Lung Transplantation in the Rat: I. Technique and Survival
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ABSTRACT For immunogenetic and economical reasons, an operative technique for lung transplantation in the rat was developed. With the use of an operation microscope and 8-0, 9-0, and 10-0 sutures, the left pulmonary artery, vein, and bronchus were anastomosed. The mean operation time was 4 hours; the mean graft ischemia time, 87 minutes. After frequent failures initially, due to anesthetic problems and lack of experience with microsurgical operative technique, a final peroperative survival of about 80% was obtained. Postoperative mortality approximated 50%. The main cause of postoperative death was thrombus formation at the site of an anastomosis. In a final series of 28 isogeneic transplantations, proper data for a one-month follow-up was obtained from 36% of the animals that underwent operation, a percentage comparable with initial canine pulmonary and rat renal transplantation studies. Left lung transplantation in the rat proved to be technically feasible and may provide an immunogenetically well defined and economically advantageous animal model in lung transplantation research.

To date, clinical lung transplantation has not been as successful as kidney, heart, or liver transplantation. Causes of complications that interfere with clinical success have been reported [1]: (1) scarcity of suitable donor lungs; (2) complications of the bronchus anastomosis; and (3) frequent rejection and infection. Concerning the third point, Benfield [2] noted that "the information regarding the immunological characterization of the lung allograft response is still primitive." Most canine lung allograft experiments lack a well-defined immunogenetic background, which restricts conclusions [3].

Materials and Methods
Random-bred Wistar rats weighing 200 to 350 gm were used in the first series of 90 transplantations, and inbred WAG and BN rats weighing 250 to 300 gm were used in the second series of 39 transplantations (28 isografts, WAG→WAG; 11 allografts, BN→WAG). All WAG and most BN rats were specific-pathogen free (SPF) when obtained a few weeks before operation. The Wistar rats and a few BN rats were raised under normal laboratory conditions.

The left lung was selected for transplantation because the lungs of the rat consist of one whole lung on the left side and four smaller lobes on the right side. Figure 1 shows schematically the anatomy of the respiratory tract in the rat.

All animals were administered atropine, 0.25 mg per kilogram of body weight intramuscularly, as premedication. For the animals used as
recipients, initially pentobarbital was used as an anesthetic, administered in the tail vein (30 mg per kilogram) or intraperitoneally. However, we switched to anesthesia with Fluothane (halothane) after 20 transplantations because the duration of anesthesia was hard to control and there was a high mortality at an early postoperative stage. Anesthesia with Fluothane (5% Fluothane and a 2:1 mixture of nitrogen dioxide and oxygen) is initiated with a mask. Intubation is performed with an intravenous catheter 15 cm long and with an outer diameter of 1.8 mm. Transillumination of the neck affords direct vision of the glottis and facilitates quick intubation [11]. Artificial ventilation is effected with a Keuskamp infant ventilator (50 strokes per minute; 20/0 or 20/5 cm H₂O). No muscle relaxant is given. Anesthesia is maintained with the same gas mixture and 0.5% Fluothane. For the donor animals, anesthesia is obtained by injecting chloral hydrate (400 mg per kilogram) intraperitoneally. Then, intubation is performed.

Operative Technique

Recipient Preparation. With the animal positioned as for a left thoracotomy, the left chest is shaved and cleaned with Joflon. A clean, nonsterile technique is used. A thoracotomy is performed in the third or fourth intercostal space. The left inferior pulmonary ligament is divided, and the left lung is retracted outside the chest cavity. Lobectomy of the right postcaval lobe is performed (except in the first 40 transplantations). Using an operation microscope (Zeiss OPMI 7-D), the hilar structures are dissected. Hemostasis is obtained by bipolar coagulation. The bronchus is constricted with a 2.0 suture loop and transected for as long a length as possible. Both the pulmonary artery and vein are occluded with a single vascular clamp and divided. An ordinary vessel clamp with modified curves is used, the jaws of which are protected by silicone rubber tubes. A little platform is placed in such a way that it covers the heart and deadens the movements of the heart beat and the ventilated right lung (Fig 2).

Graft Harvest. After the abdomen has been opened, the diaphragm is removed from its costal attachment. The ribs are divided on both
sides of the spine, and the whole anterior chest wall is removed. The lungs are slightly inflated by a continuous stream of compressed air. Heparin, 1,000 U, is given intravenously. After careful dissection of the left pulmonary artery, vein, and bronchus, the vein is divided. The common pulmonary artery is cannulated, and the lungs are perfused with a 4°C perfusate of lactated saline solution and procaine (2 gm per liter), after which the left pulmonary artery is divided. The perfusion pressures range from 20 to 40 cm H₂O. The bronchus is constricted while the lungs are inflated (except in the first 20 transplantations), and pneumonectomy is completed by division of the bronchus. The length of the vessel cuffs depends on the length needed for the recipient. The bronchus stump is kept as short as possible.

