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Obliterative lesions in small airways in an immunosuppressed porcine heterotopic bronchial allograft model

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Abstract We have recently developed a heterotopic large-animal model for research into obliterative lesions in small airways caused by allograft rejection. In this model, the small airways of subcutaneously implanted allografts gradually obliterate, whereas autografts remain patent. Twenty lung fragments and 20 segments of bronchi were implanted in domestic pigs weighing 20 kg from non-related donors. The histology of five animals receiving daily cyclosporine A (CsA) (10 mg/kg), azathioprine (2 mg/kg) and methylprednisolone (20 mg), Group C, was compared with that of six animals without immunosuppression, Group A. Four animals received monotherapy with CsA (10 mg/kg) or methylprednisolone (3 mg/kg). The histological findings were graded from 0 to 3 on the basis of implants harvested repeatedly over 3 months. Epithelial destruction and bronchial obliteration was rapid and permanent in all the allografts. Inflammation and fibrosis of the bronchial wall was less prominent in Group C than in Group A and the onset of fibrosis was delayed. Cartilage degeneration and pericartilaginous inflammation were significantly less severe in Group C ($P < 0.05$). Monotherapy was less potent than triple therapy. This large-animal model is useful for studying the effects of immunosuppressive drugs on obliterative airway disease.

Key words Lung transplantation · Chronic allograft rejection · Obliterative bronchiolitis · Cyclosporine A

Introduction

Obliterative bronchiolitis (OB), which is believed to be a consequence of chronic airway rejection, entails increased mortality and has a prevalence of more than 50% 5 years after lung transplantation [7]. So far, there has been a lack of any proper large-animal model for elucidating the pathogenesis of OB, although various rodent models with lung or tracheal transplants have been described [2, 5, 6, 8, 11]. Our group has recently developed a simplified, reproducible pig model with subcutaneous lung tissue and bronchial implants [4]. Ischemic epithelial damage and the subsequent minor obliteration of the bronchial lumen occurring with autograft implants are seen to normalize after the first month. The implants remain vital, with functioning mucus glands, for at least 6 months and the bronchi look normal, apart from mild to moderate fibrosis and mild cartilage destruction in the lung tissue implants. Occasional mononuclear cells are present. Rejection and rapid progressive obliteration of the bronchial lumen occur in non-immunosuppressed allograft implants.

The aim of the present work was to further improve our large-animal model and to study the effects of cyclosporine A (CsA), azathioprine, and methylprednisolone on the development of obliterative lesions.

Materials and methods

Animals and graft harvesting

The animals received humane care in compliance with the Principles of laboratory animal care (NIH publication 86–23, revised 1985) and specific national legislation.

Ketamine sulfate (10–15 mg/kg), azaperone (10–15 mg/kg), atropine sulfate (0.05 mg/kg), sodium pentobarbital (6–12 mg/kg), and pancuronium bromide (2–4 mg) were used to anesthetize 15 domestic pigs weighing 20 kg. The animals were then intubated and ventilated with 40% oxygen and enflurane. Left thoracotomy was performed and the caudal lobe removed for the preparation of implants.

Study design

Twenty lung tissue fragments of 1 cm^3 with bronchial structures (LB), and 20 pieces of terminal bronchi (B) 1–2 mm in diameter and 1–1.5 cm in length were transplanted subcutaneously into the ventral side of each pig from a non-related donor. No immunosuppression was used in Group A (six animals), whereas Group C (five animals) received daily 10 mg/kg oral CsA (Sandimmun), 2 mg/kg oral azathioprine, and 20 mg oral methylprednisolone for 3 months. Monotherapy allograft groups of two animals each received daily 10 mg/kg oral CsA (Sandimmun), in the case of Group D, and daily 3 mg/kg oral methylprednisolone, in that of Group E. The follow-up was at least 3 months. Implants were harvested serially for histological analysis with hematoxylin and eosin and Masson's trichrome stainings twice a week during the first 2 weeks and weekly or every other week thereafter. Individual histological findings were graded from 0 to 3. Blood samples for CsA whole blood levels were taken several times during the follow-up, 2–3 h after the last dose.

