PROTOCOL FOR ANIMAL USE AND CARE
Email to: campusvet@ucdavis.edu

CNPRC

PROTOCOL: 10401
EXPIRES: 2/27/04

Investigator

Last Name: ___________________________  Last Name: ___________________________
First: _______________________________  First: _______________________________
Middle: _____________________________  Middle: _____________________________
email: ______________________________  email: _____________________________
Department: _________________________  Department: _________________________
Phone / Fax: _________________________  Phone: _____________________________
After hrs. #: _________________________  After hrs. #: _________________________

Species (common names): ___________________________  Number: ___________________________
Macaca mulatta (Rhesus monkey)  12 maximum total  CRPC

Source: ___________________________

Project Title: Pig to Rhesus monkey as an animal model for xenotransplantation

Overnight housing location: _______________  Day use only: _______________
CRPRC  CRPRC

Animals will be maintained by: [ X ] Vivarium [ ] Investigator (If investigator maintained, attach husbandry SOP’s.)

Procedures: Provide a one or two sentence layman’s description of the procedures employed on the animals in this project. This information will help the animal care staff understand any conditions they may encounter while caring for your animals.

Pig blood vessel, liver and insulin-making cells will be obtained from collaborating groups and injected into the blood stream of Rhesus monkeys. Porcine hearts or partial liver lobes will be harvested from young piglets at Stanford University, transported to the CRPRC and transplanted to the abdomens of Rhesus monkeys. Blood will be collected from the non-immunosuppressed monkeys to determine antibody response to the pig cells and vascularized grafts in this primate model of human xenograft transplantation.

Special Husbandry Requirements: Describe any special requirements your animals have with respect to food, water, temperature, humidity, light cycles, caging type, bedding, or any other conditions of husbandry.

Standard CRPRC care.

Other instructions for animal care staff: (check applicable entries)

Sick Animals  Dead Animals  Pest Control

[ x ] Call Investigator  [ x ] Call Investigator  [ ] Call Investigator
[ x ] Clinician to treat  [ ] Save for Investigator  [ x ] OK to use pesticides
[ x ] Terminate  [ ] Bag for disposal  [ ] No Pesticides in animal area
[ ] Necropsy  [ x ] Necropsy

Hazardous Materials (only if in the animal room):

Infectious Agents?  [ ] Yes  [ x ] No  Agent(s):
Radioisotopes?  [ ] Yes  [ x ] No  Agent(s):
Chemical Carcinogens?  [ ] Yes  [ x ] No  Agent(s):
Toxic Chemicals?  [ ] Yes  [ x ] No  Agent(s):
Summary of Procedures:

a) Briefly describe the overall intent of the study. Include in your description a statement of your hypothesis, the objectives and significance of the study. Your target audience is a faculty member from a discipline unrelated to yours. Do not use jargon.

Our hypothesis is that, as in humans, the antibody and gene response of primates (represented in our study by Rhesus monkeys) to swine cells and tissues will be encoded by a restricted number of activated genes; our initial data from 1 Rhesus monkey (and several cynomolgus monkeys) exposed to porcine cells indicates that this is so. We wish to further identify and characterize the antibody response, using both pig cells and vascularized organ grafts, to mimic the transplant situations that would occur clinically in humans. Once the set of responsive genes are identified, we can use that information to help control the rejection response in primates by targeting for destruction only those B cells which make the rejection-causing antibodies directed by these genes.

b) Procedures employed in this project:

Please check the appropriate boxes if any of these procedures will be employed in your project:

- [ ] Monoclonal Antibody Production **
- [ ] Polyclonal Antibody Production **
- [ ] LD 50 or ID50 studies.
- [x] Catheters, blood collection, intubation
- [ ] Prolonged restraint (8 hrs+)
- [x] Fasting prior to a procedure.
- [ ] Food or water restriction
- [x] Non-recovery surgical procedures
- [x] Survival surgical procedures
- [ ] Multiple survival surgery
- [ ] Induced illness, intoxication, or disease
- [ ] Special diets; food or water treatment.
- [x] Trapping, banding or marking wild animals
- [ ] Behavioral modification.
- [ ] Aversive conditioning.
- [ ] Induced death as an endpoint (see i below)

** If this protocol only describes antibody production, you may use the attached antibody production page in lieu of completing section c below.
describe the use of animals in your project in detail, with special reference to any of procedures checked above. Include any physical, chemical or biological agents that may be administered. List each study group, and describe all the specific procedures that will be performed on each animal in each study group. Use terminology that will be understood by individuals outside your field of expertise. (Note: This cell will expand to whatever length you require. You may make this section as long as you wish, but try to be concise. Some projects may require one or two pages.)

