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Matthew J. Bowles
Alan G. Pockley
Richard F. M. Wood

Effect of anti-CD4 monoclonal antibody administration on rat small bowel allograft survival and circulating leukocyte populations

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M.J. Bowles · A. G. Pockley
R. F. M. Wood (✉)
Section of Surgery,
Division of Clinical Sciences (NGH),
Clinical Sciences Centre,
Northern General Hospital,
Herries Road, Sheffield S5 7AU, UK
e-mail: r.f.m.wood@sheffield.ac.uk
Fax: + 44-114-261-9246

Abstract This study assessed the effect of an anti-rat CD4 monoclonal antibody (OX38) on heterotopic small bowel allograft rejection. Fully allogeneic small bowel transplants were performed in the PVG-to-DA-rat strain combination. Animals received either i) short course (days –1, 0 and 1) of 1 mg/kg per day OX38, ii) short course of 5 mg/kg per day or iii) extended course (days –2, –1, 0, 1, 2 and twice weekly thereafter) of 1 mg/kg per day. Both the high dose (13 days) and extended low-dose (12 days) courses prolonged graft survival compared to untreated control animals (7 days). The low-dose, short-course treatment had no effect. Similar regimens were given to animals that did not receive transplants and in which peripheral blood $CD4^+$ cell counts fell to between 20 and 55% of pretreatment levels and 20–30% of binding sites were blocked. In summary, anti-CD4 monoclonal antibody therapy delayed rejection of rat small bowel allografts; however, long-term survival was not achieved.

Key words Small bowel transplantation · Monoclonal antibody · Rat · Rejection · Flow cytometry

Introduction

Although cyclosporin A and, more recently, tacrolimus have improved the clinical success of small bowel transplantation, rejection remains a major problem. Data from the International Intestinal Transplant Registry indicate that 47% of small bowel transplant recipients succumb to sepsis and 10% to lymphoproliferative disorders [6]. More specific inhibition of the immune response is required if small bowel transplantation is to achieve the levels of success seen in renal, hepatic and cardiac transplantation.

The pivotal role of $CD4^+$ T lymphocytes in the allograft rejection response [12] renders this cell population an attractive target for immunosuppression using anti-CD4 monoclonal antibody (mAb). The ability of anti-CD4 mAbs to delay the rejection of cardiac [8, 23, 25], renal [5, 26] and skin [14] allografts has been demonstrated in experimental animal models. Such approaches have been of variable efficacy in human cardiac and renal transplantation [17, 19], although more recent clinical trials have been more encouraging [2, 15].

This study assessed the effects of anti-CD4 mAb protocols administered in the immediate perioperative period on recipient survival in an experimental rat model of small bowel transplantation. We also investigated the possible mechanisms underlying the influence of mAb on recipient survival by determining the effects of treatment on peripheral blood leukocyte populations and the degree of in vivo binding of OX38 mAb to $CD4^+$ target cells.

Materials and methods

Preparation of anti-CD4 monoclonal antibody (OX38)

OX38 hybridomas (ECACC, Salisbury, U.K.) were cultured in serum free culture medium (Hyclone Europe, Cramlington, U.K.) containing penicillin (100 U/ml), streptomycin (100 μg/ml) and

L-glutamine (2 mM; Life Technologies, Paisley, U.K.) at 37 °C, 100% humidity and 5% CO_2. OX38 was purified from culture supernatants by protein A sepharose affinity chromatography (Sigma Chemical Co., Poole, UK), and the concentration was measured using an enzyme immunoassay. Briefly, 96 well microtitre plates were coated with rabbit polyclonal anti-murine IgG,A,M antibody (100 µl–1/10,000 dilution, SERT 100, Serotec, Kidlington, UK). Plates were washed with phosphate-buffered saline (PBS) containing 0.1% v/v Tween 20 (PBS/T) and blocked with PBS/T containing 1% w/v bovine serum albumin. Bound mAb was detected using a biotinylated sheep anti-murine immunoglobulin polyclonal antibody (Amersham Life Science, Little Chalfont, UK), a streptavidin/biotin/horseradish peroxidase enzyme complex (Amersham) and 5-amino salicylic acid-based substrate (Sigma). Absorbance changes were measured at 450 nm using a Titertek Multiskan MCC/340 microplate spectrophotometer. The concentration of purified OX38 was determined by reference to a dose response curve generated using a standard OX38 preparation (Serotec) and ASSAYZAP data analysis software (BIOSOFT, Cambridge, U.K.). Purified OX38 was diluted with sterile PBS such that the required doses for in vivo administration were contained in 0.3–0.4 ml.

