Urol Res (2000) 28:69–74

ORIGINAL PAPER

H. Harada · H. Ishikura · I. Nakagawa · J. Shindou
M. Murakami · T. Uede · T. Koyanagi · T. Yoshiki

Abortive alloantigen presentation by donor dendritic cells leads to donor-specific tolerance: a study with a preoperative CTLA4Ig inoculation

Received: 12 June 1999 / Accepted: 1 October 1999

Abstract Donor dendritic cells (DCs) within allografts initiate the induction of an allospecific T cell response, while an abortive alloantigen presentation by DCs may induce allospecific unresponsiveness. We thus investigated the tolerogenic effect of donor DCs that were made incompetent in alloantigen presentation by treatment of CTLA4Ig. When we treated rats with donor DCs (2×10^6/rat i.v.) on the preoperative day, nine rejected allografts in an accelerated manner (5.0 ± 2.2 vs. 8.2 ± 1.6 days in the control group). Preoperative inoculation of DCs pulsed with CTLA4Ig, a procedure which suppresses an allogeneic mixed lymphocyte reaction (MLR), also provoked an accelerated rejection (5.6 ± 1.7 days). When DCs and CTLA4Ig (500 μg/rat i.p. on days −9, −7 and −5) were concomitantly inoculated, allograft survival was significantly prolonged (>38.7 ± 40.0 days); a preoperative CTLA4Ig inoculation alone failed to do so (7.5 ± 1.2 days). Long-term graft survivors tolerated skin grafts from the donor but not from those from a third party. These results indicate that abortive alloantigen presentation by donor DCs, upon which an accessory signal pathway is suppressed by CTLA4Ig, leads to prolonged graft survival and donor-specific tolerance.

Key words Kidney transplantation · Dendritic cell · Antigen presentation · CTLA4Ig · Donor-specific tolerance

H. Harada · J. Shindou
Departments of Urology and Pathology,
Hokkaido University, Sapporo, Japan

H. Ishikura (✉)
Department of Pathology,
Chiba University School of Medicine,
Inohana 1–8-1, Chuo-ku, Chiba, 260–8670, Japan
e-mail: hishik@med.m.chiba-u.ac.jp
Tel.: +81-11-706-5050, Fax: +81-11-706-7825

I. Nakagawa · M. Murakami · T. Uede
Institute of Immunological Science,
Hokkaido University, Sapporo, Japan

T. Yoshiki
Department of Pathology,
Hokkaido University, Sapporo, Japan

T. Koyanagi
Department of Urology,
Hokkaido University, Sapporo, Japan

Introduction

Intravenous administration of donor cells can often induce prolongation of allografts [12], but, in some instances, sensitization to the donor occurs and graft rejection is accelerated [1]. Although the mechanism of this ambiguous effect is unclear, the presence of non-professional antigen presenting cells (APCs) such as B cells in the inoculated cell populations have to be considered. Antigen presentation to T cells through non-professional APC may result in an antigen-specific T cell unresponsiveness, through absent or incomplete interaction between costimulatory signals such as CD80/86 molecules on APCs and CD28 on T cells [13]. On the contrary, APCs such as dendritic cells (DCs) potently and consistently stimulate T cells.

DCs are characterized by the expression of MHC class I, II, and costimulatory molecules [4]. Signals from DCs are indispensable in alloantigen recognition; DCs have been regarded as major passenger leukocytes in engrafted organs and these cells migrate into host lymphoid organs to provoke an allospecific T cell response (passenger cell theory) [6]. Thus, the injection of DCs, not whole blood cells, T cells, B cells, or adherent cells, restored a strong allospecific response to passenger cell-depleted allografts [7].

CTLA4Ig is a recombinant protein composed of the extracellular domain of a counter receptor to CD 80/86 and the Fc portion of IgG. CTLA4Ig has a 20-fold high affinity to its ligand, CD80, compared to that exhibited by CD28, and this protein has successfully prolonged graft survival [2, 11, 16]. Accordingly, if CTLA4Ig is admixed with DCs, the blockade of costimulatory

signals to T cells occurs [9]. As systematic CTLA4Ig administration has been done postoperatively in most of previous studies, where several types of APCs of donor or recipient origin are intermingled in vivo, the following questions have remained unanswered: on which APCs, donor or host, does CTLA4Ig work? And on what type of cells, APCs of DC or non-professional origin or other types of cells, does CTLA4Ig work? We conducted a study, therefore, focusing on the effectiveness of donor DCs with abortive alloantigen presentation, by preoperatively injecting donor DCs in combination with CTLA4Ig.