Twelve Rhesus monkeys, juvenile adult males, 3 - 5 kg, in total will be used over a 3 year period. These experiments are designed to: 1. compare the immunoglobulin gene usage and humoral immune response in primates transplanted with porcine cells and vascularized organs amongst themselves, and 2. to compare the results to human patient data. We do not expect to obtain a statistically significant sample size in each group. Pig organs mentioned for groups 3 and 4 will be harvested at Stanford University by Dr. and his team, and transported to the CRPRC the day of the transplant. Dr. has an IACUC approved protocol in place at Stanford University for his work under this protocol. For all groups, Cephazolin will be administered perioperatively for 5 days to prevent infection. Children's chewable vitamins will also be administered prior to surgery, and will be continued after the procedures, to maintain hematocrit levels during the blood collection periods. For each blood collection in each group, the monkey will be sedated with IM ketamine (at 10 mg/kg) and the blood sampled from either the femoral or saphenous vein, by CRPRC staff using routine CRPRC techniques. The blood will then be processed by the CRPRC staff to separate serum, and to isolate peripheral blood leukocytes (PBLs), both of which will be shipped to USC/CHLA by FedEx.

Group 1. Two monkeys will be immunized with porcine endothelial cells (blood vessel cells) and hepatocytes (liver cells). Each animal will be fasted overnight. Prior to induction of general anesthesia, each animal will be sedated with ketamine (with a single IM injection of 10 mg/kg) and premedicated with atropine sulfate (at 0.02 to 0.04 mg/kg IM). General anesthesia will be induced by mask inhalation of isoflurane. The monkey will be intubated and maintained on 1 to 4% isoflurane. The monkey will be placed in dorsal recumbency and attached to extensive monitoring equipment (for blood pressure, heart rate and quality [ECG], percent O2 saturation, temperature, etc). Using sterile technique, a 3 to 6 cm midline incision will be made into the abdomen of the monkey. Prior to injection of the cells, the distal end of the spleen will be identified and brought to the incision. A small sample of spleen, approx. 3 mm x 3 mm x 2mm, will be collected by using 3.0 PDS suture material to ligate and transect the most distal tip of the spleen, which will be the sample collected. Any additional blood loss from the ligated splenic tip will be controlled by electrocautery. Additionally, between 1 to 2 mesenteric lymph node(s) will also be collected (depending on their size - sufficient tissue for experiments may be collected from just one lymph node) by dissection of the node(s) from mesenteric tissue, with cautery for hemostasis. These samples will be subdivided for routine histology, mRNA extraction, and immunohistochemistry to detect the presence of the B cells we are attempting to expand with this experiment, as well as the presence of any pig cells from the injection(s) (in later samples). Following collection of these samples, a loop of small intestine will be brought to the surgical field. A large jejunal vein will be identified and catheterized with a 25-gauge Angiocath-type catheter. The cells (a mixture of hepatocytes and endothelial cells, 100 million cells in 10 to 20 mls of physiologically buffered sterile saline [PBS]) will be infused through sterile tubing into the jejunal catheter, at a rate of 1 to 2 mls/min. During infusion, the monkey will be monitored for signs of anaphylaxis (decreased blood pressure, increased or erratic heart rate, irregular breathing). Any change in these parameters will result in cessation of cell infusion if/untill the monkey's condition can be stabilized. If the monkey's signs cannot be returned to normal, the cell infusion will be terminated, the jejunal catheter removed, the abdominal incision closed and the monkey recovered to continue the rest of the study. If the monkey's vital signs return to normal, the infusion will continue at a slower rate (0.25 to 0.5 ml/min) until complete. At the end of the infusion, the catheter is removed and all sites (spleen, site