**Implantation of the Graft: Vessel Anastomoses.** The graft is placed on the platform in the thorax of the recipient and covered with gauze. The graft and the operation field are frequently rinsed with cold saline solution. The venous anastomosis has to be made first. Two stay sutures are placed on the posterior wall, opposite each other by about 160 degrees, and fixed to two tiny eyes attached to the clamp (Fig 3). First the posterior wall is closed with a continuous suture. After one knot with the stay suture, the anterior wall is closed in the same way. The pulmonary artery anastomosis is made accordingly, after which the clamp is opened and perfusion restored. To suture the vessels, Ethylon 8-0 (BV-2 needle) was used initially, and later in the series, 9-0 (BV-4) or preferably 10-0 (BV-6). Initial leaking of the anastomosis is controlled by counterpressure with two cotton pledgets. In case of persistent bleeding, additional sutures are placed and several milliliters of Haemaccel is infused into the femoral vein as volume replacement. No heparin is given to the recipient animal.

**Bronchus Anastomosis.** After the vessel anastomoses are completed, both bronchus stumps are dissected to the appropriate lengths (as short as possible on the donor side) and brought together in such a way that both stumps telescope over the length of one cartilage (Fig 4). First the cartilages of the stumps are fixed with three or four interrupted sutures

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Fig 3. The vessel anastomoses: both vessels are held in a single clamp to which two eyes of wire are attached, to fix the stay sutures. The venous anastomosis is partly finished using a continuous suture. The ligated bronchus stumps both of donor and recipient are shown.

Fig 4. The bronchus anastomosis with the telescoping technique. Interrupted sutures are used.

Fig 5. The three anastomoses prior to closure of the thorax. No important constriction is observed at the site of the anastomoses. (v = pulmonary vein; b = bronchus; a = pulmonary artery.)
(Ethylon 8-0) and then the paries membranae with another six to eight interrupted sutures (Ethylon 8-0 or 9-0). After removal of both constricting loops, ventilation of the grafts starts. Temporary hyperinflation (to 30/15 cm H₂O) is necessary to remove partial atelectasis and to check air leakage from the anastomosis.

Figure 5 shows the three anastomoses prior to closure of the thorax. The thorax is closed in four layers. Negative pressure drainage is maintained until the animal is conscious. Terramycin (oxytetracycline) is given in the drinking water during the first postoperative week.

**Results**

We did 129 left lung transplantations. In the first 20 transplantations, the lung was not kept inflated after harvesting. In this series, rather high ventilation pressures were required to remove the atelectasis after transplantation. In the following series of transplantations we kept the graft inflated by ligating the bronchus, and ventilation of the graft was restored far more easily. In the later series, the right postcaval lobe was resected to prevent herniation to the left hemithorax, which caused problems with the interpretation of chest roentgenograms and technetium 99m perfusion scintigrams of the lungs [12, this issue]. Operation times ranged from 3½ to 5 hours (mean, 4 hours). Graft ischemia time ranged from 52 to 149 minutes (mean, 87 minutes). No important shift toward shorter operation or ischemia time was observed during the course of the experiments.

Figure 6 shows the perioperative survival in consecutive groups of 15 animals each. There is steady progress during the course of the first 75 operations. Then a plateau is reached at about the 80% level. The causes of perioperative death (Table 1) changed during the series. During the first 30 transplantations, failing microsurgical techniques were the main cause of death, especially tearing of the very thin venous vessel wall, besides death from anesthesia. Later, there were bleeding problems from the vessel anastomoses. Among the total of 129 animals, 84 (65%) survived the operation and 45 (35%) died postoperatively.