Material and methods

Animals

Inbred rat strains, LEW/Hkm ($RT1^l$), WKAH/Hkm ($RT1^k$) and ACI/Hkm ($RT1^a$), maintained and fed at the Center for Animal Experimentation, Hokkaido University School of Medicine in accordance with the Guide for Care and Use of Laboratory Animals, Hokkaido University School of Medicine, 1988 were used. Only males (200–300 g) were used in the present study. Principles of laboratory animal care (NIH publication No. 86-23, revised 1984) were also followed.

Reagents

Murine CTLA4-human IgG (CTLA4Ig) hybrid protein was produced, using the E1- and E3-deleted recombinant adenovirus vector, Adex1CACTLA4IgG [10]. Briefly, recombinant CTLA4Ig proteins were obtained from culture supernatants of CTLA4Ig gene-transfected COS7 cells, using protein A-sepharose 4B conjugate (Zymed, San Francisco, Calif., USA). Purified human IgG (hIgG) (O.E.M. Concepts Inc., Toms River, N.J., USA) were used as control proteins. The CTLA4Ig and hIgG were diluted in pyrogen-free phosphate-buffered saline (PBS). The OX-6 (anti rat MHC class II), OX-62 (anti-rat dendritic cell), G4.18 (anti-rat CD3) were purchased from PharMingen (San Diego, Calif., USA); RLN-9D3 (anti-rat pan B cells) was purchased from Seikagaku Co. (Tokyo, Japan). Mouse anti-rat intercellular adhesion molecule-1 (ICAM-1) mAb, 1A29, was a gift from Dr. M. Miyasaka, Osaka University School of Medicine [17]. The FITC-conjugated rabbit anti mouse IgG Fc (Cappel, Durham, N.C., USA) and FITC-conjugated anti-human IgG (Cappel) were used as second antibodies in the flow cytometric analysis.

Dendritic cells

The purification of rat DCs was done according to Knight et al. [5] and Havenith et al. [3], but with some modifications. Briefly, spleen cells of LEW rats were prepared by incubating minced spleen with 150 U/ml collagenase (Wako Pure Chemical Industries, Osaka, Japan) and 10 U/ml DNAse (Promaga, Madison, Wis. USA) in RPMI-1640 (GIBCO, Grand Island, N.Y., USA), containing 10% heat-inactivated fetal bovine serum (FBS) (JRH Bioscience, Lenexa, Kan., USA). The spleen cell suspension was cultured overnight on 100 mm^2-tissue culture plastic dishes at the concentration of 1×10^8 cells/dish in 5×10^5 M 2-mercaptoethnol (Wako) in RPMI-1640 medium with 10% FBS. The non-adherent cells were collected to put on the 14.5% Nycodenz (Nycomed, Oslo, Norway) gradient. After centrifugation for 25 min at 600 g at room temperature, cells were collected from the interphase. These low density cells were composed of dendritic cells (70–80%), macrophages (20–30%) and B cells ($< 5\%$) (unpublished observation). To eliminate macrophages and B cells, low density cells were resuspended in Hanks' salt solution containing 3% bovine serum albumin then incubated in a 100 mm^2 plastic dish for 60 min at 37 °C according to Severson et al. [14]. Non-adherent cells, with a viability exceeding 95% were used as freshly isolated DCs.

Flow cytometry

Freshly isolated DCs were subjected to analysis using a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif., USA). DCs were first incubated with either OX-6, CTLA4Ig, 1A29, G4.18, RLN-9D3 or OX-62, followed by FITC-labeled isotype-specific second antibodies. Prior to the scanning, cells were stained with propidum iodide (Sigma, St. Louis, MO, USA), then, positive cells were gated out as dead cells.

Pulsing dendritic cells with CTLA4Ig

The LEW DCs were incubated for 60 min on ice with variable concentrations of CTLA4Ig. To eliminate excess CTLA4Ig, the DC preparation was washed four times with PBS. Human IgG was used for the isotype-matched controls.