of mesenteric lymph nodes, jejunal vein) inspected for any hemorrhage. Once the surgeon is satisfied that hemostasis has been achieved, the abdomen will be routinely closed and the animal recovered. Post-operative pain, if it occurs, will be controlled by IM injections of oxymorphone, at 0.15 mg/kg up to 3 times per day for the first few days post-surgery. Past this timepoint, discomfort and inflammation may be treated with either ketoprofen (at 2 mg/kg PO SID) or ibuprofen (at 7 mg/kg PO BID); steroids will not be used in this protocol due to their interference with immune function. Blood samples, for serum and peripheral blood lymphocytes (PBLs), 5 to 10 ml total volume (2.5 to 5 ml in heparin tubes and 2.5 to 5 ml in serum separator tubes), will be collected preinjection and at 10 and 14 days postinjection. If sufficiently elevated antiporcine antibodies are detected by day 10, additional blood samples will be collected at 21, 30, 45, 60, 75 and 90 days postinjection. The maximum allowable amount to be sampled from an individual animal of this size (which is 10.5 ml per week or 21.0 ml every 2 weeks or 42.0 mls per month) will not be exceeded; collection volumes will be adjusted downward if necessary to obtain sufficient samples at the necessary timepoints; in our hands, 5 ml per timepoint divided (2.5 ml each in heparin and serum separator tubes) has been sufficient for our experimental needs. If no or low antiporcine antibodies are detected at day 10, the injection of cells will be repeated, and, again, the blood tested following the above post first injection schedule. It is expected that a maximum of 3 surgeries, involving 3 pig cell injections with simultaneous sampling of monkey lymph node and spleen, will be necessary to produce a sufficient antibody response in each animal. Blood samples will be collected in glass tubes (serum tubes) for antibody studies and in preservative free heparin tubes (for the isolation of PBLs and for flow cytometry). Blood samples will be processed to isolate PBLs and separate serum at CRPRC, and the products transported by courier to Childrens Hospital in Los Angeles. The porcine endothelial cells and hepatocytes will be obtained from Multicell Technologies (a private company), prepared at Childrens Hospital in Los Angeles, and transported to the CRPRC by courier. At each injection, another sample of spleen and mesenteric lymph nodes will be obtained for routine histology, mRNA extraction,
and immunohistochemistry, as described above. At the end of 90 days, the animal will be euthanized and necropsied, with a final blood sample drawn and samples taken from multiple lymphoid tissues (spleen, peripheral and mesenteric lymph nodes, and thymus) as well as liver (to examine for the persistence of pig cells by immunohistochemistry).

Group 2. Two monkeys will be anesthetized and immunized with fetal porcine islet cells (insulin-making cells), 15,000 clusters/kg body weight in 10 mls of PBS. Preparation of the monkeys for surgery, the procedure itself, aftercare (including post-operative pain control) and blood and tissue sampling will be as explained for Group 1. If no antiporcine antibodies are detected 10 days following injection of the cells, the injection procedure will be repeated. As for Group 1, it is expected that a maximum of 3 surgeries, involving 3 pig islet injections with simultaneous sampling of monkey lymph node and spleen, will be necessary to produce a sufficient antibody response in each animal. The porcine islet cells will be provided by and prepared at Children's Hospital Los Angeles. All blood collection volumes will be 5 to 10 mls, and will occur preoperatively, and at post-first injection at days 10, 14, 21, 30, 45, 60, 75 and 90 days. The maximum allowable amount to be sampled from an individual animal of this size (which is 10.5 ml per week or 21.0 ml every 2 weeks or 42.0 mls per month) will not be exceeded; collection volumes will be adjusted downward if necessary to obtain sufficient samples at the necessary timepoints; in our hands, 5 ml per timepoint divided (2.5 ml each in heparin and serum separator tubes) has been sufficient for our experimental needs.

