMARKS: Incidental/age/strain associated findings are not reported. Pulmonary interstitial edema is encountered in a number of situations in which there is an increase in capillary hydrostatic pressure without increased permeability of the blood/air barrier. This is because pulmonary interstitial space acts as a physiologic sump to prevent fluid from entering alveolar spaces and can handle as much as 10-fold increase in volume as long as the fluid accumulates slowly in the presence of normal alveolar epithelium. The most common cause of this change is some form of cardiac failure. Other possibilities are shock and renal disease. There are no findings in this animal to cause me to suspect such other causes of interstitial edema as acute infectious pneumonia. Interstitial edema is not the cause of death. The hepatic fatty change could be at least partially the result of fasting. If this animal is diabetic, this is an additional potential contributing factor

to hepatic fatty change. The specific cause for the change seen in follicular centers in spleen and lymph nodes is uncertain. It can occur with age, stress and some immunologic manipulations; however, I am not aware of these animals being given immune therapy intended to affect B cells. The renal changes are too mild to have caused death. There is little inflammation in affected areas. Small islets of Langerhans are noted in sections of pancreas. The suspected islet cells in the liver could be stained for presence of beta granules to confirm their nature. Since the surgeon removed liver for this purpose, I have not repeated this research procedure. A source of the blood coughed up at time of extubation was not noted grossly or microscopically. Could the coughing have been associated with regurgitation of the mucinous red material noted in the gastric lumen ? It is not unusual to find no morphologic evidence of cause of death in animals that die during surgery or recovery from anesthesia.

Veterinary Pathologist

HISTORY: This is animal number CP6J. She had an islet cell transplant in September 2003 but insufficient islet mass reconstituted in the monkey. The procedure was repeated. She was reinduced with recombinant ATG + deoxyspergualyn (anti-T cell treatment?) on 3/23/04 and taken to surgery 3/25/04 for islet cell transfusion to liver. The islet cell suspension is infused via a cannulated mesenteric artery. She was breathing unassisted post-anaesthesia. She started coughing blood on extubation and died at 10:07 am.

GROSS PATHOLOGY: Post mortem interval is 1 hour, 15 minutes. There is an 11 cm stapled abdominal midline incision. There is an opening on the right side of the thorax and abdomen and a right lobe of the liver is absent. There are 19 ml of serous, red fluid with some small clots in right side of the thorax, 32 ml on this fluid on the left side and 9 ml in the abdominal cavity. The stomach contains clear, red, mucinous material. There is no evidence of hemorrhage into the lungs or trachea. There is no blood in the oral cavity or the nasopharynx.

Individual Animal Data
COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

UAB accession no. 20867

GROSS EXAM: Abnormal

ECTO/ENDOPARASITES: ☐

POLYMERASE CHAIN REACTION: ☐
Helicobacter bilis *Helicobacter hepaticus*

BACTERIAL CULTURES: ☐
Nasopharynx ☐

Liver ☐

Cecum ☐

Other ☐

MYCOPLASMA CULTURES: ☐
M. pulmonis

SEROLOGIES (See attached report for test results): Not Performed
(AGENT ABBREVIATIONS: CARB = cilia-associated respiratory bacillus, CMV = mouse or guinea pig cytomegalovirus, CPIL = *Clostridium piliforme*, ECUN = *Encephalitozoon cuniculi*, H-1 = Toolan H-1, KRV = Kilham rat virus, LCM = lymphocytic choriomeningitis, MAD = mouse adenovirus, MHV = mouse hepatitis virus, MRV = mouse rotavirus, MTV = mouse thymic virus, MVM = minute virus of mice, MPV = mouse parvoviruses, POX = mouse pox, PVM = pneumonia virus of mice, RCV = rabbit coronavirus, RRV = rabbit rotavirus, SDAV = sialodacryoadenitis virus, SV5 = simian virus 5, TCUN = *Treponema cuniculi*.)

BLOCKS:10 OTHER TESTS:

DIAGNOSES (ONLY POSITIVE FINDINGS REPORTED):
Lungs, interstitial edema, moderate with airway macrophages some having small numbers of brown, cytoplasmic granules; Liver, hepatocellular vacuolation, moderate and cells resembling islet cells in sinusoids; Spleen and lymph nodes (abdominal), follicular hyalinosis and hypocellularity of follicular centers; Kidney, interstitial nephritis, multifocal, slight with interstial fibrosis, tubular atrophy and thickening of glomerular (Bowman's) capsules and proliferation of glomerular parietal epithelium in affected foci.

REMARKS: Pulmonary interstitial edema is encountered in a number of situations in which there is an increase in capillary hydrostatic pressure without increased permeability of the blood/air barrier. This is because pulmonary interstitial space acts as a physiologic sump to prevent fluid from entering alveolar spaces and can handle as much as 10-fold increase in volume as long as the fluid accumulates slowly in the presence of normal alveolar epithelium. The most common cause of this change is some form of cardiac failure. Other possibilities are shock and renal disease. There are no findings in this animal to cause me to suspect such other causes of interstitial edema as acute infectious pneumonia. Interstitial edema is not the cause of death. The hepatic fatty change could be at least partially the result of fasting. If this animal is diabetic, this is an additional potential contributing factor to hepatic fatty change. The specific cause for the change seen in follicular centers in spleen and lymph nodes is uncertain. It can occur with age, stress and some immunologic manipulations; however, I am not aware of these animals being given immune therapy intended to affect B cells. The renal changes are too mild to have caused death. There is little inflammation in affected areas. Small islets of Langerhans are noted in sections of pancreas. The suspected islet cells in the liver could be stained for presence of beta granules to confirm their nature. Since the surgeon removed liver for this purpose, I have not repeated this research procedure. A source of the blood coughed up at time of extubation was not noted grossly or microscopically. Could the coughing have been associated with regurgitation of the mucinous red material noted in the gastric lumen ? It is not unusual to find no morphologic evidence of cause of death in animals that die during surgery or recovery from anesthesia.

