Transplant Int (1992) 5 [Suppl 1]: S313–S317

TRANSPLANT
International

Inhibition of rejection of hamster-to-rat heart xenografts

R. Hasan, J. van den Bogaerde, J. Forty, L. Wright, J. Wallwork, and D. J. G. White

Papworth Hospital, Cambridge, UK

Abstract. Prolonged survival of concordant organ xenografts as typified by hamster-to-rat heart transplants is difficult to produce. Studies have revealed that T cells are not primarily involved in rejecting such xenografts and that the rat recipients produce high titres of lytic anti-hamster antibodies. In this study, 200 hamster-to-rat cardiac xenografts performed in 30 different experiments revealed that cyclophosphamide (CyP) and cyclosporin A (CyA) could inhibit this antibody production. CyP alone was relatively ineffective in prolonging graft survival (the median survival time was 14 days versus 3 days in untreated controls). Combining CyP and CyA virtually abolished rejection in this model. Four critically timed doses of CyP combined with continuous CyA resulted in recipients not producing anti-hamster antibodies, despite cessation of CyP therapy, and prolonged graft survival time (median survival time was more than 100 days). Cessation of CyA at 60 and 100 days resulted in the rejection of the xenografts and the appearance of the rat anti-hamster antibodies. Xenografts in recipients given only one or two doses of CyP (and continuous CyA) had a median survival time of 7 and 12 days respectively. However xenograft rejection in rats given only 1 or 2 doses of CyP could be averted by complement depletion using a 3-week course of cobra venom factor (CoF) starting on day 4 or day 7 post-transplantation respectively. Discontinuation of CoF after 3 weeks did not result in graft rejection. These results showed that immunosuppressive therapies directed at inhibiting antibody production may be of value in preventing rejection of concordant xenografts. Short-term complement depletion could rescue xenografts from rejection such that rescued grafts appear to be accommodated.

Key words: Hamster-to-rat xenograft – Cyclophosphamide – Cyclosporin A – Cobra venom factor – Complement – Concordant xenograft

Offprint requests to: R. Hasan, Department of Surgery, Addenbrookes Hospital, Hills Road, Cambridge CB2 2QQ, UK

Xenografts have been categorized as discordant or concordant [5]. The discordant category is that species combination in which rejection of organ xenografts is hyperacute, with vascular lesions similar to those seen in second-set allografts. In the concordant category, rejection occurs at a tempo and with morphological characteristics similar to first-set allografts. In this study the immunologic processes involved in the rejection of concordant hamster-to-rat xenografts were investigated.

Current immunosuppressive regimes used in transplantation are designed to inhibit the predominantly cellular processes which are responsible for first-set rejection of allografts. The application of these protocols in both experimental and clinical xenografting has been disappointing [24, 30]. While graft prolongation has been achieved in combinations where donor and recipient were phylogenetically very closely related such as wolf to dog [9, 12], goat to sheep [25], hare to rabbit [8] and chimpanzee to man [29], results in more distantly related species though still concordant such as hamster to rat [33], monkey to baboon [6] or baboon to man [32] have been disappointing. Those regimes which do produce prolongation of such xenografts rely on immune ablative procedures, the mechanisms of which cannot be fully analyzed because of their multifunctional nature [15, 16]. The transplantation of organs from hamster to rat has been extensively studied as a model of concordant xenograft rejection [7, 14, 16, 21, 37]. Previous workers have not been able to achieve long term xenograft survival consistently in this species combination, in spite of the ablation of T cell mediated immune responses [11, 36]. However, the depletion of complement in rat recipients of hamster heart in combination with continuous cyclosporin A (CyA) therapy has resulted in some long term survival [37]. Additionally, the kinetics of the anti-hamster antibody response in untreated or T cell deficient recipients [17] in conjunction with the demonstration of binding of these antibodies to the rejected grafts, has suggested an important role for antibody as well as complement in the rejection of these concordant xenografts. These data indicate that inhibition of anti-graft antibody production might extend xenograft survival in this model.