Group 3. Under general anesthesia, 4 monkeys will receive a heterotopic heart transplant (placed in the monkeys' abdomen) from an approximately 2 day old neonatal pig. The monkeys will be prepared for surgery as for Group 1. The donor pig will be prepared and the donor heart and both kidneys will be harvested at Stanford University, and the organs transported to the CRPRC on ice in appropriate medium and an approved container. Once at the CRPRC and in the surgical suite, the porcine kidney's renal arteries and veins will be cannulated and connected to sterile, heparinized surgical tubing in preparation for connection to the monkey's circulatory system to allow for ex vivo perfusion of the pig kidneys, to remove circulating anti-pig antibodies. The anesthetized monkey (for procedures, refer to Group 1) will receive femoral arterial and venous catheters. Using sterile technique, the kidneys will be attached to the monkey's circulation (femoral artery to renal artery and renal vein to femoral vein). This circuit will be maintained for 15 to 30 minutes, with careful attention to the monkey's blood pressure and maintenance of same with crystal colloid solutions (such as Hetastarch or Dextran 40, which are artificial solutions that mimic the blood's own proteins and function to increase blood pressure) - the use of fresh frozen plasma and whole blood must be avoided, except in an emergency, due to the reintroduction of the antibodies that we are attempting to eliminate. Any decrease in blood pressure which cannot be compensated for by administration of fluids and/or decrease in perfusion rate will result in disconnection of the kidneys from the circuit and control return of all blood to the monkey's body. This filtering step is considered necessary in order to deplete the recipient monkey of circulating xenobodies to the pig's cells, which would cause hyperacute rejection of the heart within minutes after placement of the graft. Filtering of primate blood in this manner has been described in numerous previous publications, with no major adverse effects noted other than decreases in blood pressure that were alleviated by supplementary fluids. Regardless of the success of the kidney circuit, the transplant will be continued, providing the monkey is stable and judged able to tolerate the continued surgery. If it is deemed too dangerous to continue the surgery, due to decreased blood pressure or unstable vital signs, the monkey will be recovered. If the animal was successfully attached to the kidney circuit, it will be entered in to the study and blood collections begun 10 days post-kidney filtering. In such a situation, we will not be able to repeat the blood filtering and transplant in that particular monkey, as exposure to the porcine kidneys would have sensitized it to pig antigens, which would result in hyperacute rejection of any future transplant, thereby disrupting the timeline of our study. However, samples from such a monkey may be useful, as it was exposed to pig antigens and may mount an immune response to those antigens, which we can study. To complete the transplant procedure, the aorta of the harvested pig heart will be anastomosed to the abdominal aorta of the monkey, end-to-side, and the pulmonary artery of the heart will be anastomosed end-to-side to the inferior vena cava of the monkey. The heart will be restarted by perfusion and bathing in warm saline. Following final inspection of the anastomoses, the abdomen will be closed routinely and the monkey recovered. Transplantation of hearts into the abdomens of monkeys is also an established transplant practice, and has been performed in several different laboratories. The most common adverse effect/complication at surgery appears from the literature to be leaking at the vascular sutures. Drs are experienced vascular surgeons, and are confident that these anastomoses can be successfully carried out. The majority of the adverse effects post-surgery that have arisen from similar procedures involved infection due to massive amounts of immunosuppressive medications, which we will not be using in our experiments. The pig hearts will be small enough that they should not create a "pool" for the monkey's blood circulation or cause a decrease in blood pressure that could not be corrected by additional fluids. There is no record in the literature of any anaphylactic-type reaction to a graft placement, or any complications other than those already mentioned. From personal correspondence with the lab, we understand that cynomolagus monkeys have survived for many days, one up to 78 days, with a heteropic heart in its abdomen, some with significant necrosis due to rejection despite immunosuppressive therapy. In these studies as well, post-operative complications were attributable to immunosuppression and not specifically the presence of the failed graft. The monkey's condition will be monitored closely the first few hours to days post-surgery. Post-operative pain will be controlled by IM injections of oxymorphone, at 0.15 mg/kg up to 3 times per day for the first few days post-surgery. Past this timepoint, discomfort and inflammation may be treated with either
ketoprofen (at 2 mg/kg PO SID) or ibuprofen (at 7 mg/kg PO BID). For the first few days post-surgery, the monkey will be sedated daily with ketamine (10 mg/kg IM) for palpation of the neonatal heart in the monkey's abdomen: cessation of beat will be correlated with rejection. These sedation/palpations may not be necessary if the heart is declared rejected (no beats) prior to incision closure or prior to the monkey's recovery from anesthesia. The heart will provide a continual source of antigens (it will shed protein necessary for antibody production from the graft). We will not attempt to prolong survival of the graft beyond normal rejection times with immunosuppressive agents as we are attempting to establish the nature of the host immune response. In rodents, heterotopic heart xenografts fail at approximately 4 days post transplant and the continued presence of the graft is well tolerated. The use of a heart from as young a pig as possible will decrease the amount of tissue present in the monkey's abdomen, while still providing an intact vascularized organ to mimic that which would be seen by whole organ transplant into a human patient. We anticipate clinical signs of rejection of the heterotopic heart graft to be no more severe than mild depression, anorexia, fever, or general malaise. If medicinal therapy does not sufficiently relieve the monkey's discomfort, as judged by the presiding veterinarian, the monkey will be returned to surgery and the graft removed or, if the monkey's vital signs can not be stabilized to allow for another surgery to remove the graft, the monkey will be humanely euthanized (using B euthanasia solution or pentobarbital, to effect). Providing the monkeys tolerate the graft, blood for antiporcine antibody levels and PBLs, 5 to 10 ml total at each collection, will be collected preoperatively, and at 10, 14, 21, 30, 45, 60, 75 and 90 days following surgery. The maximum allowable amount to be sampled from an individual animal of this size (which is 10.5 ml per week or 21.0 ml every 2 weeks or 42.0 mls per month) will not be exceeded; collection volumes will be adjusted downward if necessary to obtain sufficient samples at the necessary timepoints; in our hands, 5 ml per point divided (2.5 ml each in heparin and serum separator tubes) has been sufficient for our experimental needs. If a graft must be removed from a monkey, blood collections will continue on the same schedule from day 0 being the day the heart graft was placed. If the hearts survive, and the animals show an acceptable level of physical response to the rejecting graft (see i. Adverse Effects), the animals will be maintained for a maximum of 90 days. If a monkey had its graft removed, it will also be euthanized at 90 days following transplantation. If a monkey underwent the kidney blood-filtering procedure but did not have a heart graft placed, it will also be euthanized at 90 days post-procedure.