Small bowel transplantation

Fully allogeneic heterotopic small bowel transplantation was performed between adult male PVG ($RT1^c$) donor and DA ($RT1^a$) recipient rats (B & K Universal, Hull, UK), essentially as previously described [16]. Animals were housed under standard conditions and were given normal rat chow and water ad libitum. All animals received care and were treated in compliance with the Animals (Scientific Procedures) Act 1986.

The small bowel immediately distal to the duodeno-jejunal junction to just proximal to the ileo-caecal junction was isolated on a vascular pedicle comprising the superior mesenteric artery on an aortic cuff and the portal vein. The vasculature was flushed with Marshall's perfusion solution, and the bowel lumen was cleared with chlorhexidine solution (Baxter Healthcare, Thetford, UK). The graft was stored in Marshall's solution at 4 °C until transplanted. Aorto-aortic and porto-caval anastomoses were performed in the recipient, and the small bowel graft was transplanted in a heterotopic position with cutaneous stomata at both luminal ends. Animals were monitored regularly and sacrificed if necessary. The survival time was recorded as the number of days from the transplantation to the day of sacrifice or to the day prior to death if the animal died overnight. All animals with transplants displayed gross macroscopic evidence of graft rejection at post-mortem examination.

Monoclonal antibody treatment

Animals with transplants (5–7 per group) underwent one of three OX38 mAb treatment regimens: i) short course (days –1, 0 and 1) at 1 mg/kg per day, ii) short course at 5 mg/kg per day or iii) an extended course (days –2, –1, 0, 1, 2 and twice weekly thereafter) at 1 mg/kg per day. Day 0 was the day of transplantation, and OX38 was administered by intravenous injection into the tail vein or penile vein. Control animals received PBS in place of the OX38 mAb.

Similar regimens were administered to three groups of animals that did not receive transplants (2 per group) to assess the effect of administered OX38 mAb on circulating leukocyte populations and in vivo binding to $CD4^+$ cells. Control animals received PBS in place of OX38 mAb.

Peripheral blood analysis and cell population studies (animals without transplants)

Heparinised blood was obtained on days –1, 0, 1, 2, 3, 4, 7 and 8, and the total leukocyte count, differential leukocyte count, cell sub-populations and in vivo mAb binding of OX38 were monitored. Leukocytes were counted using an improved Neubauer chamber, and the proportions of neutrophils, lymphocytes, monocytes and eosinophils were determined using Giemsa (Sigma Chemical Co.) stained blood smears. Absolute counts of individual cell subpopulations were calculated from the total leukocyte number.

Flow cytometric analysis

The effect of mAb administration on circulating leukocyte subsets and its in vivo binding to target cells was determined using whole blood flow cytometry, based on a previously described technique [28]. Briefly, 10 µl of heparinised blood was incubated with 20 µl of pooled rat serum (Serotec) for 15 min at room temperature. After washing, cells were incubated sequentially with the appropriate mAb combination (OX19-PE/OX33-FITC; OX35-biotin/OX19-PE; OX8-FITC/OX19-PE; 3 µl). OX19 binds to the CD5 antigen expressed on all T cells, and OX33 binds to the leukocyte common antigen expressed on B cells. OX35 binds to an epitope on the CD4 antigen that is distinct to that bound by OX38, and OX8 binds to the CD8 antigen, which defines a subset of T cells and which is also expressed on natural killer cells. The binding of biotinylated OX35 was determined by subsequent incubation with 3 µl streptavidin/ FITC conjugate (Serotec). Cells were washed following every incubation with antibody, and erythrocytes were lysed using Erythrolyse (Serotec). All mAbs were obtained from Serotec except for OX35, which was purified from hybridoma culture supernatants (ECACC) and subsequently biotinylated using a standard protocol (Amersham Life Science).

Stained cell suspensions were analysed on a FACScan flow cytometer (Becton Dickinson UK, Cowley, UK). The forward and side scatter and green and red fluorescence data for 5000 cells within a live gate placed around the lymphocyte and monocyte regions were acquired using 'Consort 30' software (Becton Dickinson UK). The combination of mAbs used for double staining enabled the relative numbers of T and B cells and the proportions of the former that were $CD4^+$ and $CD8^+$ to be calculated. The absolute counts of cell subpopulations were calculated from the total and differential leukocyte counts.