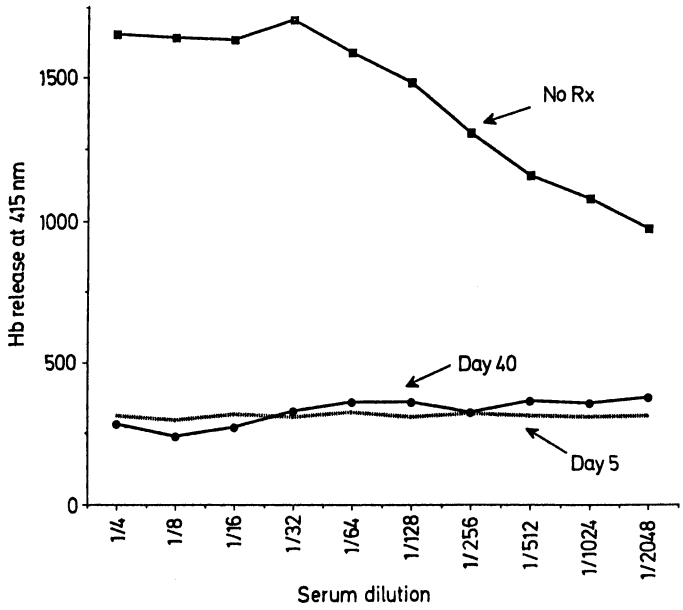

Fig. 1. Lytic anti-hamster antibody in cyclophosphamide and cyclosporin A treated rat recipients of a hamster heart xenograft on days 5 and 40 compared with antibody levels on day 5 in an untreated recipient

This study was designed to investigate the possibility of suppressing pharmacologically anti-hamster antibody production in rat recipients of hamster heart xenografts using cyclophosphamide (CyP) because of its known ability to inhibit antibody production via selective action on rapidly proliferating B cell blasts [34]. We also explored the feasibility of rescuing concordant xenografts from rejection.

Materials and methods

Animals. Syrian hamsters (80–110 g) were used as heart donors (Wright Brothers, Essex, UK) and PVG rats (220–250 g) as recipients (Bantin and Kingman, Hull, UK).

Cardiac transplants. Heterotopic cardiac transplants were placed into the neck of recipients vascularizing the xenograft on a pedicle of the external jugular and carotid vessels using the cuff technique as previously described [13].

Immunosuppressive agents. Cyclophosphamide (CyP) was freshly prepared in distilled water at 10 mg/ml from 100 mg vials (Degussa Pharmaceuticals, Cambridge), injected intraperitonealy and the excess discarded. CyA (a gift from Dr. J. F. Borel, Sandoz Ltd, Basel, Switzerland) was dissolved in olive oil and administered intramuscularly at 20 mg/kg on alternate days (i.e. 10 mg/kg daily). Cobra venom factor (CoF) was prepared as previously described in aliquots of 0.5 mg/ml and administered intramuscularly at a dose of 0.5 mg/kg on alternate days (i.e. 0.25 mg/kg daily). Cobra venom was prepared as previously described in 0.5 mg/ml aliquots and injected intramuscularly at 0.5 mg/kg on alternate days (i.e. 0.25 mg/kg daily) [37].

Antibody titres. Lytic antibody titers were measured in the serum of recipient rats, using hamster red blood cells as targets and baby rabbit serum as a source of complement. Cell lysis was assayed by haemoglobin release into complement fixation diluent as measured on a spectrophotometer [37].

Statistical analysis. Groups of survivors were compared using the Mann Whitney test [18], with significance achieved with P value less than 0.01.

Results

Untreated rats rejected hamster hearts in a median of 3 days (group 1, Table 1). Very high titres of anti-hamster antibodies in excess of 1/2048 were detected in these animals (Fig. 1). Animals receiving CyP only at a dose of 40 mg/kg on day 1 followed by 20 mg/kg twice weekly for 4 weeks ($n = 10$), (group 2, Table 1) survived significantly longer (median survival time of 14 days) than the untreated group ($P < 0.01$). In this group, five animals rejected their xenografts while three died with beating hearts during therapy. The remaining two recipients completed the course of treatment (28 days), but died 1 week later with beating xenografts.