Statistics

The Mann-Whitney U test, Z adjusted for ties (Statistica v. 4.5; Stat Soft, Tulsa, Okla., USA), was used to evaluate significant differences between the Group A and Group C allografts. Values of $P < 0.05$ were considered statistically significant.

Results

The mean CsA whole blood level was 555 ± 311 ng/ml in Group C and 853 ± 203 ng/ml in Group D.

Epithelial destruction

Damage to the bronchial epithelium was permanent and total within 14 days in all the allograft implants.

Luminal obliteration

Gradual occlusion of the bronchial lumen was total in all the allograft implants within 21 days except in Group C, where total obliteration was delayed by 1 week in the B implants and by 2 weeks in the LB implants. In Group C the onset of obliteration in the B implants was delayed to 10 days (average grade 1 ± 1.2) compared with onset at 7 days (average grade 0.4 ± 0.7) in the B implants of Group A.

Mural fibrosis

The onset of mural fibrosis was seen at 3 days in Group A and reached its maximum average grade of 2.4 ± 0.7 in the B implants and 2.6 ± 0.4 in the LB implants at 1 month. In Group C, the onset was at 7 days and the highest average grade of 2.0 ± 0.5 in the B implants and 2.0 ± 0.9 in the LB implants was seen at 21 days. After reaching the maximum score, intramural fibrosis decreased slightly for 1–1.5 months but reached its maximum score again at the end of the follow-up.

Cartilage degeneration

Destruction of the bronchial cartilage progressed rapidly in all the allografts within the first 2 weeks, being already present at 3 days. It reached an average grade of 3.0 ± 0 in 2.5 months with both kinds of implants in Group A, but the immunosuppression used in Group C reduced cartilage degeneration to a highest average grade of 1.5 ± 0.7 in the B implants and 2.2 ± 0.3 in the LB implants. A significant difference ($P < 0.05$) between Group A and Group C was reached with both implants (Fig. 1). Both monotherapy groups showed some effect on cartilage degeneration, this being stronger in Group D than in Group E, which reached almost the same grade as in Group A.

Mononuclear inflammation

Mild pericartilagineous accumulation of mononuclear cells was present at 3 days in Group A, and increased throughout the follow-up, reaching an average grade of 3.0 ± 0 in the B implants within 3 months and an average of 2.8 ± 0.3 in the LB implants within 1.5 months. In Group C, mild pericartilagineous inflammation was present at 7 days in the B implants and at 3 days in the LB implant and reached its highest average grades of 1.3 ± 0.3 and 1.4 ± 0.8, respectively, at 1 month. The difference between Group A and Group C reached statistical significance ($P < 0.05$) in both implants (Fig. 2). Some effect on pericartilagineous inflammation was seen in the monotherapy groups, this being more distinct in Group E than in Group D.

Mild intramural inflammation was seen in all the allografts at 3 days and was most prominent within

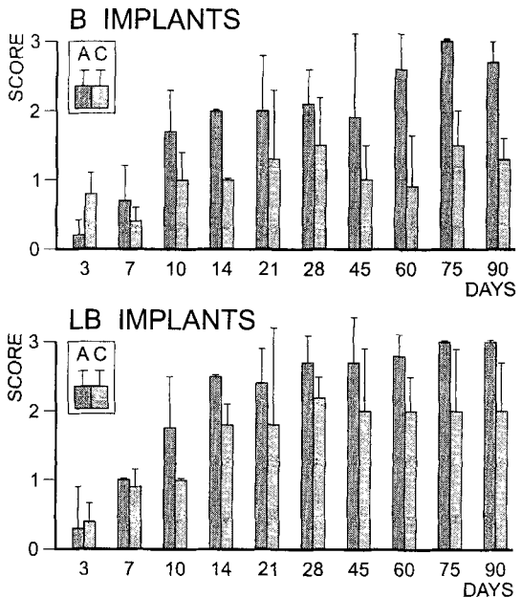

Fig. 1 Cartilage degeneration (mean, SD) was severe in both the bronchial *(B)* and lung tissue *(LB)* implants of Group A allografts. A statistically significant decrease ($P < 0.05$) was achieved with immunosuppression (cyclosporine A, azathioprine, and methylprednisolone) in Group C