OX38 binding capacity of $CD4^+$ cells

In rats, the CD4 antigen is present on monocytes as well as the subset of T cells that it defines. The presence of residual binding sites on peripheral blood lymphocytes and monocytes after in vivo mAb treatment was assessed by flow cytometry on the basis of in vitro OX38 binding. A whole blood staining technique was used in which the cells were incubated with a previously titrated saturating amount of biotinylated OX38, followed by streptavidin/FITC conjugate.

The fluorescent intensities of the lymphocyte and monocyte populations were determined and converted to the number of biotinylated mAb molecules bound to the cell surface using Quantum Simply Cellular precalibrated microbeads (Sigma Chemical Co.) which were stained and analysed concurrently with the blood samples. Comparing the fluorescent intensity of cells from treated animals to that of cells from PBS-treated rats (which by definition had

100% free antibody binding sites) gave a measurement of the residual OX38 mAb binding capacity.

Statistical analysis

The survival times of animals from different groups were compared using the Wilcoxon's rank sum test. Differences in the survival of two groups were considered to be of statistical significance when P values were less than or equal to 0.05.

Results

Survival (animals with transplants)

Untreated control animals succumbed to the effects of graft rejection at median of 7 days after transplantation (Fig. 1). Treatment with 1 mg/kg per day OX38 for 3 days had no effect on survival, whereas administration of the same dose for 5 days perioperatively and then twice weekly thereafter increased the median survival to 12 days. This was significantly longer than both the control ($P < 0.02$) and low-dose, short-course ($P < 0.01$) groups (Fig. 1). Animals given the higher dose of OX38 (5 mg/kg per day) for 3 days survived for a median of 13 days (Fig. 1), which was also significantly longer than the control ($P < 0.05$) and low-dose, short-course ($P < 0.05$) groups. It is clear from these findings that significantly prolonged survival could be achieved by either prolonging the time course of OX38 administration or increasing the antibody dose.

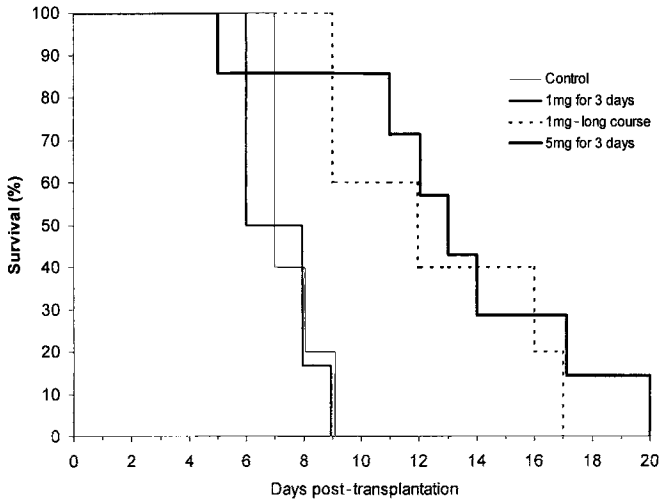

Fig. 1 Effect of OX38 monoclonal antibody (mAb) treatment on recipient survival after heterotopic small bowel transplantation. OX38 was given at a dose of 1 or 5 mg/kg per day to recipient rats for 3 perioperative days or 1 mg/kg per day for 5 perioperative days and twice weekly thereafter. Control animals received phosphate-buffered saline in place of the OX38 mAb. Each group comprised 5–7 technically successful transplants. Both the high-dose and low-dose long-course groups exhibited significantly longer survival than the control and low-dose, short-course groups

Table 1 Baseline cell populations in the peripheral blood of adult male DA rats that did not receive transplants. Data are mean ± standard deviation of 2 animals

Cell population	Cell count ($\times 10^9$/l)
Leukocytes (total)	14.6 ± 2.8
Neutrophils	4.3 ± 1.8
Monocytes	0.5 ± 0.3
Lymphocytes (total)	9.8 ± 1.8
B cells	3.3 ± 0.8
T cells (total)	6.5 ± 1.3
$CD4^+$ T cells	4.9 ± 1.0
$CD8^+$ T cells	1.5 ± 0.3

Peripheral blood cell populations (animals without transplants)

The baseline leukocyte populations in the peripheral blood of DA rats that did not receive transplants are summarised in Table 1. OX38 treatment induced gradual falls in the total lymphocyte and monocyte counts, with the high-dose, short-course group exhibiting a more marked early decrease than the low-dose groups (Fig. 2). As expected, OX38 treatment had no effect on peripheral blood neutrophil and B cell counts (data not shown).