Mixed lymphocyte reaction (MLR)

The LEW DCs, spleen cells (SPCs) and lymphnode cells (LNCs) were used as stimulators. The WKAH (syngeneic), LEW and ACI (allogeneic) LNCs were used as responders. The LEW DCs pulsed with CTLA4Ig ex vivo were used as stimulators in one experiment. Responder cells were added at 1×10^5/well. Stimulator cells, 1.25×10^4 to 2×10^5/well in cases of SPC and LNC, and 1.25×10^3 to 2×10^4/well in case of DC, were treated with 25 mg/ml of mitomycin C, and mixed with responder cells. MLR was done in a 96-well microtiter culture plate in RPMI-1640, containing 10% FBS (JRH Biosciences Lenexa, Kan., USA) and 5×10^5 M 2-mercaptoethanol (Wako Pure Chemical Industries Osaka, Japan) for 96 h in a humidified 5% CO_2 atmosphere at 37 °C. Various concentrations of CTLA4Ig were added to the medium in a separate experiment. Then, [methyl-^{3}H]thymidine, 0.5 μm Ci (Amersham Life Science, Bucks, UK), was added to each well 16 h before the cells were harvested, with a cell harvester. Radioactivity was measured in a liquid scintillation counter. All experiments were done in triplicate.

Experimental design

Seven experimental groups were prepared to test the hypothesis that donor DCs are required in preoperative CTLA4Ig-induced graft prolongation. In Group I, the LEW kidney was transplanted to recipient WKAH, without any further treatment. In Group II, CTLA4Ig at 500 μg/rat was administered i.p. on 9, 7 and 5 days prior to transplantation (days −9, −7 and −5). In Group III, donor DCs (2×10^6/rat) were given i.v. on day −9. In Group IV, donor DCs pulsed with 500 μg/ml of CTLA4Ig were given i.v. on day −9. In Group V, donor DCs pulsed with human IgG were given i.v. on day −9. In Group VI, donor DCs (2×10^6/rat) were administered i.v. on day −9, and i.p. administration of CTLA4Ig was given on days −9, −7 and −5. In Group VII, donor DCs (2×10^6/rat) were administered i.v. on day −9, and human IgG was administered i.p. on days −9, −7 and −5.

Rat renal transplantation

Allogeneic renal transplantation in the rat was done as described [8] but with some modifications, in the combination of LEW and WKAH as donors and recipients, respectively. Native kidneys in the recipients were removed, and graft survival time was evaluated. Animals who died within the first 24 h were regarded as a technical failure and excluded from the study. Serial graft biopsy was done for histological analysis in randomly selected long-survivors in Group VI.

Rat skin transplantation

In Group VI, in which long term-survivors were present, skin transplantation was carried out to determine if the graft acceptance was donor-specific. Tail skin tissues, 10 mm in diameter, from three rat strains (LEW, allogeneic donor; WKAH, syngeneic, and ACI, allogeneic third-party) were transplanted to the dorsum of long-surviving WKAH rats. For each recipient, two pieces of skin tissue from a donor strain were transplanted. Thus, a recipient had a total of six skin grafts on the dorsum. The graft was considered to be rejected at the time of complete necrosis.

Statistics

Graft and MLR survival were statistically evaluated using Student's *t*-test. A $P < 0.05$ value was considered to have statistical significance.

Results

Phenotype and allostimulatory activity of dendritic cells

They strongly expressed MHC class II, CD 80/86, ICAM-1 and rat dendritic cell marker OX-62 antigens (Fig. 1). A small number of B cells were present. Dendritic cells were much more potent in inducing primary allogeneic responses in MLR than were LNCs or SPCs; the scintillation counts were approximately 20 times higher in DCs than in LNCs or SPCs (Fig. 2). Dendritic cells also stimulated autologous MLR. These data strongly suggest that splenic non-adherent cells of low density prepared in this study were indeed CD80/86-positive DCs, with strong allostimulatory activity.

Suppression of allogeneic mixed lymphocyte reaction by CTLA4Ig

Next we examined the suppressive effect of purified CTLA4Ig in allogeneic MLR. A MLR was carried out using the combination of mitomycin C-treated LEW DCs (2×10^4/well) as stimulators and WKAH LNCs (1×10^5/well) as responders. The CTLA4Ig or control human IgG was added at the beginning of co-culture, at final concentrations of 0 to 500 μg/ml. The CTLA4Ig in the culture media suppressed MLR activity, in a dose-dependent manner (Fig. 3a). Stimulator DCs were coated with CTLA4Ig and MLR was then carried out. Allostimulatory activity of DCs was reduced by coating with CTLA4Ig, in a dose-dependent manner (Fig. 3b).