Group 4. Under general anesthesia, this group of 4 monkeys will receive a heterotopic partial liver lobe transplant of approximately 40 g, placed in the monkey's abdomen, each graft harvested from 1 neonatal pig. Prior to the transplant, the kidneys from the pig will be surgically removed en bloc, as well as the entire liver, and transported to the CRPRC from Stanford University in appropriate transport media in an approved container. Each monkey will have its blood filtered through the pig kidneys (refer to Group 3). Meanwhile, the harvested pig liver will be cut-down as back-table work once the liver is transported to the CRPRC. The dissected partial lobe graft will then be heterotopically implanted in the abdomen of the monkey, using the monkey's abdominal aorta and inferior vena cava and the graft's supra-hepatic vena cava, celiac trunk, and superior mesenteric vein. (Again, as for Group 3, the transplant procedure will not continue if the monkey's vital signs cannot be stabilized post-kidney perfusion.) The implanted graft will be reperfused and observed for hemorrhage. Dr has practiced this procedure repeatedly in neonatal pigs, and feels confident that the liver lobe dissection will not result in massive hemorrhage, or "oozing" of blood from the liver surface. Rejection is expected within a few minutes to several hours or days. As for Group 3, the status of the monkey will be evaluated with the rejecting graft in situ. If it is determined that the presence of the graft does not pose an immediate health risk, based on stable vital signs post-reperfusion, the incision will be closed (triple layer closure) and the monkey recovered. The monkey's condition will be monitored closely the first few hours to days post-surgery, with routine post-operative pain controlled by oxymorphone at 0.15 mg/kg, IM. Any adverse effects (see i, Adverse Effects) will be initially medically treated, using either ketoprofen (at 2 mg/kg PO SID) or ibuprofen (at 7 mg/kg PO BID); steroids will not be used in this protocol due to their interference with immune function. As for Group 3, we expect clinical signs from a rejected partial liver lobe of this size to be minimal. However, there is less information available in the literature regarding heterotopic liver transplants, as compared to the more common heart grafts. If medicinal therapy does not sufficiently relieve the monkey's discomfort, as judged by the presiding veterinarian, the monkey will be returned to surgery and the graft removed or, if the monkey's vital signs can not be stabilized to allow for another surgery to remove the graft, the monkey will be humanely euthanized (using B euthanasia solution or pentobarbital, to effect). All blood collection and follow-up periods will be the same as for Group 3. All monkeys will be euthanized 90 days following the initial surgery or blood-filtering through pig kidneys.

