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CD4 and CD8 monoclonal antibody therapy in canine renal allografts

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Abstract Therapy with CD4 and CD8 monoclonal antibodies was evaluated in dogs which received double-haplotype MHC-mismatched renal allografts. Neither CD4 nor CD8 monoclonal antibodies given alone prolonged allografts survival (creatinine $\geqq$ 300 μmol/l) beyond 7 days. However, combined therapy with CD4 and CD8 antibodies given up to day 10 did prolong allograft survival to a median of 14 days. A longer (21 day) course of CD4 and CD8 antibodies did not extend allograft survival further. The effect of prolonged antibody therapy was restricted by the occurrence of both an antiglobulin response and an anaphylactoid reaction to the monoclonal antibody preparation. When the CD4 and CD8 antibodies were combined with a pan-T-cell -depleting Thy-1 antibody, the survival of double-haplotype mismatched allografts was further prolonged (median 16 days). The median survival of single-haplotype mismatched renal allografts on this triple therapy was 21 days, with one surviving to day 36.

Key words CD4 · CD8 · Immune suppression · Canine model

Introduction

The potential of CD4 and CD8 monoclonal antibodies to act in the induction of tolerance has been well demonstrated in the rodent [1, 2] but remains to be fully explored in preclinical models for possible use in the clinic. To accomplish this, a selection of monoclonal antibodies has been raised in rats against canine leucocytes, and their efficacy evaluated in the treatment of mismatched canine renal allografts.

In the mouse a hierarchy of therapy for tolerance induction has been defined according to the strain combination, the MHC disparity and the nature of the allograft. Tolerance to skin grafts across a multiple non-MHC minor antigen mismatch (B10.BR onto CBA/Ca) can be achieved using a combination of CD4 and CD8 antibodies without T-cell depletion [3]. A full MHC mismatch (CBA/Ca H-2^k mice grafted with BALB/c H-2^d skin) requires both depleting and non-depleting CD4 and CD8 antibodies [4]. A heart graft is less immunogenic than a skin graft, and tolerance across the same full MHC mismatch can be achieved with non-depleting CD4 and CD8 antibodies alone [5].

This paper describes initial experiments in a canine renal allograft model using combinations of CD4 and CD8 monoclonal antibodies, with T-cell depletion effected by a depleting Thy-1 antibody, Thy-1 being expressed on all peripheral T cells in the dog. Initial experiments used short courses of antibody for the immunosuppression of renal allografts across a two-haplotype mismatch (defined by mixed leucocyte culture, MLC).

Table 1 Results (n.d. not determined, *MAb* monoclonal antibody)

Group	Therapy[a]	Treatment protocol[b]			Day of rejection	Dog. no.	Adverse reaction	Last day of free MAb[c]		Day antiglobulin detected		
		CD4	CD8	Thy-1				CD4	CD8	CD4	CD8	Thy-1
A	CD4 alone	−2 to 10	–	–	5	AP23	–	8		None		
					7	CY20	–	9		6		
B	CD8 alone	–	−2 to 10	–	5	AS4[d]	6		4		4	
					7	CY25[d]	7		3		4	
C	CD4+CD8	−2 to 10	−2 to 10	–	10	CY33	–	8	7	None	13	
	10 days post-op				13	CB18[d]	–	12	12	13	11	
	therapy				15	AP30	–	13	9	5	4	
					18	AS7[d]	–	12	13	None	13	
D	CD4+CD8	−2 to 21	−2 to 21	–	12	AP34	10	9	7	11	9	
	21 days postop				12	CY38	–	15	15	15	15	
	therapy											
E	Thy-1 alone	–	–	−2, −1	7	AP27	–					5
					9	CY39	–					6
F	Thy-1,	−2 to 10	−2 to 10	−2, −1	12	AP28	8	6	6	n.d.	n.d.	n.d.
	CD4+CD8				16	AP31	10	8	6	15	11	10
					25	CY37	–	13	11	10	4	None
G	Thy-1,	−2 to 10	−2 to 10	−2, −1	11	AP49	6[e]	6	3	7	5	5
	CD4+CD8				19	AB26	6[e]	6	>2	<10	<10	10
	Single-haplotype				24	AB29	–	9	12	14	14	14
	mismatch				36	AB27	–	6	12	<10	8	10

[a] All donor-recipient combinations were double-haplotype mismatches except group G

[b] Monoclonal antibody therapy was continued to the day indicated, unless an adverse reaction occurred. Transplantation was done on day 0

[c] No free Thy-1 antibody was detectable in any dog even as early as 1 h following injection

[d] Received the CD8 monoclonal antibody YCATE 60.3 in addition to YCATE 55.9, the remainder received YCATE 55.9 alone

[e] Received CD4 alone from day 6 due to adverse reaction to CD8

Materials and methods

Mongrel dogs were selected on the basis of strong proliferative responses in MLC [6]. In all but one experimental group (group G, see Table 1), the donor-recipient combinations were mismatched for both MHC haplotypes. Dogs were operated upon in pairs, each undergoing bilateral nephrectomy and exchanging a single kidney. The day of rejection was defined as the day the creatinine level exceeded 300 μmol/l (3.3 mg/dl), and the dogs were killed when they became symptomatic; untreated controls had rejected by day 7.