OX38 treatment reduced total T cell counts, an effect that persisted until the final time point studied (day 8; Fig. 3). The $CD4^+$ T cell count in the high-dose, short-course group fell by nearly 50% after the first injection, and by day 4 the $CD4^+$ cell count was less than 25% of the baseline value for this group (Fig. 3). The falls in the $CD4^+$ T cell counts in the low-dose OX38 groups were less dramatic in the early post-treatment period, although reduced $CD4^+$ cell counts persisted in all treatment groups until day 8. As would be expected, OX38 had no effect on peripheral blood $CD8^+$ cell counts (data not shown), and the falls in total lymphocyte counts resulted from the reduced numbers of $CD4^+$ T cells.

Monoclonal antibody binding capacities (in vivo receptor occupancy)

The residual antibody binding capacity of $CD4^+$ T cells in the low-dose, short-course group was approximately 80% on days 0, 1 and 2 and returned to nearly 100% on day 3 (Fig. 4). The binding capacity in the low-dose, long-course group was maintained at approximately 80% during the antibody administration period

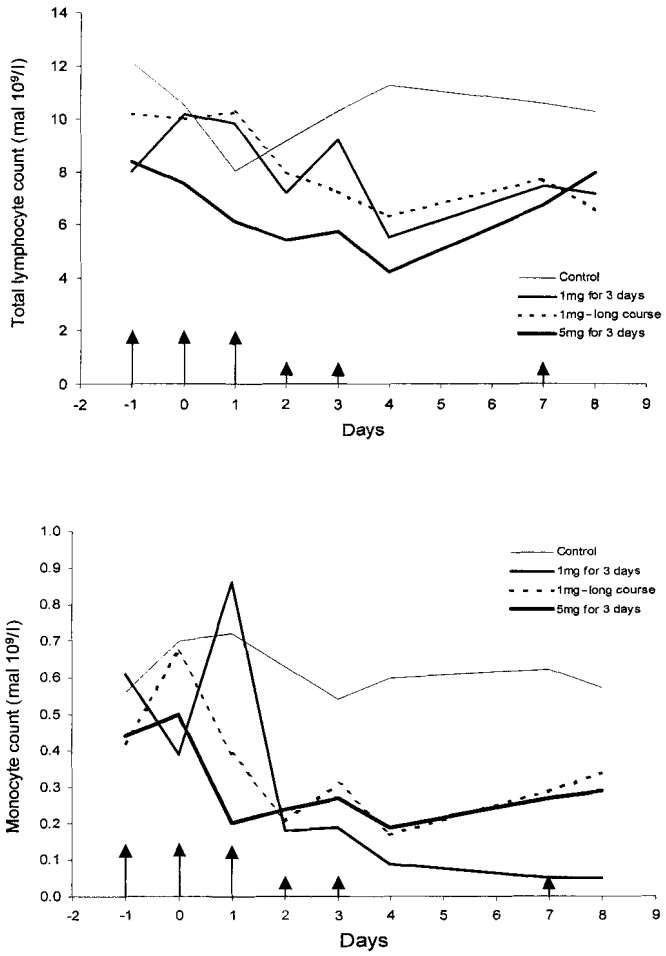

Fig. 2 Effect of OX38 monoclonal antibody (mAb) treatment on peripheral blood lymphocyte (*upper*) and monocyte (*lower*) counts in adult male DA rats that did not receive transplants. The x-axis designation is consistent with the timing of injections in the transplant groups. Day 0 (the day of the second injection) is equivalent to the day on which the transplant procedure was performed, except for the low-dose, long-course group for which day 1 is equivalent to the day of transplantation. Control animals received phosphate-buffered saline in place of the OX38 mAb. The *large arrows* indicate the timing of the intravenous injections and the *small arrows* the subsequent injections in the long-course treatment group. Each line represents the mean of data from 2 animals with the exception of the control group, for which data were interpolated between days 1 and 3, due to insufficient sample to perform all analyses on day 2