d) Study Groups and Numbers: Define, in the form of a table, the numbers of animals to be used in each experimental group described above. The table may be presented on a separate page as an attachment to this protocol if you prefer. The Normal format should be three columns: Study Group, Procedure, Number of animals. The number of rows should follow from the number of study groups; you may add as many rows as you require. The chart must fully account for the number of animals you intend to use under this protocol. Assign each group to an invasiveness category according to the chart below.

<table>
<thead>
<tr>
<th>Group</th>
<th>Procedures / Drugs</th>
<th>Number of Animals</th>
<th>Category</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Immunization with endothelial cells/hepaotocytes</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>Immunization with islet cells</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Heterotopic heart transplant</td>
<td>4</td>
<td>3</td>
</tr>
</tbody>
</table>
Heterotopic partial liver lobe transplant

The primate is necessary for the study of porcine to primate xenograft responses prior to study on human beings. We are using the minimum number of animals possible in each group to qualify the antibody and gene response. We are not attempting to achieve significant numbers.

Categories of invasiveness

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
</table>
| 1        | Little or no discomfort or stress  
**Examples:** domestic flocks or herds being maintained in simulated or actual commercial production management systems; the short-term and skillful restraint of animals for purposes of observation or physical examination; blood sampling; injection of material in amounts that will not cause adverse reactions by the following routes: intravenous, subcutaneous, intramuscular, intraperitoneal, or oral. |
| 2        | Minor stress or pain of short duration  
**Examples:** cannulation or catheterization of blood vessels or body cavities under anesthesia; minor surgical procedures under anesthesia, such as biopsies or laparoscopy; short periods of restraint beyond that required for simple observation or examination, but consistent with minimal distress |
| 3        | Moderate to severe distress  
**Examples:** major surgical procedures conducted under general anesthesia, with subsequent recovery; prolonged (several hours or more) periods of physical restraint; induction of behavioral stresses such as maternal deprivation |
| 4        | Severe pain near, at or above the pain tolerance threshold  
**Examples:** exposure to noxious stimuli or agents whose effects are unknown; exposure to drugs, chemicals, or infectious agents at levels that markedly impair physiological systems and which cause death, severe pain, or extreme distress: Surgical experiments which have a high degree of invasiveness. |

Further descriptions of these categories are included in the instructions following this document.

e) **Rationale for species and numbers:** How did you determine that 1) the species choice was appropriate and 2) the number of animals in each study groups was the minimum number necessary to achieve sound scientific results?

   The primate is necessary for the study of porcine to primate xenograft responses prior to study on human beings. We are using the minimum number of animals possible in each group to qualify the antibody and gene response. We are not attempting to achieve significant numbers.

f) **Surgery:** If the project involves survival surgery, where will the surgery be conducted?

   **Building:** CRPC  
   **Room:** Surgical suite  
   **Who will be the surgeon?**

   g) **Anesthetics, Analgesics, Tranquilizers, Neuromuscular blocking agents:**

   Post procedural analgesics should be given whenever there is possibility of pain or discomfort that is more than slight or momentary. If postoperative analgesics are not to be given, justify the practice under part (i) below.

   Provide the following information about any of these drugs that you intend to use in this project.

