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Factors relevant for the induction of rejection by indirect recognition in a rat heart allograft model: effect of CTLA4Ig treatment on indirect alloactivation induced by immunization with donor MHC I peptides

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Abstract We have defined factors relevant for the induction of rejection by indirect recognition in a rat heart allograft model and analyzed the influence of CTLA4Ig treatment on indirect alloactivation induced by donor MHC I peptides in a DA → LEW heart allograft model. Indirect allorecognition of MHC I led to accelerated graft rejection and was accompanied by the induction of anti-peptide antibodies and donor peptide-activated T cells. In an attempt to block the B7-induced costimulatory signal of T cell activation, CTLA4Ig was administered to graft recipients in addition to MHC I peptide treatment. CTLA4Ig therapy, however, was not effective in preventing the humoral or cellular anti-donor immune response, nor did it prevent accelerated graft rejection.

Key words
Allorecognition · MHC · Graft rejection · CTLA4Ig · Indirect alloactivation

Introduction

Recent studies provided evidence that indirect recognition of donor MHC antigens plays a role in allograft rejection [1, 3, 6, 7]. Donor MHC antigens are taken up by antigen-presenting cells (APC), digested into small peptides and expressed on the cell surface in association with self-MHC before they are presented to T cells. In order to be activated, T cells must receive a second signal which is provided by costimulatory molecules [4]. The most potent costimulatory signal is delivered by the B7 molecule. CTLA4Ig has been developed as a recombinant fusion molecule that competes in the interaction between the CD28 antigen on T cells and B7 on APC [2, 8]. We studied the potential of CTLA4Ig to suppress the indirect route of alloactivation.

Materials and methods

Two peptides corresponding to the hypervariable α_1 and α_2 domains of the donor MHC class I (RT1-A^a) antigen were synthesized. Their sequence was α_1: HN-Pro-Glu-Tyr-Trp-Glu-Gln-Gln-Thr-Arg-Ile-Ala-Lys-Glu-T rp-Glu-Gln-Ile-Tyr-Arg-Val-Asp-Leu-Arg-Thr-OH; α_2: H_2N-Thr-Arg-Asn-Lys-Trp-Glu-Arg-Ala-Arg-Tyr-Ala-Gl u-Arg-Leu-Arg-Ala-Tyr-Leu-Glu-Gly-Thr-Cys-OH [1]. Porcine neuropeptide Y served as a negative control. Its sequence was: HN-Pro-Ala-Glu-Asp-Leu-Ala-Arg-Tyr-Tyr-Ser-Ala-Leu-Arg-H is-Tyr-Ile-Asn-Leu-Ile-Thr-Arg-Gln-Arg-Tyr-amide.

All recipients were treated twice, 1 and 2 months before transplantation. Group 1 was immunized s.c. with a mixture of $\alpha_1 + \alpha_2$ peptide (combined 100 µg + 100 µg) in complete and incomplete Freund's adjuvant (FA). Other recipients received FA only (group 2), neuropeptide Y in FA (group 3) or $\alpha_1 + \alpha_2$ peptide in FA combined with CTLA4Ig (group 4). CTLA4Ig (50 µg) was administered i.p. 1 day before and on days 1, 4, 8, and 12 after the first and second peptide immunization. The controls in group 5 received CTLA4Ig and FA only.

Anti-peptide antibodies in the sera of immunized animals were assessed by ELISA on microtiter plates coated with $\alpha_1 + \alpha_2$ peptide (0.5 µg/well), incubated with 50 µl of test sera, and developed with mouse F(ab')2 anti-rat Ig-antipeptid plus P-nitrophenyl phosphate substrate.

For the detection of T cell sensitization, 5×10^4 peripheral blood mononuclear cells of unimmunized or peptide-immunized Lewis rats were incubated with 10 µg of $\alpha_1 + \alpha_2$ peptide in RPMI + 5% fetal calf serum. After 3 days, 20 µl ^{3}H-thymidine (5 Ci/mmol) was added. Counts per minute were measured and the index of stimulation was calculated.

