

Acta Medica Okayama

Volume 48, Issue 1

1994

Article 11

FEBRUARY 1994

Unresponsiveness of antidonor cytotoxic T cells in a long-term stable renal transplant recipient.

Takuzo Fujiwara*

Kenichi Sakagami†

Shinya Saito‡

Masashi Uda**

Kunzo Orita††

*Center for Adult Diseases,

†Center for Adult Diseases,

‡Center for Adult Diseases,

**Okayama University,

††Okayama University,

Unresponsiveness of antidonor cytotoxic T cells in a long-term stable renal transplant recipient.*

Takuzo Fujiwara, Kenichi Sakagami, Shinya Saito, Masashi Uda, and Kunzo Orita

Abstract

The antidonor immune response was examined in a one haplotype-mismatched renal transplant recipient with an allograft that had been well-functioning for more than 10 years. Although the relative response of the mixed lymphocyte reaction (MLR) was (45.8)% and the MLR responder cells stimulated by donor cells produced measurable amounts of interleukin-2 (IL-2) (11.6 U/ml), the cytotoxic T lymphocytes (CTL) could not be generated against donor cells, even with exogenous IL-2. These results indicate that antidonor CTL precursors were either deleted or inactivated in this recipient.

KEYWORDS: renal transplantation, long-term stable recipient, cytotoxic T lymphocytes

*PMID: 8191919 [PubMed - indexed for MEDLINE]

Copyright (C) OKAYAMA UNIVERSITY MEDICAL SCHOOL

— Brief Note —

Unresponsiveness of Antidonor Cytotoxic T Cells in a Long-Term Stable Renal Transplant Recipient †TAKUZO FUJIWARA*, KENICHI SAKAGAMI, SHINYA SAITO, MASASHI UDA^a AND KUNZO ORITA^aDepartment of Transplantation Surgery, Center for Adult Diseases, Kurashiki 710, Japan and ^aFirst Department of Surgery, Okayama University Medical School, Okayama 700, Japan

The antidonor immune response was examined in a one haplotype-mismatched renal transplant recipient with an allograft that had been well-functioning for more than 10 years. Although the relative response of the mixed lymphocyte reaction (MLR) was (45.8) % and the MLR responder cells stimulated by donor cells produced measurable amounts of interleukin-2 (IL-2) (11.6 U/ml), the cytotoxic T lymphocytes (CTL) could not be generated against donor cells, even with exogenous IL-2. These results indicate that antidonor CTL precursors were either deleted or inactivated in this recipient.

Key words: renal transplantation, long-term stable recipient, cytotoxic T lymphocytes

Organ transplantation between genetically disparate individuals presently requires nonspecific immunosuppressants to prevent rejection. The use of such agents risks morbidity and mortality from a range of associated adverse effects including increased susceptibility to bacterial and viral infections. On the other hand, the immunological tolerance to alloantigens has been successfully induced in laboratory animals to prevent organ transplantation rejection without long-term use of immunosuppressants. Previous donor-specific transfusions (DSTs) (1), antithymocyte globulin (2), total lymphoid irradiation (3), or administration of cyclosporine (4) can result in permanent acceptance of allografts in some rat strain combinations. However, information concerning transplantation tolerance in human beings is very limited and is mostly derived from case reports. Several investigators have reported donor-specific immune hypo-

responsiveness in recipients with well-functioning kidney grafts at various times after transplantation. The response of peripheral blood lymphocytes (PBLs) to donor alloantigens in these recipients was inhibited in mixed lymphocyte reaction (MLR) (5, 6) or cell-mediated lympholysis assay (CML) (7-9), while the response to third-party cells was nearly normal.

In this study, the *in vitro* immune response to a living-related kidney transplant was examined in a patient who had been preconditioned with DSTs and had maintained a well-functioning kidney graft for more than 10 years after transplantation to better understand the mechanisms underlying the antidonor immune response.

MLR and CML were carried out using PBLs isolated by density gradient centrifugation on Ficoll-Conray. In brief, MLR was set up with 5×10^4 responder cells and 5×10^4 stimulator cells treated with mitomycin C (MMC) in 0.2 ml of RPMI 1640 HEPES medium (GIBCO Laboratories, Grand Island, N.Y.) supplemented with heat-inactivated fetal calf serum (culture medium). Following 6 days of culture, the proliferation of responder cells was assessed by ³H-thymidine incorporation measured in counts per min (cpm). The relative response was calculated by the following formula:

$$\text{Relative response} = \frac{\text{donor cpm} - \text{autologous cpm}}{\text{third-party cpm} - \text{autologous cpm}} \times 100.$$

CML was carried out on day 7 of mixed lymphocyte culture with 1×10^5 responder and 5×10^4 MMC-treated stimulator cells, using the 6-h ⁵¹Cr-release test with ⁵¹Cr-labeled phytohemagglutinin lymphoblast targets. Spontaneous (SP) and maximum (MA) release were determined by incubating target cells in medium alone and

* To whom correspondence should be addressed.

