Transpl Int (1994) 7 [Suppl 1]: S629–S633

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Inhibition of IL-2 synthesis by donor-specific suppressor T cells in a renal transplant recipient

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Abstract A study was conducted to elucidate the mechanism of donor-specific Mixed Lymphocyte Reaction (MLR and Cell Mediated Lymphotoxicity (CML) unresponsiveness in a renal transplant recipient with a long-term well-functioning kidney. The peripheral blood lymphocytes (PBL) of the recipient, who had not shown rejection since his transplantation 5 years previously, and those of his mother (donor), his father and two healthy third parties were examined. MLR, CML, semimicro MLR in a double chamber, interleukin-2 (IL-2) synthesis assay and limiting dilution assay were performed. This recipient showed donor-specific MLR and CML unresponsiveness. IL-2 assay showed that the PBL of the recipient produced less IL-2 against the donor than against the father and the third parties. The addition of exogenous recombinant IL-2 (rIL-2; Takeda Co.) to the priming MLR caused a recovery of CML against the donor. A limiting dilution assay indicated that cytotoxic T cell precursor (CTLp) frequencies against the donor and father did not differ. The suppressor assay in a double chamber indicated that the PBL of the recipient stimulated by the donor PBL had a non-specific suppressive effect on MLR, CML and IL-2 synthesis of the PBL across the Major Histocompatibility Complex (MHC) barrier. This suppressive effect was abolished by OKT3 or OKT8 monoclonal antibody and complement. Thus, the recipient had donor-specific suppressor T cells that produced a humoral non-specific suppressive factor only when stimulated by the donor PBL, and this factor suppressed MLR and CML by inhibiting IL-2 synthesis of the PBL.

Key words Renal transplantation
Donor-specific unresponsiveness
Donor-specific suppressor T cells
IL-2

Introduction

In transplant therapy, rejection is still the most serious obstacle to overcome. Many forms of immunosuppressive therapy have been developed, but most of them are non-specific and have many side-effects. The ideal way to control rejection has been considered to be donor-specific immunosuppression. Donor-specific immunological unresponsiveness is often observed in renal transplant recipients with a well-functioning kidney. We rationalized that if the mechanism of donor-specific immunological unresponsiveness in renal transplant recipients could be

clarified, then it might provide some clues to the ideal form of immunosuppression.

Materials and methods

Peripheral blood lymphocytes (PBL) were obtained from the venous blood of a recipient, donor (mother), father and two healthy third parties by the Ficoll gradient method. PBL were frozen and thawed before each assay. The Medium used was 1640 RPMI (GIBCO) supplemented with 20% human AB serum and 1000 U/ml penicillin. Rabbit complement was purchased from Pel-Freez inc. OKT3, 4 and 8 antibodies were from Ortho Diagnostic system inc.

MLR

Effector (1×10^5/100 µl) and stimulator cells (1×10^5/100 µl, 20-Gy irradiated) were plated in a 96-well flat bottom plate in triplicate. The cells were cultured for 6 days in a 5% CO_2 humidified atmosphere. We added 0.5 µ Ci [^{3}H]-Tdr to each well during the last 16 h and harvested on the 6th day.

CML

Effector (1×10^6/ml) and stimulator cells (1×10^6/ml, 20-Gy irradiated) were cultured in a Falcon 3033 tube for 5 days in a 5% CO_2 humidified atmosphere. Target cells were PHA blasts of the donor, father and third parties cells. Target cells were labelled with ^{51}Cr on the 5th day. Effector cells and target cells were incubated in a 96-well round bottom plate for 4 h. We collected 100 µl of supernatant and the release of ^{51}Cr was measured by a gamma counter. CML was evaluated by the percentage specific lysis (PSL). PSL was calculated as follows:

$$\text{PSL} = \frac{(\text{experimental lysis-spontaneous lysis})}{(\text{maximum lysis-spontaneous lysis})} \times 100(\%)$$

Limiting dilution assay for cytotoxic T cell precursor (CTLp) frequency

A graded number of the recipient cells (from 2×104/100 µl to 312/100 µl) were plated in a 96-well flat bottom plate in 24 wells, respectively. T-irradiated stimulator cells (5×10^4/100 µl, 20 Gy) were plated in each well. The plates were incubated for 7 days in a 5% CO_2 humidified atmosphere. ^{51}Cr-labelled target cells (1×10^4/100 µl) were plated in each well. The release of ^{51}Cr was measured and a value exceeding the mean + 3 SD of spontaneous release were considered to be positive. CTLp (Cytotoxic-T-cell-precursor) was calculated from the number in the negative well by a Poisson distribution analysis.