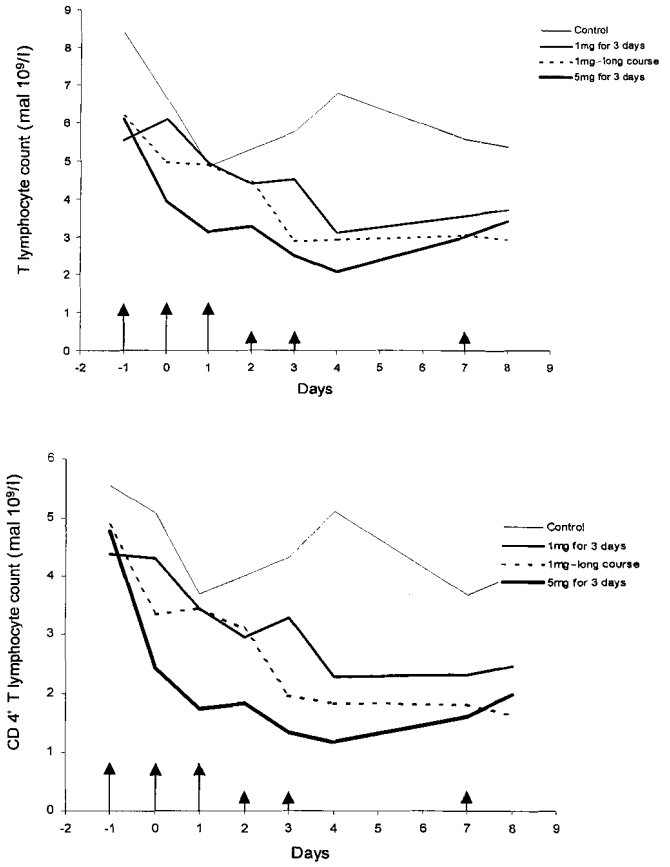

Fig. 3 Effect of OX38 monoclonal antibody (mAb) treatment on peripheral blood T lymphocyte (*upper*) and CD4⁺ lymphocyte (*lower*) counts in adult male DA rats that did not receive transplants. The x-axis designation is consistent with the timing of injections in the transplanted groups. Day 0 (the day of the second injection) is equivalent to the day on which the transplant procedure was performed, except for the low-dose, long-course group for which day 1 is equivalent to the day of transplantation. Control animals received phosphate-buffered saline in place of the OX38 mAb. The *large arrows* indicate the timing of the first three intravenous injections and the *small arrows* the subsequent injections in the extended treatment group. Each line represents the mean of data from 2 animals with the exception of the control group, for which data were interpolated between days 1 and 3, due to insufficient sample to perform all analyses on day 2

(Fig. 4). Administration of higher OX38 mAb doses (5 mg/kg per day) was marginally more effective at blocking in vitro binding sites (Fig. 4). The in vitro monoclonal antibody binding capacity of monocyte was somewhat variable, and there was no appreciable blockade of OX38 binding sites on monocytes, except in the high-dose treatment group (Fig. 4).

Discussion

The potential efficacy of anti-CD4 mAb has been demonstrated in a number of experimental transplantation models including rat cardiac, renal and skin grafts [14, 22, 23, 25, 26]. Efficacy appears to be influenced by the animal strains used and organs transplanted, as anti-CD4 prevents cardiac graft rejection in low, but not high responder combinations [1, 25, 27] and has differential effects on heart, kidney and liver graft survival in Lewis recipients of ACI grafts [29].