<table>
<thead>
<tr>
<th>Species</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Route</th>
<th>When and how often will it be given?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primate</td>
<td>Ketamine</td>
<td>10</td>
<td>IM</td>
<td>For sedation on days blood to be drawn</td>
</tr>
<tr>
<td>Primate</td>
<td>Ketamine plus Atropine</td>
<td>10 .02-.04 IM</td>
<td>For premeds the morning of surgery</td>
<td></td>
</tr>
<tr>
<td>Primate</td>
<td>Isoflurane</td>
<td>1-4%</td>
<td>inhaled</td>
<td>Induction and maintenance of anesthesia during surgery</td>
</tr>
<tr>
<td>Primate</td>
<td>Oxymorphone</td>
<td>.15</td>
<td>IM</td>
<td>As needed up to TID for the first few days post-surgery</td>
</tr>
<tr>
<td>Primate</td>
<td>Ketoprofen</td>
<td>2</td>
<td>orally</td>
<td>As needed SID for inflammation and/or pain relief post-surgery</td>
</tr>
<tr>
<td>Primate</td>
<td></td>
<td>7</td>
<td>Orally</td>
<td>“ “</td>
</tr>
</tbody>
</table>

   h) **Neuromuscular blocking agents** can conceal inadequate anesthesia and therefore require special justification. If you are using a neuromuscular blocking agent, please complete the following:
Why do you need to use a neuromuscular blocking agent?

What physiologic parameters are monitored during the procedure to assess adequacy of anesthesia?

Under what circumstances will incremental doses of anesthetics-analgesics be administered?

i) Adverse effects:
Description any potential adverse effects of the experiment on the animals (such as pain, discomfort; reduced growth, fever, anemia, neurological deficits; behavioral abnormalities or other clinical symptoms of acute or chronic distress or nutritional deficiency)

Possible complications of the injection of porcine cells (Groups 1 and 2) include fever and nausea. Possible clinical signs of acute organ rejection (Groups 3 and 4) include: fever, malaise, vomiting, abdominal cramping, abdominal pain, inappetence, weight loss; we anticipate these to be mild. Transient postoperative pain is expected.

How will the signs listed above be ameliorated or alleviated? If signs are not to be alleviated or ameliorated by means of post-operative analgesics or other means, explain why this is necessary.

Fever and nausea following cell injection (Groups 1 and 2) should be transient. Mild pain/discomfort due to the surgical procedures themselves will be controlled using oxymorphone for the first few days post-surgery. Ketoprofen and/or ibuprofen will be used after that time to ameliorate inflammation and relieve any discomfort due to rejection of the heart or liver grafts, and subsequent necrosis/breakdown of the pig tissue. If moderate to severe signs of rejection occur, which cannot be treated successfully with NSAIDs, the monkey will either: 1) be returned to surgery for removal of the graft or, 2) if it is decided the monkey is not a good candidate for another surgery (due to unstable vital signs), it will be euthanized.

Note: if any unanticipated adverse effects not described above do occur during the course of the study, a complete description of those effects and the steps taken to mitigate them must be submitted to the committee as an amendment to this protocol.

Is death an endpoint in your experimental procedure?  [  ] Yes    [ X] No

(Note: “Death as an endpoint” refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation). If death is an endpoint, explain why it is not possible to euthanize the animals at an earlier point in the study. If you can euthanize the animals at an earlier point, describe the clinical signs which will dictate that an animal will be euthanized.

j) Literature search for alternatives and unnecessary duplication:

Federal law specifically requires this section. You are required to conduct a literature search to determine that either 1) there are no alternative methodologies by which to conduct this class/lab, or 2) there are alternative methodologies, but these are not appropriate for your particular class/lab. “Alternative methodologies” refers to reduction, replacement, and refinement (the three R’s) of animal use, not just animal replacement. You must also show that this use of animals is not unnecessarily duplicative of other studies.

UC Davis provides on-line access to a number of databases that can be used to search for alternatives. Visit http://trc.ucdavis.edu/jawelsh/Databases/Databases_Med_Vet_Researchers.htm (email: jawelsh@ucdavis.edu) or http://www.vetmed.ucdavis.edu/Animal_Alternatives/main.htm (email: mwwood@ucdavis.edu)

What was the date on which you conducted this search?  11/07/02

List the databases searched or other sources consulted (there should be more than one). Include the years covered by the search.