The production of monoclonal antibodies has been described elsewhere [6]. Thy-1 antibody (YKIX 337.217, rat IgG_{2b}) depletes peripheral T cells, while CD4 antibody (YKIX 302.9, rat IgG_{2a}) immunomodulates its target antigen; CD8 antibodies (YCATE 55.9 and YCATE 60.3, both rat IgG_1) are also depleting [7]. Monoclonal antibodies were given singly or in combination by slow intravenous injection. CD4 and CD8 therapy was commended 2 days prior to transplantation and continued to day 10 or day 21 according to the protocol (Table 1); Thy-1 therapy was given on the 2 pre-operative days only. Therapy was stopped prematurely upon the occurrence of an anaphylactoid reaction (ataxia, vomiting, loss of consciousness) following injection. Trough levels of free antibody and antiglobulin production (dog anti-rat antibody) were monitored daily [8, 9].

Results

Table 1 illustrates the results. Neither CD4 nor CD8 monoclonal antibodies given alone prolonged renal allograft survival (group A and B), with rejection occurring while the recipients were still on therapy. Ten days of combined therapy with CD4 and CD8 antibodies delayed rejection until after therapy had ceased (group C). However, when CD4 plus CD8 therapy was extended (group D), no further prolongation of survival was achieved. One dog in this group experienced an anaphylactoid reaction following antibody administration on day 10, requiring the cessation of therapy; this reaction corresponded with the development of an antiglobulin response. The other dog rejected the kidney in spite of good levels of circulating antibody and no antiglobulin response.

Pretransplant T-cell depletion alone (group E), in which circulating T-cell numbers were reduced by over 95% using the Thy-1 antibody, resulted in prolonged survival in one of two dogs. When T-cell depletion was

combined with CD4 and CD8 antibodies in double-haplotype mismatched dogs, survival was prolonged further (group F), with one dog surviving to day 25. In the single-haplotype mismatched combination (group G), further prolongation was achieved, with one dog surviving to day 36.

Discussion

These experiments with highly mismatched mongrel dogs show that neither CD4 nor CD8 monoclonal antibodies alone prolong survival, but that combination therapy is immunosuppressive. In the rodent such monoclonal antibody combinations are not just immunosuppressive but tolerogenic, and our observation confirms that the immunological challenge posed by a canine renal allograft is greater even than fully MHC-mismatched vascularised organ or skin allograft models in the rodent [4]. Such observations support our contention that formal evaluation in a preclinical large animal model is required before extending observations from the rodent directly into the clinic. As in the highly mismatched rodent allograft models, better results are achieved in the canine renal allograft model when T-cell depletion is included in the therapeutic regimen. Moreover, when donor and recipient differ by just a single haplotype, the combined depleting and non-depleting antibody combination is even more effective.

While the immunosuppressive properties of the CD4, CD8 and Thy-1 antibody combinations have been demonstrated, tolerance was not induced. Aside from the magnitude of the immunological challenge, another possible reason is that the duration of therapy was too short. If tolerisation occurs when the recipient T cells notice antigen in the presence of CD4 and CD8 blockade, a larger proportion of cells will be tolerised by a longer period of antibody therapy, particularly if it is accepted that a critical number of T cells must be blocked before tolerance is induced, as has been suggested from adoptive transfer experiments in T-cell-depleted mice [2]. Prior T-cell depletion will facilitate this process of tolerisation by reducing the absolute number of T cells. Hence, longer courses of CD4 and CD8 monoclonal antibody therapy together with lymphocyte depletion are desirable. However, effective prolonged antibody therapy was restricted both by the occurrence of anaphylactoid reactions to components in the monoclonal antibody preparation (either the antibody itself or adjuvant protein impurities) and by the generation of an antiglobulin response which resulted in immune clearance of the antibody from the serum. We have evidence that the combination of CD4 and CD8 monoclonal antibodies with T-cell depletion, together with conventional immunosuppressive therapy (azathioprine, cyclosporine) will permit more prolonged therapy [6], raising future prospects for tolerance induction.

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