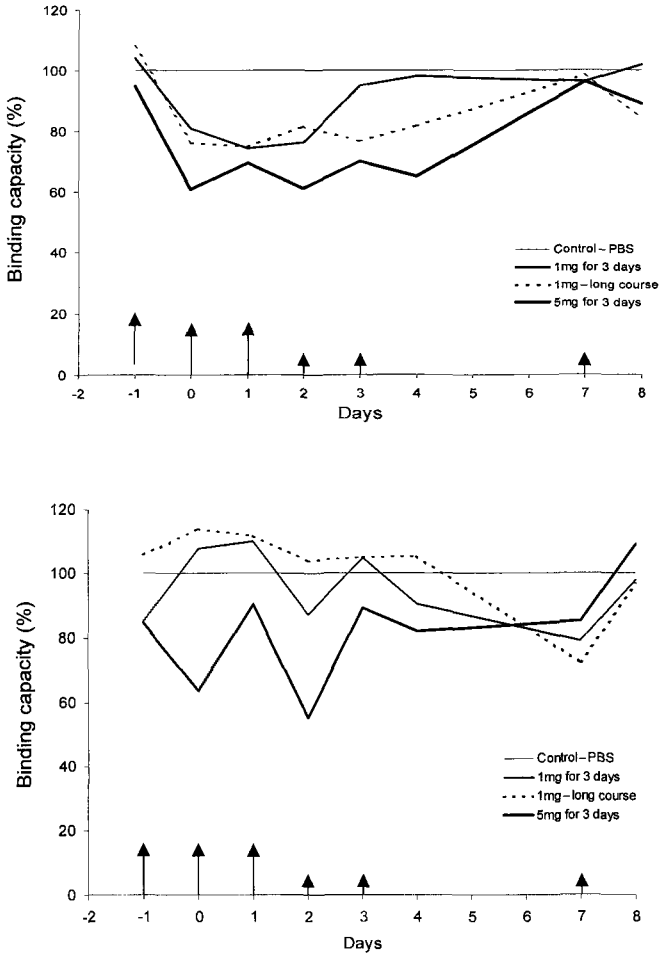

Fig. 4 In vitro OX38 binding capacity of $CD4^+$ lymphocytes (*upper*) and monocytes (*lower*) in the peripheral blood of OX38-treated adult male DA rats that did not receive transplants. The mean monoclonal antibody (mAb) binding capacity of the control (phosphate-buffered saline-treated) group on each day was designated 100%, and the binding capacities of other groups were compared to it. The x-axis scale is consistent with the timing of injections in the transplant survival studies. Day 0 (the day of the second injection) is equivalent to the day that transplantation was performed, except for the low-dose, long-course group for which day 1 is equivalent to the day of transplantation. Control animals received phosphate-buffered saline (PBS) in place of the OX38 mAb. The *large arrows* indicate the timing of the three intravenous injections of mAb and the *small arrows* the additional injections in the animals that received an extended course of low-dose (1 mg/kg per day) OX38. Data are means of two animals in each group, with the exception of the control group on day 2 when only one sample was available

Monoclonal and polyclonal antibodies have been used in experimental small bowel transplantation to some effect in the context of graft or donor pretreatment in unidirectional graft-versus-host disease models [10, 11, 24] and a murine unidirectional rejection model [18]. Few studies have evaluated the effect of systemic mAb administration to recipients of fully allogeneic small bowel allografts – a situation that is more closely analogous to the clinical situation.

OX38 administered to Lewis recipients of Brown Norway small bowel allografts on days –6 to –3 before transplantation has no effect on graft survival, although animals died of a graft-versus-host-like illness 19 days after transplantation [4]. Intravenous OX38 at 5 mg/kg per day on days –3 to 0 prolongs the survival of Lewis recipients of ACI small bowel grafts from 8.2 to 16.0 days [30]. More recent evidence suggests that OX38 can prolong small bowel graft survival when the intestine is cotransplanted with a heart [29].

The majority of studies have used anti-CD4 mAb administration protocols lasting several days prior to transplantation, and the aim of our study was to assess the effect of anti-CD4 mAb using a more clinically relevant short pretransplantation period of treatment, continued for a variable time after small bowel transplantation. Our findings indicate that prolongation of recipient survival could be achieved, provided that OX38 was given in a sufficient dose or over a prolonged post-transplantation course.

In an attempt to understand the mechanism underlying prolonged survival, we studied circulating lymphocyte subsets and in vivo CD4 antigen occupancy after mAb treatment. All three regimens used in this study reduced $CD4^+$ lymphocyte numbers over the course of several days, although the effects were quantitatively different. Our findings are broadly consistent with previous work in which OX38 was administered intraperitoneally to ACI rats [25]. However, a subsequent study has reported that a reduction from 48% to 4% has been observed after intravenous administration of BWH-4 to Lewis recipients of cardiac grafts [23].