A series of experiments were performed to verify the optimum dosage of CyP (Hasan et al., manuscript submitted) necessary to suppress antibody production. It was found that four critically timed doses of CyP (days 1, 2, 5 and 8) was sufficient to prevent rejection by the effect of suppressing anti-hamster antibodies; 60% of recipients survived long term (> 100 days) while the remaining 40% of recipients died of infection with beating xenografts (group 3, Table 1). There were no detectable anti-hamster antibodies in any rat recipient receiving CyP and CyA therapy up to 30 days after the last dose of CyP (Fig. 1).

Table 1. Survival of hamster heart xenografts in rats treated with cyclophosphamide (CyP) and cyclosporin A (CyA)

Group	Therapy	Survival in days	*N*	MST
1	Untreated	3, 3, 3, 3, 3, 3, 3, 3, 4, 4	10	3
2	CyP (4 wks)	3[a], 4[a], 11[a], 12, 14, 14, 18, 20, 35[a], 35[a]	10	14
3	CyP (one dose) + CyA	7, 7, 7, 7, 7, 7, 8, 8, 9, 9	10	7
4	CyP (two doses) + CyA	11, 11, 11, 12, 12, 12, 12, 12, 13, 13	10	12
5	CyP (four doses) + CyA	10[a], 18[a], 27[a], 61[a], (> 100 × 6)	10	> 100

[a] Recipient died with beating xenograft
MST, Median survival time

Table 2. Survival of hamster hearts in rat recipients treated with one and two doses of cyclophosphamide (CyP) and continuous cyclosporin A (CyA) when they are rescued by a 3-week course of cobra venom factor (CoF) starting on day 4 and day 7 respectively

Group	Therapy	Survival in days	*N*	MST
6	CyP (one dose) + CyA + CoF (on day 4 for 3 weeks)	4[a], 17, 18, 21, 38, (60 × 5)	10	49
7	CyP (two doses) + CyA + CoF (on day 7 for 3 weeks)	38, 40, (60 × 8)	10	60

[a] Recipient died with beating xenograft
MST, Median survival time

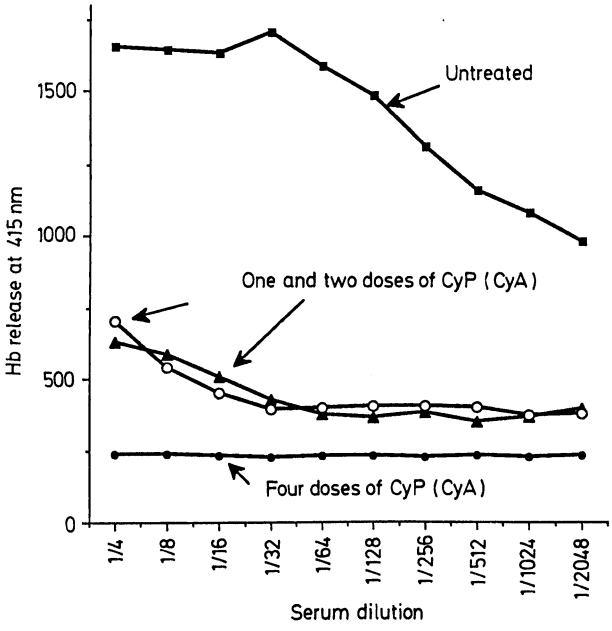

Fig. 2. Lytic anti-hamster antibody in cyclophosphamide (one and two doses) and cyclosporin A treated rat recipients of a hamster heart xenograft at the time of rejection compared with antibody levels on day 12 in recipients who had four doses of cyclophosphamide and continuous cyclosporin A. Peak lytic anti-hamster antibody in untreated rat recipients on day 5 is shown for comparison