<table>
<thead>
<tr>
<th>Database Name</th>
<th>Years Covered</th>
<th>Keywords / Search Strategy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Melvyl</td>
<td>past 20</td>
<td>Xenograft, primate, experimental model, monkey, rejection response</td>
</tr>
</tbody>
</table>
**What were your findings with respect to alternative methodologies?**

There are no alternative methods that duplicate the complex interactions between the primate host and the porcine xenograft.

Has this study been previously conducted?  [ ] Yes  [ X] No

If the study has been conducted previously, explain why it is scientifically necessary to replicate the experiment.

**k) Disposition of animals:** At what point in the study, if any, will the animals be euthanized?

Euthanasia will be performed if an animal becomes moderately to severely ill, secondary to receiving a porcine graft, or at the end of the 90 day study period for each group.

**l) Methods of euthanasia:** Even if your study does not involve killing the animals, you should show a method that you would use in the event of unanticipated injury or illness. If anesthetic overdose is the method, show the agent, dose, and route.

<table>
<thead>
<tr>
<th>Species</th>
<th>Method</th>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primate</td>
<td>Lethal injection</td>
<td>Pentobarbital or Beuthanasia solution</td>
<td>60 (to effect)</td>
<td>IV</td>
</tr>
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**m) Surplus animals:** What will you do with any animals not euthanized at the conclusion of the project?

Any suitable monkeys will be returned to the control of the CRPRC.
n) Project Roster: Please provide the names of all the individuals who will work with animals on this project. This page will not be made available to the public. Give either the University Employee ID # or a valid UC Davis email address so that we can document training and occupational health compliance for regulatory agencies. Include all investigators, student employees, post-doctoral researchers, staff research associates, post-graduate researchers and laboratory assistants who will actually work with the animals. You don't need to include the staff of the vivarium in which your animals will be housed.

The principal investigator is responsible for keeping this roster current. If any staff is added or subtracted from this project, you must amend the protocol by sending the campus veterinarian a memo describing any changes.

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<th>Last Name</th>
<th>First Name</th>
<th>Middle Name</th>
<th>UC ID Number or SSN</th>
<th>Email Address</th>
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Occupational Health Program:

Supervisors must enroll their employees in the campus Occupational Health Program if the workers are at increased risk of illness or injury (such as allergy, physical injury, or infectious disease) because of their work. Enroll workers by having them complete an "Animal Contact History Form", available from Employee Health Services (phone 752-2330). For further information, visit our web site at [http://clueless.ucdavis.edu/health/](http://clueless.ucdavis.edu/health/) or read the UC Davis Policy & Procedure Manual 290-25.

Training:

Supervisors are responsible for insuring that their employees are adequate trained, both in the specifics of their job and in the requirements of the Federal Animal Welfare Act. EH&S offers free, basic wet labs in laboratory animal handling and techniques, and lecture format classes in the requirements of the Animal Welfare Act. To schedule a class for your unit, contact EH&S at 2-2364. Autotutorials are also available on the world wide web at [http://clueless.ucdavis.edu/](http://clueless.ucdavis.edu/).
Assurances for the Humane Care and Use of Vertebrate Animals:

Principal Investigator’s Statement:

I have read and agree to abide by the UC Davis Policy and Procedure Manual section 290-30 (Animal Use and Care). This project will be conducted in accordance with the ILAR Guide for the Care and Use of Laboratory Animals, and the UC Davis Animal Welfare Assurance on file with the US Public Health Service. (These documents are available from the Campus Veterinarian and at http://ehs.ucdavis.edu/). I will abide by all Federal, state and local laws and regulations dealing with the use of animals in research.

I will advise the Animal Use and Care Administrative Advisory Committee in writing of any significant changes in the procedures or personnel involved in this project.

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<tr>
<th>Principal Investigator</th>
<th>Rank / Title</th>
<th>Date</th>
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** Conditions necessary for Committee Approval:

Final Disposition of this protocol:

- Approved
- Not Approved
- Withdrawn by Investigator

Date of Action: _____ / _____ / _____

I verify that the Institutional Animal Care and Use Committee of the University of California, Davis, acted on this protocol as shown above.

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<th>Campus Veterinarian</th>
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University of California, Davis
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