Transplant Immunology 36 (2016) 32–41

Contents lists available at ScienceDirect

Transplant Immunology

journal homepage: www.elsevier.com/locate/trim

# Difficulties, guidelines and review of developing an acute rejection model after rat intestinal transplantation☆

Ane Miren Andres [a,*], Monica Santamaria [b], Francisco Hernandez-Oliveros [a], Laura Guerra [c], Sergio Lopez [a], Pablo Stringa [d], Maria Teresa Vallejo [e], Carlota Largo [b], Jose Luis Encinas [a], Maria Soledad Garcia de las Heras [f], Manuel Lopez-Santamaria [a], Juan Antonio Tovar [a]

[a] Pediatric Surgery Department, La Paz University Hospital, Paseo La Castellana, 261, 28046 Madrid, Spain
[b] Experimental Surgery Department, La Paz University Hospital, Paseo La Castellana, 261, 28046 Madrid, Spain
[c] Pathology Department, La Paz University Hospital, Paseo La Castellana, 261, 28046 Madrid, Spain
[d] Fundacion Favaloro Buenos Aire:Experimental Transplant Department, Buenos Aires, Argentina
[e] Idipaz Institute, Immunohistochemistry Department, Paseo La Castellana, 261, 28046 Madrid, Spain
[f] King Juan Carlos University, Madrid: Histology Department, Alcorcon, Madrid, Spain

ARTICLE INFO

*Article history:*
Received 12 January 2016
Received in revised form 12 April 2016
Accepted 15 April 2016
Available online 19 April 2016

ABSTRACT

Experimental small bowel transplantation (SBT) in rats has been proven to be a useful tool for the study of ischemia-reperfusion and immunological aspects related to solid organ transplantation. However, the model is not completely refined, specialized literature is scarce and complex technical details are typically omitted or confusing. Most studies related to acute rejection (AR) use the orthotopic standard, with small sample sizes due to its high mortality, whereas those studying chronic rejection (CR) use the heterotopic standard, which allows longer term survival but does not exactly reflect the human clinical scenario. Various animal strains have been used, and the type of rejection and the timing of its analysis differ among authors. The double purpose of this study was to develop an improved unusual AR model of SBT using the heterotopic technique, and to elaborate a guide useful to implement experimental models for studying AR. We analyzed the model's technical details and expected difficulties in overcoming the learning curve for such a complex microsurgical model, identifying the potential problem areas and providing a step-by-step protocol and reference guide for future surgeons interested in the topic. We also discuss the historic and more recent options in the literature.



## 1. Background

Small bowel transplantation (SBT) continues to be an immunological enigma with a high mortality rate [1]. The mechanisms of rejection are not completely understood, and treatment is frequently empiric. Thus, animal research models are still necessary to study immunological pathways and therapeutic alternatives to those currently used.

Experimental SBT in rats has been the most commonly used model due to ethical and economic advantages [2]. However, this technique requires excellent microsurgical skills to overcome a steep learning curve before achieving survival, and worldwide only a few groups of surgeons perform it. Furthermore, mortality during the early postoperative days is high, particularly in the orthotopic model if there is no close monitoring similar to that performed on humans [3]. These complications appear to diminish in the heterotopic model [3–6], although this will never provide the same information as an orthotopic model, which is similar to that experienced in human clinical practice [3,7–9].

An ideal acute rejection (AR) model is difficult to find in the literature for several reasons: only a few groups have published their experience in rat SBT, thus sample sizes are limited; most do not provide many details about the model itself and there are no data regarding the time consumption and cost-effectiveness of the procedure, particularly when starting to reproduce it; most groups use the orthotopic model for AR whereas the heterotopic is more frequently used for chronic rejection (CR); each group uses different strains according to the availability in their respective countries—therefore histoincompatibility and the timing of rejection varies depending on each strain; and the euthanasia day varies among authors. For these reasons, it is difficult to compare the various publications and to establish conclusions before starting as a novice in the field [4–6,8–18].

☆ Grant support: FIS grant (Fondo de Investigacion Sanitaria, Spain, PI10/02507)
* Corresponding author at: Pediatric Surgery Department, Hospital Universitario La Paz, Paseo de la Castellana 261, 28046 Madrid, Spain.
*E-mail addresses:* aneandresmo@hotmail.com (A.M. Andres), monica.santamariamiro@gmail.com (M. Santamaria), fhernandezo.hulp@salud.madrid.org (F. Hernandez-Oliveros), lauraguerrap@gmail.com (L. Guerra), sergio_lopez_fdez@hotmail.com (S. Lopez), Pablo_stringa@hotmail.com (P. Stringa), teresa.vallejo@salud.madrid.org (M.T. Vallejo), carlota.largo@salud.madrid.org (C. Largo), encinas.j@tiscali.it (J.L. Encinas), soledad.garcia@urjc.es (M.S. Garcia de las Heras), mlsantamaria@salud.madrid.org (M. Lopez-Santamaria), juan.tovar@salud.madrid.org (J.A. Tovar).

http://dx.doi.org/10.1016/j.trim.2016.04.003
0966-3274/

In our hospital, where rat SBT has been performed by various surgeons for two decades, the surgical technique has been previously described [6,16,18–20]. When studying rejection, however, there are still important questions about the ideal strain, type of transplant and anastomosis, which was the reason we searched for the ideal AR model in the literature. We also previously published the major complications that occurred during the procedure, which led to finding some significant prognostic factors for success, such as the total transplant duration—particularly the warm ischemia time—and lack of postoperative bleeding, as has been described by others [7]. However, the results were limited by the surgeons who participated with varying skill levels, experience and dedication, and the procedure was not always performed for studying rejection, but also for ischemia preconditioning, bacterial translocation or technical details [17,19–21].