Interleukin-2 (IL-2) assay

The recipient PBL (1×10^6/0.8 ml) were cultured with γ-irradiated (20 Gy) PBL from the donor, father or third party 1 (1×10^6/0.8 ml). The supernatant of the culture was harvested at 72 h. We plated 100 µl of the supernatant in a graded dose (ranging from × 2 to × 128) in a 96-well flat bottom plate and incubated this with CTLL-2 (Cytotoxic-T-lymphocyte, IL_2-dependent) cells (4×10^3/100 µl) for 24 h. [^{3}H]-Tdr (0.5 µ Ci/well, Amersham) was added during the last 6 h of culture. The cellular incorporation of [^{3}H]-Tdr was assessed using a liquid scintillation counter. (Aloka, Tokyo, Japan). The amount of IL-2 was calculated by probit analysis.

IL-2 synthesis, CML and MLR suppression test in a double chamber

The double chamber we used in this experiment (Millipore Milicell) was partitioned by a membrane that permitted only the passage of humoral factors. The cell combinations in the upper chamber were: (a) X-irradiated (7.5 Gy) recipient PBL (5×10^5/150 µl) and γ-irradiated (20 Gy) donor PBL (5×10^5/150 µl), (b) X-irradiated (7.5 Gy) recipient PBL (5×10^5/150 µl) and γ-irradiated (20 Gy) third party 1 PBL (5×10^5/150 µl), (c) X-irradiated (7.5 Gy) recipient PBL (5×10^5/150 µl) and γ-irradiated (20 Gy) father PBL (5×10^5/150 µl) or (d) empty as a control. The cell combination of the lower chamber was third party 1 PBL (5×10^5/300 µl) and γ-irradiated (20 Gy) third party 2 PBL (5×10^5/300 µl). The cells were incubated at 37 °C in a 5% CO_2 humidified atmosphere. For IL-2 assay, the supernatant of the chamber was harvested on the 3rd day. For CML, the PBL in the lower chamber were used as effectors on the 5th day. The target was PHA blasts generated from PBL of the third party 2. CML was assessed by PSL. For MLR, PBL in the lower chamber were pulsed with 0.5 µ Ci of [^{3}H]-TdR 16 h before harvesting on the 6th day.

MLR suppression test with complement-dependent cytotoxicity (CDC)

The recipient PBL (3×10^6/ml) were incubated with OKT3, 4 or 8 antibody (× 40) for 1 h at 24 °C. We added 2.5 ml of rabbit complement to each tube and this was incubated for 1 h at 37 °C. After CDC treatment, the recipient cells were used for IL-2 synthesis assay, and CML and MLR suppression test in the double chamber.

Results

[^{3}H]-TdR incorporation (cpm) values by recipient PBL in MLR stimulated by the donor, father and third party 1 were 798.3 ± 753.3, 9663.3 ± 6028.9 and 19987.6 ± 3470.3, respectively (Table 1). The recipient showed donor-specific MLR unresponsiveness and a normal response against the father and third party 1. PSL of the recipient against the donor, father and third party 1 were 2.6%, 19.0% and 36.4%, respectively. The recipient also showed donor-specific CML unresponsiveness (Table 1). However, the addition of exogenous rIL-2 to the priming MLR caused a recovery of the cytotoxicity against PHA blasts of the donor PBL.

In the limiting dilution assay, CTLp frequencies of the recipient PBL against the donor and father were 1/18746 and 1/14176, respectively. There was no significant difference in CTLp frequency between the donor (mother) and father (Fig. 1).