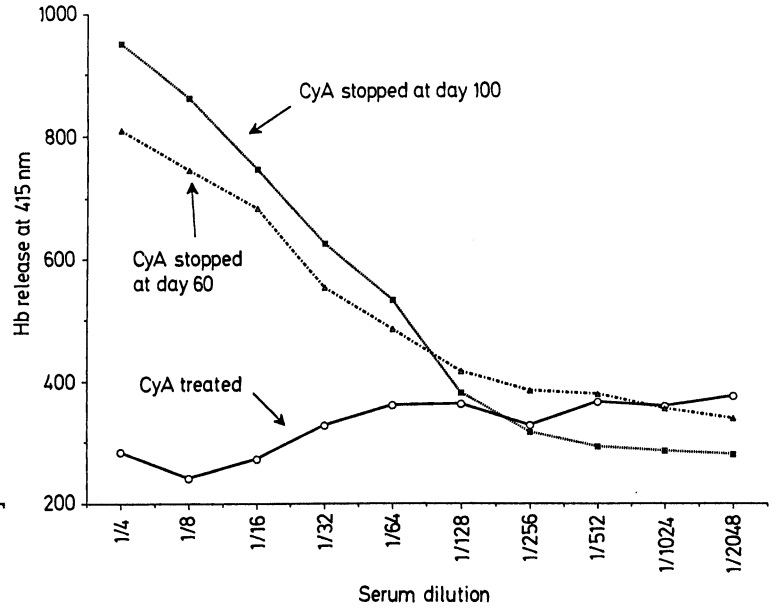

Fig. 3. Lytic antibody in rat recipients of hamster heart xenograft who had cyclosporin therapy discontinued on day 60 and day 100 post-transplantation. Antibodies were measured at the time of rejection. As a control, antibody levels were measured in a recipient rat on day 40 while it was still receiving cyclosporin

In an attempt to eliminate the infective complications, the CyP dosage was reduced to one (the day before transplantation) or two (the day before the operation and on the second post-operative day) doses only, but this was not sufficient to suppress antibody production and the xenografts survived for a median of 7 or 12 days respectively (groups 4 and 5, Table 1). Antibody in these animals was detectable at a titre of 1/16 compared to a total absence in recipients who had four doses (Fig. 2). A rescue therapy was attempted in which recipients given 1 or 2 doses of CyP as described above were treated with CoF either on day 4 (1 dose group) or day 7 (2 doses group) post-transplantation. This therapy was successful in averting the rejection. Furthermore, when CoF was discontinued after 3 weeks the grafts continued to survive (Table 2).

The role of continued CyA therapy was examined in two experiments. A group of 10 recipients (out of 13) treated with four doses of CyP, and CyA at 10 mg/kg daily survived for 60 days. These recipients then had the CyA therapy discontinued. All the transplanted hearts were rejected with a median survival time of 11 days following the discontinuation of CyA (group 1, Table 3). A second group of 10 recipients, who received the same CyP and CyA therapy, with xenografts surviving more than 100 days had the CyA therapy discontinued at day 100. All the recipients rejected the transplanted hearts with a median survival time of 19.5 days following the withdrawal of CyA therapy (group 2, Table 3). In these two groups anti-hamster antibodies could be demonstrated in the recipients at the time of rejection (Fig. 3).

Table 3. Survival of hamster heart xenografts in rats after discontinuation of cyclosporin A (CyA)

Group	Therapy	Survival in days	*N*	MST
1	CyP (4 doses) + CyA 10 mg/kg CyA stopped at day 60	8, 9, 9, 10, 11, 11, 15, 18, 20, 20	10	11
2	CyA stopped at day 100	17, 17, 17, 18, 18, 21, 21, 23, 23, 23	10	19.5

CyP, Cyclophosphamide; MST, median survival time

Discussion

The data described here demonstrated that judicious treatment of rat recipients of hamster heart xenografts with a combination of CyP and CyA inhibited the production of anti-species antibodies and produced long-term xenograft survival. Furthermore it seems likely that these two events were causally linked. Insufficient doses of cyclophosphamide produced some prolongation of graft survival but all the grafts were rejected due probably to the development of antibodies although the titres were less than those in untreated recipients. Prevention of graft rejection in the face of the imminent appearance of anti-species antibodies was achieved by a short-term depletion of complement with CoF (3-week course).