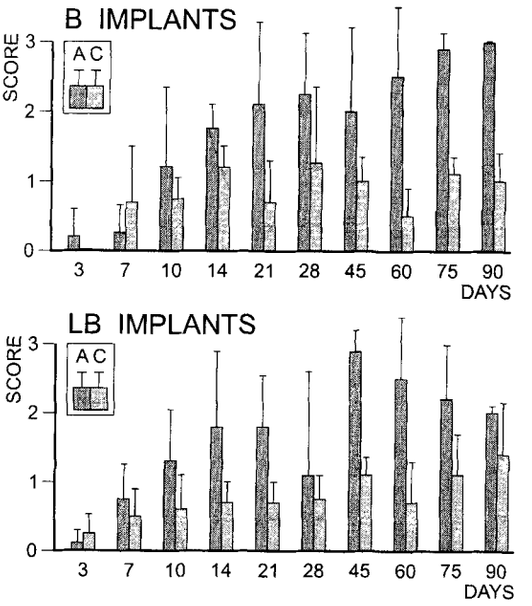

Fig. 2 Pericartilagineous inflammation (mean, SD) increased throughout the follow-up in the bronchial *(B)* implants and for 45 days in the lung tissue *(LB)* implants of Group A allografts. Immunosuppression (cyclosporine A, azathioprine, and methylprednisolone) produced a statistically significant decrease ($P < 0.05$) in this mononuclear inflammation in both the B and LP implants of Group C

10 days, reaching maximum average grades of 1.5 ± 0.6 in the B implants and 1.7 ± 0.5 in the LB implants in Group A, and 1.1 ± 0.6 and 1.0 ± 0.4, respectively, in Group C. Thereafter it decreased in all the groups and was very mild in Group C.

Discussion

Compared with previously reported large-animal models with findings of OB [3], the advantage of our heterotopic model [4] is the high number of consecutive, large-sized specimens, and the fast appearence of progressive obstruction of the bronchial lumen. The surgical procedure is not as demanding as orthotopic lung transplantation, and the implants are easily obtained and larger than samples obtained by transbronchial biopsy. Non-immunosuppressed allografts show rapid development of luminal obliteration [4], histologically similar to that seen in human OB [1], and this makes the model very suitable for research into obliterative airway disease after lung transplantation.

Triple therapy induced a statistically significant decrease in cartilage degeneration and pericartilagineous inflammation in the allografts, but there was still a difference relative to the autografts [4]. The effect on mural fibrosis was statistically non-significant, and there was also a difference with the autografts, where even moderate fibrosis of the bronchial wall is noticed in LB implants and mild fibrosis in B implants [4]. Triple therapy reduced intramural inflammation almost to the level of that of autografts, where only some occasional inflammatory cells are seen [4] but, in spite of this, no statistically significant difference was achieved between the allografts of Group A and Group C. It seems that the combination of CsA, methylprednisolone, and azathioprine had a tendency to preserve the implants, although it was unable to inhibit obliteration. Monotherapy with CsA or methylprednisolone also had some effect on the histological features, but this was not as distinct as with the triple therapy.

Based on the close regulatory relationship between fibroblasts and the respiratory epithelium and between lymphocytes and epithelial cells in the bronchial wall [9, 10], it may be that epithelial recovery in the allograft implants could inhibit bronchial obliteration. Unlike the situation in tracheal allografts in rats [5], we could not avoid epithelial destruction and consequent luminal obliteration using the present combination of immunosuppressive drugs. Koskinen et al. [5] observed a dose-dependent effect of CsA on the tracheal epithelium and obliterative changes, and found that low-dose CsA was unable to prevent obliteration. Thus, despite a

relatively high blood level, our dose of CsA may be too low, or this combination may not be effective enough to inhibit the obliteration in this accelerated model. Further experiments are needed to find a more potent drug combination or a single immunosuppressive agent [6] that can prevent the permanent epithelial destruction and obliteration of the allografted bronchi in this model.

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