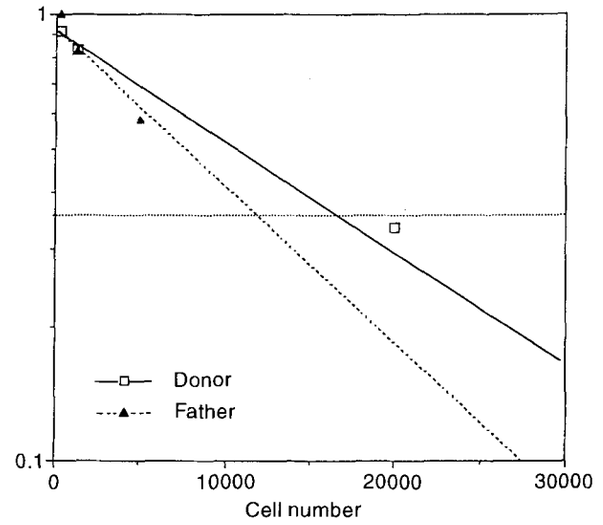

Fig. 1 Cytotoxic T cell precursor (CTLp) frequency of the recipient was measured by a limiting dilution assay. CTLp frequency against the donor (mother) and father were 1/18746 and 1/14176, respectively

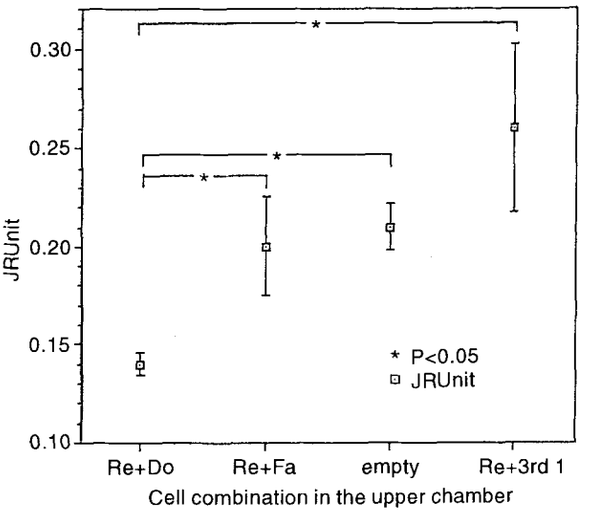

Fig. 2 Interleukin-2 (IL-2) synthesis in a double chamber MLR was measured by CTLL-2 cell after 72 h. When the cell combination in the upper chamber was recipient and donor, IL-2 was statistically lower than with other cell combinations ($P < 0.05$) (*Re* recipient, *Do* donor, *Fa* father, *3rd I* third party I)

Table 1 MLR, CML and IL-2 synthesis of the recipient

Stimulator or target	MLR (cpm ± SD)	CML (%) (E : T = 50 : 1)	IL-2 (JRU/ml)
Donor	798.3 ± 753.3	2.6	0.07
Father	9663.3 ± 6028.9	19.0	0.73
Third party 1	19987.6 ± 3470.3	36.4	1.37
Donor (0.5 JRU/ml IL-2)		17.5	

Table 2 MLR and CML in double chamber (*PSL* percentage specific lysis)

Upper chamber	Lower chamber	MLR (cpm)	% Suppression[a] of MLR	PSL (%)
Recipient + donor	Third party 1 + third party 2	9385	39.5	5.5
Recipient + third party 1	Third party 1 + third party 2	15630	−0.7	14.2
Empty	Third party 1 + third party 2	15514	–	14.1

[a] $\%\ \text{suppression} = \left(1 - \frac{\text{experimental cpm}}{\text{cpm of empty chamber}}\right) \times 100\,(\%)$