Although the reduction in $CD4^+$ T cell counts observed in the present study may be responsible for the prolonged survival in the high-dose, short-course group, it is difficult to explain the different animal survival in the low-dose, short-course and low-dose, long-course groups on this basis. Certainly, cell depletion does not appear to be essential, as $CD4^+$ or $CD8^+$ cell depletion by mAb is not necessarily the mechanism by which intestinal allograft survival is prolonged in a murine model [7]. Both depleting and nondepleting anti-CD4 mAbs can induce indefinite murine cardiac graft survival, but only if a booster dose maintains the coating of $CD4^+$ cell surfaces in the nondepleting case [3, 20, 21]. It is also difficult to explain the prolongation of graft survival on the basis of antigen blockade, as the residual binding capacity of $CD4^+$ T cells after the first 3 days of treatment were similar in all three groups.

In summary, anti-CD4 mAb therapy administered in the post-transplantation period can prolong the survival of rat small bowel allograft recipients, although its effi-

cacy is inferior to that of cyclosporin A in the same strain combination [9, 13]. Monoclonal antibody therapy may be more effective when used in combination with other agents such as CTLA4Ig which is capable of blocking costimulatory pathways and enhancing the efficacy of anti-CD4 [30], or as a short peri-transplantation course to allow the use of reduced doses of pharmacological agents.

References

1. Alters SE, Shizuru JA, Ackerman J, Grossman D, Seydel KB, Fathman CG (1991) Anti-CD4 mediates clonal anergy during transplantation tolerance induction. J Exp Med 173: 491–494
2. Cooperative Clinical Trials in Transplantation Research Group (1997) Murine OKT4A immunosuppression in cadaver donor renal allograft recipients: a cooperative clinical trials in transplantation pilot study. Transplantation 63: 1243–1251
3. Darby CR, Morris PJ, Wood KJ (1992) Evidence that long-term cardiac allograft survival induced by anti-CD4 monoclonal antibody does not require depletion of $CD4^+$ T cells. Transplantation 54: 483–490
4. Deaton DH, Posselt AM, Naji A, Barker CF (1991) Selective T-cell depletion of recipients in allogeneic small bowel transplantation (SBT) in the rat. Transplant Proc 23: 283–284
5. Delmonico FL, Knowles RW, Colvin RB, Cavender DE, Kawai T, Bedle M, Stroka D, Preffer FI, Haug C, Cosimi AB (1993) Immunosuppression of Cynomolgus renal allograft recipients with humanized OKT4A monoclonal antibodies. Transplant Proc 25: 784–785
6. Grant D (1999) Intestinal transplantation: 1997 report of the international registry. Intestinal Transplant Registry. Transplantation 67: 1061–1064
7. He G, Hart J, Kim OS, Szot GL, Siegel GL, Thistlethwaite JR, Newell KA (1999) The role of CD8 and CD4 T cells in intestinal allograft rejection: a comparison of monoclonal antibody-treated and knockout mice. Transplantation 67: 131–137
8. Herbert J, Roser B (1988) Strategies of monoclonal antibody therapy that induce permanent tolerance of organ transplants. Transplantation 46: 128S-134 S
9. Ingham Clark CL, Smith GJ, Crane PW, Price BA, Fabre JW, Lear PA, Wood RFM (1992) Reduction in graft-versus-host reactivity following small bowel transplantation by ex vivo perfusion of intestinal allografts with an anti-T cell immunotoxin. Clin Exp Immunol 88: 220–225
10. Ingham Clark CL, Grover R, Pockley AG, Wood RFM (1993) Delayed-start cyclosporine immunosuppression reduces graft cell migration after small bowel transplantation. Transplantation 56: 1284–1286
11. Johnsson C, Tufveson G (1994) The effects of various routes of administration of monoclonal anti-T cell antibodies for prevention of the graft-versus-host reaction following small bowel transplantation. Transplantation 57: 289–292
12. Krieger NR, Yin DP, Fathman CG (1996) $CD4^+$ but not $CD8^+$ cells are essential for allorejection. J Exp Med 184: 2013–2018