† Supported in part by a grant from the Okayama Kidney Foundation

target cells exposed to 1N NaOH before incubation, respectively. % Cytotoxicity was calculated according to the following formula:

$$\% \text{ Cytotoxicity} = \frac{\text{experimental cpm-SP cpm/MA cpm-SP cpm}}{\text{cpm-SP cpm}} \times 100.$$

To measure interleukin-2 (IL-2) production, supernatants of MLR on day 3 were incubated in culture medium at various dilutions for 48 h with an IL-2-dependent cell line (CTLL-2). During the final 4 h of culture, 1 μCi ^3H -thymidine was added per well. Standard curves were generated from recombinant IL-2.

The patient is a 32-year-old woman (HLA: A11, 26,

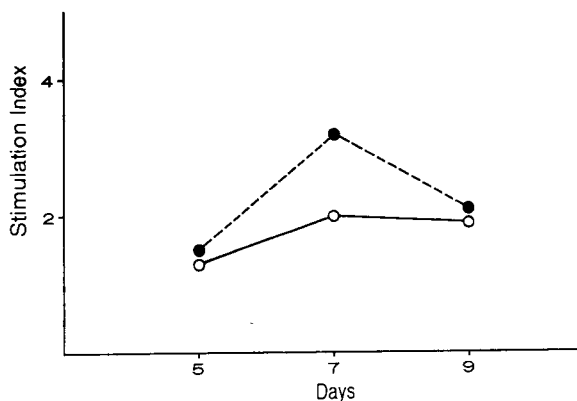


Fig. 1 The kinetics of the mixed lymphocytes reaction (MLR). Stimulation index was calculated by the following formula: stimulation index = cpm with stimulator cells/cpm with autologous cells. MLR was carried out with mitomycin C treated donor cells (○) and third-party cells (●).

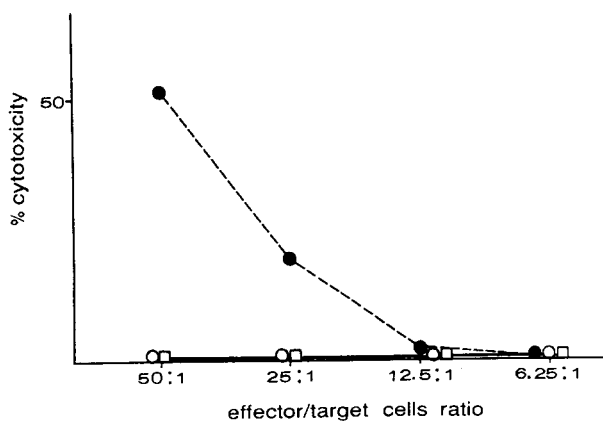


Fig. 2 Cell mediated lympholysis (CML) assay. ○—○: against donor cells, ●—●: against third-party cells. Recombinant IL-2 (50U/ml) was added to antidonor CML at the induction phase (□—□).

Stimulator cells	IL-2 bioactivity (U/ml)	
	10	20
autologous cells	(1.4)	
donor cells	(11.6)	
third-party cells	(22.1)	

Fig. 3 IL-2 production. Supernatants of the MLR on day 3 were tested their IL-2 activity by bioassay using CTLL-2 cell line. Parentheses represent IL-2 activity calculated by the standard curves using recombinant IL-2.

B7, 15, Cw3, w1, DR4, -) who received her mother's kidney (HLA: A11, 24, B7, w54, Cw3, -, DR4, -) in June, 1982. She was transfused on three occasions with 200 ml of fresh whole blood from the same donor before transplantation (DSTs). Following renal transplantation, the patient received the conventional immunosuppressive therapy consisting of methylprednisolone (MP) and azathioprine (AZP). MP was given at an initial dose of 80 mg/day and tapered to 24 mg/day at 1-month post-transplant. From 6-month after transplantation, MP was given at a dose of 12 mg/day. AZP was given at an initial dose of 100 mg/day (10). No rejection episode occurred during the observation period. At present, she is healthy and medicated with 4 and 8 mg/day MP alternately and 50 mg/day AZP. Her serum creatinine and blood urea nitrogen are 1.1 mg/dl and 14.6 mg/dl, respectively.

As shown in Fig. 1, the peaks of the alloproliferative responses to donor and to third-party cells were both seen on day 7 in the MLR kinetics. Then the relative response of the MLR was calculated with 45.6%, which was smaller than the pre-transplant value (94.5%). In the CML assay, the cytotoxic T lymphocytes (CTL) could not be generated against donor cells, although antidonor CTL was detected with a % cytotoxicity of 39.6% before transplantation. Interestingly, the addition of a high concentration of recombinant IL-2 (50 U/ml) to the CML at the induction phase did not restore the antidonor CTL generation. This unresponsiveness of CTL seems to be donor-antigen specific, since the CTL against third-party cells were induced with % cytotoxicity of 51.5% (Fig. 2). Fig. 3 shows that MLR responder cells stimulated by

donor cells made measurable amounts of IL-2 (11.6 U/ml) after 3 days of culture, which were significantly greater than those produced by the responder cells cultured with autologous cells (1.4 U/ml) but lower than those of the responder cells stimulated by third-party cells (22.1 U/ml).