## 2. Objective

Therefore, the goal of this study is threefold. First, we aim to describe an unusual AR model using the heterotopic technique, providing a step-by-step protocol and guidelines to answer the questions that could help the beginner to make the right decisions. Second, we report the difficulties in developing such a complex microsurgical model, with the aim of shortening the learning curve. Third, we summarize and discuss the historic and more recent options in the literature.

## 3. Materials and methods

An AR model was developed after SBT. We initially began with the orthotopic model, but we switched to a heterotopic model, which resulted in a higher success rate and longer-term survival. All the experiments were approved by La Paz University Hospital's animal welfare ethics committee.

### 3.1. Animals/preoperative care

A total of 320 male inbred rats weighing 250–300 g were purchased from Janvier Labs (France): 160 Brown Norway (BN) rats served as donors and 160 Lewis rats as recipients. All the procedures were performed in accordance with the principles of the federal law regarding the protection of animals (RD 56/2013). All the rodents were housed individually in standard animal facilities at La Paz University Hospital until transplantation, at a room temperature of 21 ± 2 °C, relative humidity of 45 ± 15%, maintained at a 12-hr light/dark cycle, and fed commercially available chow (Safe A04, Panlab) and tap water ad libitum. Food was withheld from the donor for 24 h prior to surgery.

### 3.2. Surgical procedures

Allogeneic SBT was performed using standard microvascular techniques as previously described [22,23].

#### 3.2.1. Anesthesia

General anesthesia was used, with sevoflurane 5% during the induction and laparotomy and 2% for the rest of the procedure, as maintenance.

#### 3.2.2. Donor operation

The procedure was clean but not sterile. Five milliliters of physiological saline was perfused subcutaneously just before the incision. A median laparotomy was performed and the entire small bowel from the ligament of Treitz to 3 cm from the ileocecal valve was prepared on a vascular pedicle consisting of the superior mesenteric artery (SMA) on an aortic cuff and the portal vein (PV). Just before removal, heparin was intravenously administered (0.2 ml 5%), the infrarenal aorta was cannulated, the infradiaphragmatic aorta was clamped and the graft was perfused with Ringer's lactate (RL) until the exiting effluent was clear through the PV. At this point the graft was removed, and the intestinal lumen was flushed with RL (4 °C). The graft was cooled with ice, as is performed in humans, and stored at 4 °C in the same solution until implantation into the recipient after 30–45 min.

#### 3.2.3. Recipient operation (SBT)

We initially began by placing a catheter in the tail vein to keep the animal hydrated during the procedure, particularly just after unclamping. This was useful to keep the animal alive in the first transplants, which had significant bleeding. Once the transplant success rate increased, with very little bleeding, a total of 5 ml of physiological saline perfused subcutaneously at the beginning of the procedure was sufficient, thus minimizing the risks of pulmonary emboli. After mobilization of the cava vein and the aorta from the surrounding connective tissue, transplantation was performed by anastomosing the graft SMA on an aortic cuff to the recipient infrarenal aorta, and anastomosing the PV to the recipient infrarenal cava vein in an end-to-side fashion with 9–0 absorbable suture (Dafilon®). Blood flow was restored after unclamping and the absence of significant bleeding was checked.

In the orthotopic model, the entire native small bowel was resected, leaving only 5 cm of jejunum and 5 cm of terminal ileum. After unclamping and restoring blood flow, both bowel ends of the graft were anastomosed with the corresponding ends of the recipient with interrupted sutures (Prolene 7/0). In the heterotopic model, the native intestine was not removed. After restoring blood flow, the bowel ends were exteriorized as ostomies on the right abdominal wall (Prolene 7/0). Finally, the wound was closed with 3/0 running sutures in two planes.

### 3.3. Postoperative care

After the procedure, the animals were resuscitated, heated with thermal blankets and placed in individual cages. During the intervention, they were subcutaneously administered tramadol 25 mg/Kg (Adolonta®) to reduce postoperative pain, and again in the following days if necessary. They were immediately offered water ad libitum and food after 24 h.

The animals were observed and weighed daily until euthanization. Their clinical status was assessed daily: appearance, posture, feeding, activity and body weight. Allograft rejection was determined clinically by palpation of induration of the abdomen and by gross examination of the exteriorized stomas. For those with significant weight loss due to low food intake, the water was replaced with 5% dextrose until they began to gain weight. Ceftriaxone 75 mg/Kg/day (saline carrier) and an extra 4–5 ml of physiological saline was subcutaneously administered daily to prevent infection and maintain hydration. Tacrolimus (TAC) (Astellas Pharma S.A. Spain) 0.5 mg/Kg/day (saline carrier) was also subcutaneously administered when indicated. Those animals with poor health, showing graft failure symptoms (e.g., antalgic posture, general discomfort, anorexia) before the scheduled day were euthanized immediately, and all the data were recorded.

### 3.4. Data collection

With the aim of describing the setup as well as the learning curve, we measured the survival of the animals after the procedure, at 24 h after the procedure and at the time of euthanasia. All the data concerning transplantation were recorded (*learning curve database*, $n = 160$ *SBT*): donor and recipient weight, data regarding the donor surgery, recipient surgery, administration of TAC, duration of anastomosis, duration of warm and cold ischemia and surgery recovery. We registered all intra- and postoperative complications, incidents and survival, as well as evolutive data in the survivors until euthanasia (e.g., daily weight, welfare and treatment toxicity). All problems and difficulties during the study were also recorded, as well as the modifications and strategies employed at each moment to improve results.