Others have demonstrated the importance of antibodies in the rejection of concordant xenografts [3, 4, 10, 19, 23]. In addition, measures designed to decrease antibody production in rats are able to produce an increase in the survival of hamster heart xenografts. These measures involve the use of splenectomy with or without cyclo-

sporin A [22] or 15-deoxyspergualin alone or combined with splenectomy or total lymphoid irradiation [19, 28, 35] but they were unsuccessful in producing long-term survival in this concordant combination (survival ranged from 7–40 days). The demonstration of some long-term survival of hamster xenografts in CyA-treated complement-depleted recipients, implicates complement in the rejection of concordant xenografts [37]. In addition, very high anti-graft antibody titers and antibody binding to transplanted hamster hearts were shown in these animals. The use of one or two doses of CyP depressed this antibody production but was not sufficient to prevent it and rejection occured. Complement depletion with CoF in such animals prevented this rejection.

This offers an exciting possibility for the future, since concordant xenograft recipients could be monitored by measuring anti-species antibodies and if they appear, then complement depletion for a short period should prevent rejection. The depletion of C3 disarms both the alternative and classical complement pathway. Both pathways have been shown to be involved in the rejection of discordant xenografts [20, 27, 31]. One question addressed by this study was whether alternative complement activation was able to cause destruction of these concordant xenografts. The data reported here showed that combination therapy with CyP and CyA completely inhibited anti-hamster antibody production, did not affect complement activity and yet produced significant prolongation of hamster heart xenograft survival. This confirmed that unlike the discordant models previously studied, [31, 20] destruction in this concordant model was not caused by the alternative pathway of complement.

These data also showed that the anti-xenograft antibody response could be inhibited by a short pulse of CyP and continuous CyA therapy. Monotherapy with CyP resulted in significant prolongation of the xenografts but was insufficient to produce long-term survival. Combined CyP and CyA was capable of producing long-term survival in this model with total absence of rat anti-hamster antibodies for the duration of CyA therapy. However, discontinuation of CyA at 60 or 100 days post-transplantation resulted in rejection of the xenografts and the emergence of anti-hamster antibodies. These results strongly suggested a major role for anti-species antibodies in the rejection of concordant xenografts and that the combined therapy of CyP and CyA produced long-term survival by suppressing this antibody production.

Data from this and other publications showing the importance of antibody-mediated rejection in "concordant" xenografts, has clouded the original distinction between concordant and discordant xenografts [5]. Although hyperacute, "antibody-driven" complement-mediated xenograft rejection is apparently unique to "discordant" combinations [26], similarities in the histological appearance and rejection mechanisms between discordant and certain concordant xenograft combinations places the value of the original "first-set allograft" (concordant) and "second-set allograft" (discordant) definition in some doubt [1]. To resolve this difficulty we would propose a subdivision of concordant xenografts into "difficult" and "easy" depending on whether antibody-mediated rejection ("difficult concordant"), or T cell mediated rejection ("easy concordant") is of primary importance. This is of practical significance, since baboon to human transplants, which represent the most likely combination for clinical xenografting in the immediate future, would appear to be "difficult concordant" xenografts according to histological and immunological data currently available [2]. By the same criteria, chimpanzee to man [29] would fall in the "easy concordant" category. The extrapolation of results obtained from rodents to clinical practice is ill advised, yet the data reported here suggest that similar studies in primates need to be undertaken to establish the possibility of using this clinically applicable therapy to prevent xenograft rejection in man and the preliminary results using the above therapeutic regimens are encourging.