IL-2 synthesis of the recipient's PBL against the donor, father and third party 1 was 0.07 JRU/ml, 0.73 JRU/ml and 1.37 JRU/ml, respectively. IL-2 synthesis was specifically donor suppressed. MLR using the cells in the lower chamber was suppressed only when the cell combination in the upper chamber was PBL of the recipient and donor. When the cell combinations in the upper chamber were (a) recipient and donor, (b) recipient and third party 1 or (d) empty upper chamber, the [^{3}H]-TdR incorporation (cpm) value by PBL in the lower chamber was 9560, 15792 and 15676, respectively (Table 2). CML was also suppressed only when the cell combination in the upper chamber was recipient and donor. When the cell combination in the upper chamber was (a), (b) or (d), the PSL against target cells was 5.5%, 14.2% and 14.1%, respectively (Table 2). IL-2 synthesis of cells in the lower chamber with the cell combination (a), (b), (c) and (d) was 0.14 ± 0.006 JRU/ml, 0.26 ± 0.042 JRU/ml, 0.20 ± 0.025 JRU/ml and 0.21 ± 0.012 JRU/ml, respectively (Fig. 2). IL-2 synthesis was also suppressed when the cell combination was (a) recipient and donor. When the recipient PBL in the upper

Table 3 MLR in double chamber with complement-dependent cytotoxicity (CDC)

Upper chamber	Lower chamber	MLR (cpm)	% suppression[a] of MLR
Recipient + donor	Third party 1 + third party 2	11 949	66.3
Recipient + third party 1	Third party 1 + third party 2	29 429	17.0
Empty	Third party 1 + third party 2	35 473	–
Recipient (OKT3 + complement) + donor	Third party 1 + third party 2	37 728	−6.4
Recipient (OKT8 + complement) + donor	Third party 1 + third party 2	35 069	1.1
Recipient (complement) + donor	Third party 1 + third party 2	27 633	22.1

[a] $\%\ \text{suppression} = \left(1 - \frac{\text{experimental cpm}}{\text{cpm of empty chamber}}\right) \times 100\,(\%)$

Table 4 MLR in double chamber with CDC

Upper chamber	Lower chamber	MLR (cpm)	% suppression[a] of MLR
Recipient + donor	Third party 1 + third party 2	37 249	35.6
Empty	Third party 1 + third party 2	–	–
Recipient (OKT4 + complement) + donor	Third party 1 + third party 2	35 113	39.3
Recipient (complement) + donor	Third party 1 + third party 2	39 986	30.8

[a] $\%\ \text{suppression} = \left(1 - \frac{\text{experimental cpm}}{\text{cpm of empty chamber}}\right) \times 100\,(\%)$

chamber were treated with complement and OKT3 or OKT8 antibody, the suppression of [^{3}H]-TdR incorporation (cpm) by the PBL in the lower chamber was abolished (Table 3). However, when the recipient's PBL were treated with complement and OKT4 antibody, or with complement alone, suppression of [^{3}H]-TdR incorporation was not abolished (Table 4).

Discussion

Donor-specific immunological unresponsiveness occurs in renal transplant recipients with well-functioning kidneys. Clonal deletion of CTLp against the donor [1], anti-idiotype antibody [3], clonal energy of T cells and donor-specific suppressor cells [2, 5] have been implicated as the causes of donor-specific unresponsiveness, but the mechanism for this is not clear. To elucidate the mechanism, we carried out the experiments described above. Our recipient showed donor-specific MLR and CML unresponsiveness. However, the addition of exogenous rIL-2 to the priming MLC caused a recovery of the cytotoxicity against the donor PBL. A limiting dilution assay showed that the CTLp frequency of the recipient PBL against the donor (mother) and the father did not differ significantly. The possibility of clonal deletion in this recipient was ruled out. In the double chamber assay, MLR, CML and IL-2 synthesis was suppressed only when the cell combination in the upper chamber was recipient and donor PBL. When the recipient PBL in the upper chamber were treated with complement and OKT3 or OKT8 antibody, suppression of MLR in the lower chamber was abolished, but complement and OKT4 antibody did not abolish MLR suppression in the lower chamber. These results indicated that the recipient PBL had donor-specific CD8-positive suppressor T cells and that these T cells produced a non-MHC-restricted IL-2 synthesis suppressive humoral factor only when stimulated by the donor PBL. Continuous contact with alloantigen has been shown necessary to induce alloantigen-specific suppressor T cells [6] and maintain alloantigen-specific unresponsiveness [4]. A similar mechanism may possibly induce this MHC non-restricted IL-2 suppressive humoral factor producing suppressor T cells.

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