13. Lear PA, Ingham Clark CL, Crane PW, Pockley AG, Wood RFM (1993) Donor cell infiltration of recipient tissue as an indicator of small bowel allograft rejection in the rat. Transplant Int 6: 85–88
14. Lehmann M, Sternkopf F, Metz F, Brock J, Döcke W, Plantikow A, Kuttler B, Hahn H, Ringel B, Volk H (1992) Induction of long-term survival of rat skin allografts by a novel, highly efficient anti-CD4 monoclonal antibody. Transplantation 54: 959–962
15. Meiser BM, Reiter C, Reichenspurner H, Uberfuhr P, Kreuzer E, Rieber EP, Riethmuller G, Reichart B (1994) Chimeric monoclonal CD4 antibody – a novel immunosuppressant for clinical heart transplantation. Transplantation 58: 419–423
16. Monchik GJ, Russell PS (1971) Transplantation of the small bowel in the rat: technical and immunological considerations. Surgery 70: 693–702
17. Morel P, Vincent C, Cordier G, Panaye G, Carosella E, Revillard JP (1990) Anti-CD4 monoclonal antibody administration in renal transplanted recipients. Clin Immunol Immunopathol 56: 311–322
18. Newell KA, He G, Hart J, Thistlethwaite JRJ (1997) Treatment with either anti-CD4 or anti-CD8 monoclonal antibodies blocks T cell-mediated rejection of intestinal allografts in mice. Transplantation 64: 959–965
19. Norman DJ, Bennett WM, Cobanoglu A, Hershberger R, Hosenpud JD, Meyer MM, Misiti J, Ott G, Ratkovec R, Shihab F, Vitow C, Barry JM (1993) Use of OKT4A (a murine monoclonal anti-CD4 antibody) in human organ transplantation: initial clinical experience. Transplant Proc 25: 361–367
20. Pearson TC, Bushell AR, Darby CR, West LJ, Morris PJ, Wood KJ (1993) Lymphocyte changes associated with prolongation of cardiac allograft survival in adult mice using and anti-CD4 monoclonal antibody. Clin Exp Immunol 92: 211–217
21. Pearson TC, Darby CR, Bushell AR, West LJ, Morris PJ, Wood KJ (1993) The assessment of transplantation tolerance induced by anti-CD4 monoclonal antibody in the murine model. Transplantation 55: 361–367
22. Sayegh MH, Kut JP, Milford EL (1989) Anti-CD4 monoclonal antibody effects cellular hyporesponsiveness and prolongs renal allograft survival in the rat. Hum Immunol 26: 131–136
23. Sayegh MH, Sablinski T, Tanaka K, Kut JP, Kwok CA, Tilney NL, Kupiec-Weglinski JW, Milford EL (1991) Effects of BWH-4 anti-CD4 monoclonal antibody on rat vascularized cardiac allografts before and after engraftment. Transplantation 51: 296–299
24. Shaffer D, Ubhi CS, Simpson MA, Gottschalk R, Milfod EL, Maki T, Monaco AP (1991) Prevention of graft-versus-host disease following small bowel transplantation with polyclonal and monoclonal anti-lymphocyte serum. Transplantation 52: 948–952
25. Shizuru JA, Seydel KB, Flavin TF, Wu AP, Kong CC, Hoyt EG, Fujimoto N, Billingham ME, Starnes VA, Fathman CG (1990) Induction of donor-specific unresponsiveness to cardiac allografts in rats by pre-transplant anti-CD4 monoclonal antibody therapy. Transplantation 50: 366–373
26. Siegling A, Lehmann M, Riedel H, Platzer C, Brock J, Emmrich F, Volk H (1994) A nondepleting anti-rat CD4 monoclonal antibody that suppresses T helper 1-like but not T helper 2-like intragraft lymphokine secretion induces long-term survival of renal allografts. Transplantation 57: 464–467

27. Sun J, Sheil AG, Wang C, Wang L, Rokahr K, Sharland A, Jung SE, Li L, McCaughan GW, Bishop GA (1996) Tolerance to rat liver allografts: IV. Acceptance depends on the quantity of donor tissue and on donor leucocytes. Transplantation 62: 1725–1730
28. Webster GA, Pockley AG (1993) Effect of red cell lysis protocols on the expression of rat peripheral blood lymphocyte subset and activation antigens. J Immunol Methods 163: 115–121
29. Yin DP, Sankary HN, Williams J, Krieger N, Fathman CG (1996) Induction of tolerance to small bowel allografts in high-responder rats by combining anti-CD4 with CTLA4Ig. Transplantation 62: 1537–1539
30. Yin DP, Sankary HN, Talor-Edwards C, Chong AS, Foster P, Shen J, Ma LL, Williams JW, Fathman CG (1998) Anti-CD4 therapy in combined heart-kidney, heart-liver, and heart-small bowel allotransplants in high-responder rats. Transplantation 66: 1–5