References

1. Bailey LL, Nehlsen-Cannarella SL (1986) Observations on cardiac xenotransplantation. Transplant Proc 18 3 [Suppl 2]: 88–92
2. Bailey Ll, Nehlsen-Cannarella SL, Concepcion W, Jolley WB (1985) Baboon-to-human cardiac xenotransplantation in a neonate. JAMA 245: 3321–3329
3. Bogman MJ, Berden JM, Hagemann JM, Maass CN, Koene RP (1980) Patterns of vascular damage in the antibody-mediated rejection of skin xenografts in the mouse. Am J Pathol 100: 727–737
4. Bouwman E, Bruin RF, Marquet RL, Jeekel J (1989) Prolongation of graft survival in hamster to rat xenografting. Transplant Proc 21: 540–541
5. Calne R (1970) Organ transplantation between widely disparate species. Transplant Proc 2: 550–553
6. Cooper DKC, Rose AG (1989) Experience with experimental xenografting in primates. In: Hardy MA (ed) Xenograft 25. Elsevier Science Publication (Biomedical Division), Amsterdam, The Netherlands
7. DeMasi R, Alqaisi M, Araneda D, Nifong W, Thomas J, Gross U, Sawson M, Thomas JM (1990) Revaluation of total lymphoid irradiation and cyclosporine therapy in the syrian hamster-to-lewis rat cardiac xenograft model. Transplantation 49: 639–662
8. Dieperink H, Steinbruchel D, Starklint H, Larsen S, Kemp E (1987) Improvement in hare-to-rabbit kidney transplant survival. Transplant Proc 19: 1140–1142
9. Duswald KH, Scheel JV, Hammer C, Brendel W (1976) Long-term graft survival in the xenogenic system wolf-dog. Res Exp Med (Berl) 167: 255–266
10. Ertel W, Reichenspurner H, Hammer C, Welz A, Uberfuhr P, Hemmer W, Reichart B, Gokel M, Brendel W (1984) Heterotransplantation in closely related species: a model for humoral rejection. Transplant Proc 16: 1258–1261
11. Gudas VM, Carmichael PG, Morris RE (1989) Comparison of the immunosuppressive and toxic effects of FK 506 and cyclosporin in xenograft recipients. Transplant Proc 21: 1072–1073
12. Hammer C, Chaussey C, Welter H, Weinbacher J, Hobel G, Brendel W (1981) Exceptionally long survival time in xenogeneic organ transplantation. Transplant Proc 13: 881–884
13. Heron I (1971) A technique for accessory cervical heart transplantation in rabbits and rats. Acta Pathol Microbiol Immunol Scand [A] 79: 366–372
14. Homan WP, Williams KA, Fabre JW, Millard PJ, Morris P (1981) Prolongation of cardiac xenograft survival in rats receiving cyclosporin A. Transplantation 31: 164–166
15. Kemp E, Dieperink H, Jensenius J, Koch C, Larsen S, Madsen H, Nielsen B, Starklint H, Steinbruchel DA (1990) Hope for successful xenografting by immunosuppression with monoclonal antibody against CD4, total lymphoid irradiation and cyclosporine. Scand J Urol Nephrol 24: 79–80