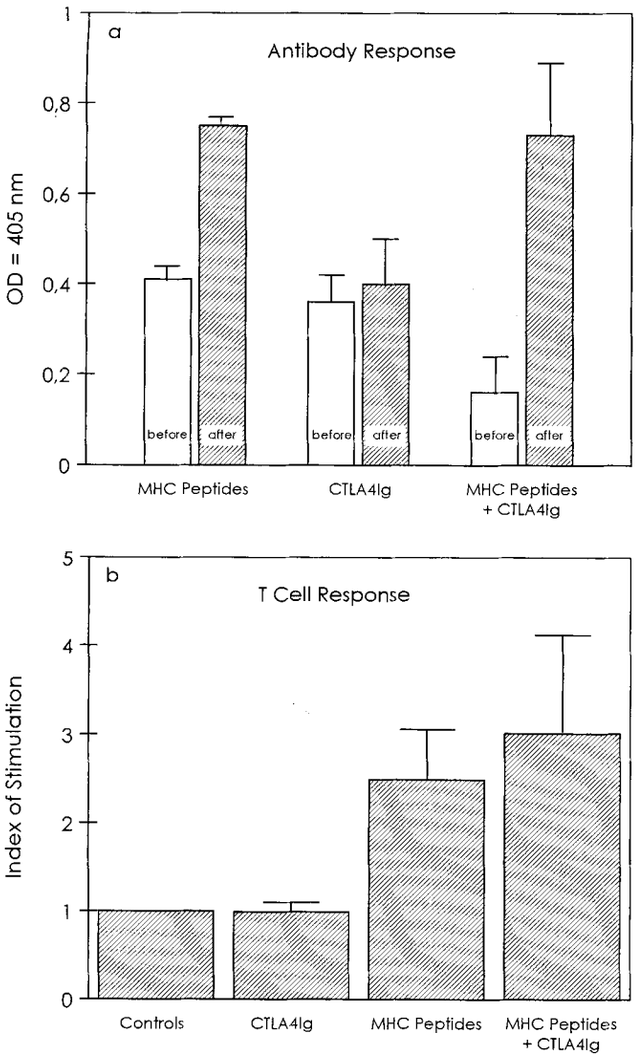

Fig. 1 a, b Humoral and cellular immune response following immunization with donor MHC class I peptides and CTLA4Ig treatment. Lewis rats were immunized with α1 + α2 peptide. **a** Anti-peptide antibody titer in serum (dilution 1:32) of four recipients before and after immunization ($P < 0.001$). CTLA4Ig treatment was not able to prevent antibody formation. **b** Peripheral mononuclear blood cells of unimmunized and peptide-immunized Lewis rats were cultured with $\alpha_1 + \alpha_2$ peptide. Cells cultured without peptide served as control. ^{3}H-Thymidine incorporation was measured and the stimulation index was calculated. Immunized animals presented a T-cell response against $\alpha_1 + \alpha_2$ peptide ($P < 0.001$). CTLA4Ig treatment in vivo was not able to prevent T cell sensitization

Donor hearts were transplanted heterotopically into the abdomen of the recipients and graft survival was monitored by electrocardiogram [5]. Rejection was defined as the moment when the transplanted heart stopped beating.

Data were compared using the Wilcoxon and Mann-Whitney U rank-sum tests.

Table 1 Graft survival times after immunization with donor MHC I peptides with or without CTLA4Ig treatment. Peptide treatment induced accelerated graft rejection ($P < 0.01$: group 1 vs 2, 3 and group 4 vs 2, 5). This effect was not abrogated by CTLA4Ig. (*FA* Freund's adjuvant)

Group	Treatment	Graft survival (days)	Mean ± SD
1	Donor MHC I peptides + FA	4, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 5, 6, 6, 6, 6	5.18 ± 0.53
2	FA	6, 6, 6, 7, 7, 7, 7, 7, 8, 8, 8, 8, 9, 11	7.5 ± 1.34
3	Neuropeptide Y + FA	6, 6, 6, 7, 7, 8	6.67 ± 0.82
4	Donor MHC I peptides + FA + CTLA4Ig	3, 5, 5, 5, 5, 6, 6, 6, 6	5.22 ± 0.97
5	CTLA4Ig + FA	7, 7, 7, 7, 7, 7, 7, 7, 7	7 ± 0

Results and discussion

Pretreatment of recipients with donor MHC I peptides elicited antibody and T cell responses (Fig. 1) and induced accelerated allograft rejection (Table 1). These results confirm previous data obtained in similar experimental systems [1, 3, 7].

T-cell alloactivation requires two signals [4]. One signal is provided by foreign MHC molecules and the second signal is provided by costimulatory molecules. The recognition of foreign MHC in the absence of a costimulatory signal renders T cells unreactive [4]. An important costimulatory signal is provided by the B7 molecule. We attempted to block the B7 pathway by treatment with CTLA4Ig. The doses of CTLA4Ig corresponded to those reported in previous experiments [8]. Contrary to our expectation, CTLA4Ig treatment did not abrogate the graft-accelerating effect of MHC peptides (Table 1). Even CTLA4Ig-treated animals developed anti-peptide antibodies and donor peptide-activated T cells (Fig. 1).

These results led us to conclude that, under the experimental conditions used in this study, CTLA4Ig is not able to abrogate indirect alloactivation and graft rejection induced by donor MHC I peptides.

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