Taken together, although the alloproliferative response to donor cells was decreased in this recipient, the CTL against donor alloantigens could not be induced. The unresponsiveness of antidonor CTL was not due to IL-2 production, because the addition of exogenous IL-2 to the CML did not restore % cytotoxicity against donor cells and the supernatants of antidonor MLR contained measurable amounts of IL-2. On transplantation tolerance in mice models, Holan *et al.* indicated that in the strain combinations where transplantation tolerance was very stable and easy to induce, transplantation tolerance was difficult to overcome it with IL-2, and the converse was also true (11). Therefore, it might be speculated that donor specific immune hyporesponsiveness was maintained in this patient.

The unresponsiveness of antidonor CTL generation observed in this recipient might be explained by any of three possible mechanisms: a) deletion of CTL precursors against donor alloantigens (12), b) active regulation by suppressor cells (8, 13) or serum immune-inhibiting factors (14), and c) interruption of the IL-2 pathway in antidonor CTL generation (15, 16). To determine which mechanism(s) mediates specific immune hyporesponsiveness in the long-term stable recipient, further studies including limiting dilution assay and IL-2 receptor expression in MLC are required on a greater number of these recipients.

Although the present data were derived from only one patient, these approaches might be useful to better understand clinical transplantation tolerance. It might provide a way to minimize the use of immunosuppressive agents including steroid withdrawal to evaluate the specific immune response in stable recipients precisely and in detail.

References

1. Fabre JW and Morris PJ: The effect of donor strain blood pretreatment on renal allograft rejection in rats. *Transplantation* (1972) **14**, 608-617.
2. Kilshaw PJ, Brent L and Pinto M: Suppressor T cells in mice made unresponsive to H-2 incompatible skin allografts. *Nature* (1975) **244**, 489-491.
3. Slavin S, Reitz B, Bieber CP, Kaplan HS and Strober S: Transplantation tolerance in adult rats using total lymphoid irradiation: Permanent survival of skin, heart and marrow allografts. *J Exp Med* (1978) **147**, 700-709.
4. White DJG and Lim SML: The induction of tolerance by cyclosporine. *Transplantation* (1988) **46**, 118S-121S.
5. Flechner SM, Kerman RH, Buren CTV, Epps L and Kahan BD: The use of cyclosporine in living-related renal transplantation: Donor-specific hyporesponsiveness and steroid withdrawal. *Transplantation* (1984) **38**, 685-691.
6. Reinsmon NL, Kaufman D, Matas A, Sutherland DER, Najarian JS and Bach FH: A new *in vitro* approach to determine acquired tolerance in long-term kidney allograft recipients. *Transplantation* (1990) **50**, 783-790.
7. Golomy É, Persijn G, Blokland E, D'Amario J and Rood JJV: Cell mediated lympholysis studies in renal allograft recipients. *Transplantation* (1981) **31**, 210-217.
8. Seki Y, Sakagami K, Takeuchi H, Tanaka K, Namba A, Fuchimoto S and Orita K: Evidence for donor-specific cell-mediated lympholysis unresponsiveness and suppressor cells in well functioning kidney transplants. *Transplant Proc* (1983) **15**, 2131-2135.
9. Pfeffer PF, Thfosby E and Hirschberg H: Donor-specific decreased cell-mediated cytotoxicity in recipients of well functioning, one HLA haplotype-mismatched kidney allografts. *Transplantation* (1983) **35**, 156-160.
10. Sakagami K, Saito S, Shiozaki S, Fujiwara T, Haisa M, Niguma T, Kusaka S, Uda M, Matsuno T, Takasu S, Yerdel MA, Matsuoka J, Tanaka S and Orita K: Renal transplantation from HLA-haploidentical living-related donors: The effects of donor-specific blood transfusions and different immunosuppressive regimens. *Acta Med Okayama* (1992) **46**, 1-5.
11. Holan V, Lamb JR and Malkovsky M: Immunological tolerance and lymphokines. *Crit Rev Immunol* (1991) **10**, 481-493.
12. Hadley GA, Anderson CB and Mohanakumar T: Selective loss of functional antidonor cytotoxic T cell precursors following donor-specific blood transfusions in long-term renal allograft recipients. *Transplantation* (1992) **54**, 333-337.
13. Charpentier BM, Lang P, Martin B and Fries D: Specific recipient-donor unresponsiveness mediated by a suppressor cell system in human kidney allograft tolerance. *Transplantation* (1982) **33**, 470-477.
14. Miyajima T, Higuchi R, Kashiwabara H, Yokoyama T and Fujimoto S: Anti-idiotypic antibodies in a patient with a functioning renal graft. *Nature* (1980) **283**, 306-308.
15. Dallman MJ, Shino O, Page TH, Wood KJ and Morris PJ: Peripheral tolerance to alloantigens results from altered regulation of the interleukin 2 pathway. *J Exp Med* (1991) **173**, 79-87.
16. Field EH and Becker GC: Blocking of mixed lymphocyte reaction by spleen cells from total lymphoid irradiated mice involves interruption of the IL-2 pathway. *J Immunol* (1992) **148**, 354-359.

Received October 5, 1993; accepted November 5, 1993.