16. Knetchle SJ, Halperin EC, Bollinger RR (1987) Xenograft survival in two species combination using total lymphoid irradiation and cyclosporin. Transplantation 43: 173–175
17. Lim SML, Li SQ, Wee A, Chong SM, Hu A, Rauff A, White DJG (1991) Both concordant and discordant heart xenografts are rejected by athymic(nude) rats with the same tempo as in T cell competent animals. Transplant Proc 23: 581–582
18. Mann HB, Whitney DR (1947) On a test of whether one of two random variables is statistically larger than the other. Ann Math Statist 18: 50–60
19. Marchman WAD, DeMasi R, Taylor D, Carrobi A, Larkin E, Alqaisi M, Thomas F (1991) Therapy with 15-deoxyspergualin and total lymphoid irradiation blocks xenograft rejection and antibody formation after xenografting. Transplant Proc 23: 210–211
20. Miyagawa S, Hirose H, Shirakura R, Yoshihumi N, Nakata S, Kawashima Y, Seya T, Matsumoto M, Uenaka A, Kimtamura H (1988) The mechanism of discordant xenograft rejection. Transplantation 46: 825–830
21. Moden M, Valdivia LA, Gotoh M, Hasuike Y, Kubota N, Kanai T, Okamura J, Mori T (1987) Hamster-to-rat orthotopic liver transplant. Transplantation 43: 745–746
22. Monden M, Valdivia LA, Goton M (1989) A crucial effect of splenectomy on prolonging cardic xenograft survival in combination with cyclosporine. Surgery 105: 535–542
23. Nakajima K, Sakamoto K, Ochiai T, Asano T, Isono K (1989) Effects of 15-deoxyspergualin and FK506 on the histology and survival of hamster-to-rat cardiac xenotransplantation. Transplant Proc 21: 546–548
24. Nakajima K, Sakamoto K, Ochiai T, Nagata M, Asano T, Isono K (1988) Prolongation of cardiac xenograft survival in rats treated with 15-deoxyspergualin alone and in combination with FK506. Transplantation 45: 1146–1148
25. Perper RJ, May J, Way L, Najarian JS (1965) Experimental renal heterotransplantation in closely related species. Fed Proc 24: 573
26. Perper RJ, Najarian JS (1966) Experimental renal heterotransplantation I. in widely divergent species. Transplantation 4: 377–388
27. Platt JL, Vercellotti GM, Dalmasso AP, Matas AJ, Bolman RM, Najarian JS, Bach FH (1990) Transplantation of discordant xenografts: a review of progress. Immunol Today 11: 450–456
28. Pruitt SK, Halperin EC, Bollinger RR (1991) The effect of 15-deoxyspergualin on hamster-to-rat cardiac xenograft survival. Transplant Proc 23: 585–586
29. Reemtsma K, McCracken DH, Schilegel JU, Pearl MA, Pearce CW, DeWitt CW, Smith PE, Hewitt RL, Flinner RL, Oscar-Creech Jr (1964) Renal heterotransplantation in man. Ann Surg 160: 384–410
30. Sakakibara N, Click RE, Condie RM, Jamieson SW (1989) Rejection/acceptance of xenografts. Transplant Proc 21: 524–526
31. Schilling A, Land W, Pratschke E, Pielsticker K, Brendel W (1976) Dominant role of complement in the hyperacute xenograft rejection reaction. Surg Gynecol Obstet 142: 29–32
32. Starzl TE, Marchioro TL, Peters GN, Kirckpatrick CH, Wilson WEC, Porter KA, Rifkind D, Ogden DA, Hitchcock CR, Waddell WR (1964) Renal heterotransplantation from baboon to man: experience with 6 cases. Transplantation 2: 752–776
33. Thomas FT, DeMasi RJ, Araneda D, Marchman W, Alqaisi M, Larkin EW, Condie RM, Carobbi A, Thomas JM (1990) Comparative efficacy of immunosuppressive drugs in xenografting. Transplant Proc 22: 1083–1085
34. Turk JL, Poulter LW (1972) Effects of cyclophosphamide on lymphoid tissue labelled with 5-iodo-2-deoxyuridine ^{125}I and Cr-51. Int Arch Allergy 43: 620–629
35. Valdivia LA, Monden M, Gotoh M, Nakano Y, Tono T, Mori T (1990) Evidence that deoxyspergualin prevents sensitization and first-set cardiac xenograft rejection in rats by suppression of antibody formation. Transplantation 50: 132–136
36. van den Bogaerde JB, White DJG, Roser B, Kampinga JR, Aspinall R (1990) In vitro and in vivo effects of monoclonal antibodies against T-cells subsets in allogenic and xenogeneic responses in the rat. Transplantation 50: 915–920
37. van den Bogaerde JB, Aspinall R, Wright L, Wang MW, Carey N, White DGJ (1991) The induction of long term survival of hamster heart xenografts in rats. Transplantation 53: 15–20