Veterinary Pathologist

REPORT SUMMARY

COMPARATIVE PATHOLOGY LABORATORY
ANIMAL RESOURCES PROGRAM
UNIVERSITY OF ALABAMA AT BIRMINGHAM

20901
Accession No.

HS ☐ DX ☒ RES ☐
Necropsy ☒ Biopsy ☐

4/18/04
Date Received

Clinician

4/27/04
Final Report Date

INVESTIGATOR

Name: Dept: Account No.

Phone: Contact: Contact Phone:

REASON SUBMITTED – REQUESTED SERVICE: Diagnostic necropsy

SOURCE

Vendor: Building: Cubicle:
Site: Date Obtained: 4/13/00 Room: Isolator:

DESCRIPTION

Genus & Species: M. nemestrina
Strain:

Color	brown
Age (mo)	85
Sex:	M
Body Wt. (g)	6750
ID No.	
Physical Exam	Abnormal
Arrival Status:	Dead
If Euthanized, Method	Pentobar overdose IC
Gross Lesions:	Yes
Photographs:	No

NECROPSY

Date: 4/18/04 Time: 10 AM Prosector:
Fixative: 10% Neutral Buffered Formalin Pathologist

DIAGNOSES (Only Positive Findings Reported):
Hypoglycemic episode, severe; emaciation.

REMARKS: Incidental/age/strain associated findings are not reported. Histologic examination of tissues was not pursued in this animal because the reported diarrhea and wasting are among the expected outcomes of this experimental manipulation as are the blood cell values noted in the history. The monkey's measured serum glucose at time of euthanasia was .8mmol/L or 14.5 mg/dL. Given that blood glucose drops 10%/hour when a sample is held at room temperature, the maximum that his glucose could have been with a 4 hour delay before sample testing (ignoring the fact that the blood was refrigerated that entire period which would decrease the glucose loss/hour) is 1.1mmol/L or 20 mg/dL. Causes of hypoglycemia are legion, but we have seen monkeys in better health than this animal that have had repeated episodes of hypoglycemia for no discernible reason. These animals were repeatedly resuscitated and would be clinically healthy until the next episode. Thus, this monkey's hypoglycemia could be part of his experimental disease or not.

Veterinary Pathologist

HISTORY: Animal APIK was infected with experimental strain SHIV- 89.6P IV approximately 2 years ago (4/2/02). He behaved normally yesterday (4/17/04), ate his morning biscuits and eagerly accepted treats. He was found moribund at 9:15 am on 4/18. He was lying ventrum down with some skin discoloration on down side. His abdomen was bloated and his rectal temperature too low to register on clinical thermometer. On physical exam by clinical veterinarian at 10 am he was unresponsive to external stimuli, had an undetectable femoral pulse, HR of 80 and jaws firmly clamped together. His bladder was full (abdominal bloating) but was easily expressed manually and urine was unremarkable. Some loose stools were expressed during abdominal palpation, but there was no diarrhea in the cage. The investigator reports that the monkey had chronic diarrhea and had lost about 2 kg in the last few months. A comparison between a CBC done at time of experimental inoculation and on 4/13/04 showed that his hematocrit, hemoglobin, platelet count, white cell count and % lymphocytes had all decreased. Lymphocytes were much decreased. % Polymorphonuclear cells were markedly increased. Blood was collected for CBC and serum and the monkey was euthanized rather than resuscitated in keeping with investigator's usual procedure.

GROSS PATHOLOGY: Animal is markedly thin with the spinous processes of the lumbar vertebrae prominent and easily palpable in their entirety. The ilium - crest, wing and gluteal surface - is clearly visible beneath the skin. On the dorsum from the midthoracic region to the base of the tail the monkey is sparsely haired (partial alopecia). Oral mucous membranes are white. Jaws are firmly clamped. Considerable force is needed to open the mouth. Teeth are unremarkable. There is a hair ball in the right cheek pouch. Skin turgor is good. There is an 8 cm diameter hemorrhage into the subcutis of the medial aspect of the right thigh; the hemorrhage extends anteriorly into the inguinal area and posteriorly to cover the scrotum and the perineum. A smaller area of hemorrhage is in the subcutis over the femoral triangle of the left thigh. There is marked muscle atrophy and no subcutaneous or intraabdominal adipose tissue. There is blood in the pericardial sac, brown discoloration (euthanasia solution) on the left side of the pericardium, clotted blood between the left lung lobes and multiple needle sticks (pinpoint wounds) through the pericardium and the left medial and anterior lung lobes. The left lung lobes are reddened and heavier than expected and bloody froth is in the trachea. The peritoneal cavity contains 10 to 20 ml of clear colorless fluid. Stomach, small intestine and colon are empty of ingesta/fecal material. Cecum contains some flecks of green material and hair. There are two easily reduced colonic intussusceptions. There is no discoloration, swelling or fibrin to suggest that these were other than terminal events. The urinary bladder contains approx. 290 ml of clear yellow urine. The urethra is unobstructed by direct observation.