The causes of postoperative death are summarized in Table 2. Eight animals died within 2

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<td>Tearing of vessel wall</td>
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<td>Failing bronchus anastomosis</td>
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<td>Clamp failure</td>
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<td>Complications associated with operation</td>
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hours after operation, most likely of complications associated with the operation, such as pneumothorax, tracheal obstruction by mucus, severe loss of blood, complete atelectasis of the right lung (cause unknown), and left and right lung infarction. The main cause of late postoperative death was thrombosis of the pulmonary vessels, usually within ten days postoperatively. A common finding at postmortem examination was a thrombus occluding the left pulmonary vein at the site of the anastomosis and a completely infarcted graft. Sometimes a thrombus was situated in the pulmonary artery, but the graft was not infarcted. Postmortem examination did not always reveal where the thrombus formation had initiated, e.g., when partial infarction of the left lung was seen with a thrombus attached to both anastomoses. Four animals died during anesthesia with ether, which was needed for lung perfusion scintigraphy or chest roentgenography. In 3 animals the left lung was affected (pneumonia, infarction of the lung, and atelectasis).

Postoperative survival in the series of random-bred Wistar rats and isografted WAG rats differed slightly. Figure 7 shows the postoperative survival of 53 Wistar rats. On the ninth postoperative day, 44% of the animals were still alive, and only a few more died thereafter. The postoperative survival of 22 isografted, SPF-raised WAG rats is shown in Figure 8. On the ninth postoperative day, 11 of them were still alive, after which only 1 animal died. The remaining 10 animals survived until they were killed six to ten weeks after transplantation. Of the 9 allografted animals surviving operation, 3 died during the first postoperative week. Six animals (67%) survived for four to seven days, after which they were killed.

Comment
In studies concerning lung transplantation, the dog has been used as the main experimental animal, chiefly for historical reasons but also because lung transplantation in smaller animals is technically more difficult. Anesthesia and artificial ventilation cause problems even in relatively large rats, as indicated by Asimacopoulos and associates [10]. In our animals, 31% of the deaths most probably were related to anesthesia. Transillumination of the trachea facilitates intubation and makes it a safe technique in the rat [11]. Quick recovery from anesthesia is mandatory in laboratory circumstances, where no intensive postoperative care is available. This caused problems in the initial experiments in which pentobarbital was used as an anesthetic; the period of anesthesia frequently was prolonged after the operation. In the later series, we used Fluothane and the rats became conscious and active immediately after the operation. If the graft was not kept inflated during the implantation period, ventilation of the donor lungs after transplantation became difficult and required high inflation pressures. Although the same problem was experienced in dogs [13], it was found to be more severe in rats.

Organ transplantation in the rat was made possible by the rapid development of microsurgery in the last decade and was due to the availability of very thin suture materials. The intensive training needed for microsurgical procedures is illustrated by the steadily improving perioperative survival in the course of our
experiments from 35 to 80% (see Fig 6). Increased experience resulted in fewer surgical failures, but did not shorten the operative time. Our results improved because of more careful handling and the use of thinner suture material—10-0 instead of 8-0. Most probably a fair perioperative survival can be reached sooner by experienced and skilled microsurgeons imitating the described technique. The perioperative survival that can be expected is higher than the 50% reported by Asimacopoulos and associates [10] and is comparable with the perioperative survival of 76% in rat kidney transplantation, which was reported by Tinbergen [14]. The perioperative survival after lung transplantation in dogs is on the same order [15].

The postoperative complications add to the important overall mortality. Thrombosis of the vessel anastomosis (mainly the venous) occurred in about 50% of the animals. The same percentage was reported in the early period of canine lung transplantation [16–18]. The atrial cuff technique [18] was successfully applied in dogs to reduce this complication. However, in rats the technique does not seem feasible. An adequate anticoagulation therapy, as has been applied to the dog model, might improve the postoperative survival. In human and canine lung transplantations, the bronchus anastomosis frequently causes postoperative complications [1, 15]. Optimal techniques for the bronchus anastomosis as reported in the dog—a short bronchus stump of the graft [19] and the telescoping technique [20]—were both feasible in the rat. No postoperative complications of the bronchus anastomosis have been experienced in these series.

Comparison of Figures 6 and 7 suggests a slight improvement in postoperative survival in the SPF-raised isogeneic rats, possibly due to better lung conditions in these rats. However, since the SPF rats were of a later series, it might also be due to an increased surgical experience and the use of thinner suture materials. Lung transplantation in the isogeneic rats resulted in an overall one-month survival of 36%. Renal transplantation with a contralateral nephrectomy in isogeneic rats resulted in a two-week survival of 39% [14]. In a series of canine left lung autografts, 45% of the animals that were operated on provided proper follow-up data [15].

From the results obtained in our study we conclude that lung transplantation in the rat is technically feasible and that perioperative and postoperative survivals are comparable with those obtained in the dog and other transplantation models in the rat. The rat model is immunogenetically and bacteriologically better defined and cheaper than the canine model. Therefore, it may be the better alternative in lung transplantation research.

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References