Acceleration of allograft rejection by pre-injection of donor DCs and rescue of accelerated rejection by addition of CTLA4Ig (Table 1)

Allografts in Group I were rejected within 8.2 ± 1.6 days. In Group II, we inoculated CTLA4Ig preoperatively to act as controls for Group VI, resulting in an unaltered graft survival (7.5 ± 1.2 day). We next asked if preoperative transfusion of donor DCs would accelerate graft rejection (Group III); graft survival was significantly shortened (5.0 ± 2.2 days), when compared with that in Group I. When donor DCs coated with CTLA4Ig were transfused preoperatively (Group IV), allograft survival remained shortened (5.6 ± 1.7 days), as it did in the control allografts in Group V (4.0 ± 1.1 days). A significantly prolonged survival time, >38.7 ± 40.0 days, occurred in Group VI, where a combined preoperative administration of donor DCs and CTLA4Ig was given. The graft prolongation was specific for the combination of DC and CTLA4Ig, because another combination, DC and human IgG (Group VII), did not exhibit this effect (5.3 ± 4.1 days).

Allogeneic skin transplantation to long-surviving, renal allograft recipients (Table 2)

Skin transplantation was done to the long-surviving recipients in Group VI. Three recipient rats (WKAH)

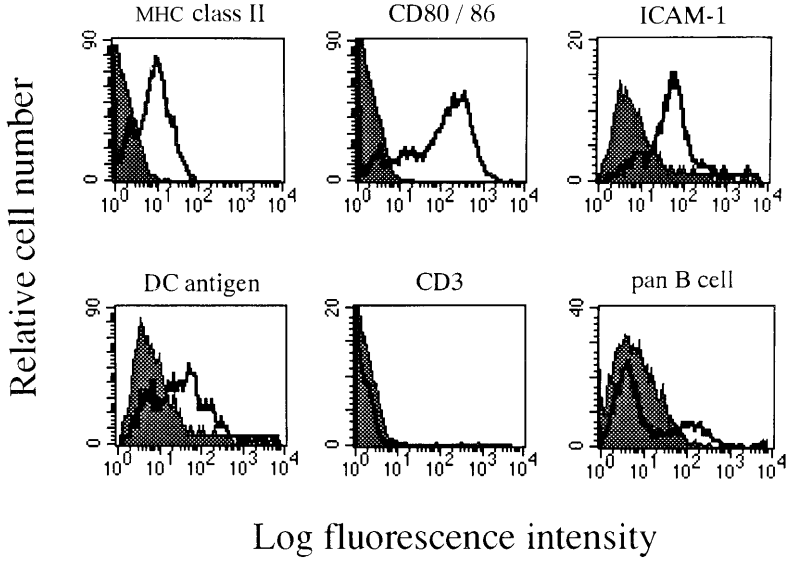

Fig. 1 Surface antigen profile of purified dendritic cells (*DCs*) from the spleen of the rat. This is representative of three independent experiments

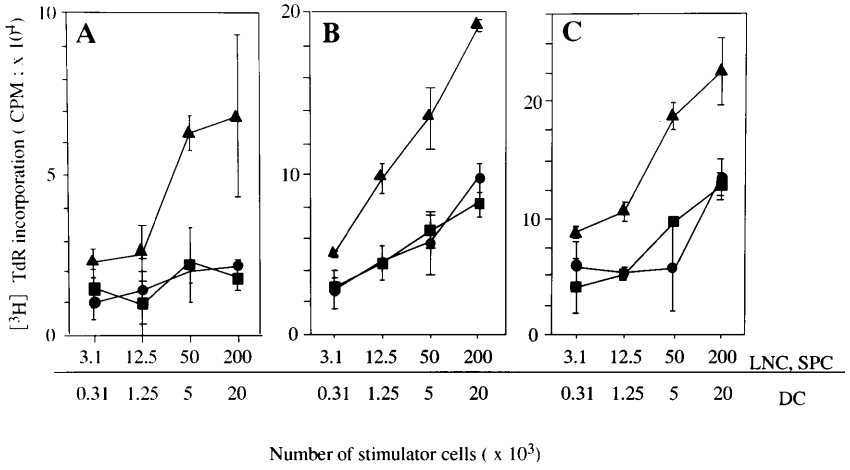

Fig. 2A–C Stimulatory activity of dendritic cells (DCs) to autologous and allogeneic T cells detected by the mixed lymphocyte reaction (MLR). *Filled triangles*, mitomycin C-treated DCs; *filled circles*, lymphnode cells (*LNC*) and *filled squares*, spleen cells (*SPC*) of LEW origin were stimulatory cells. Responder cells were LNCs from (**A**) LEW syngeneic, (**B**) WKAH allogeneic, and (**C**) ACI allogeneic third-party

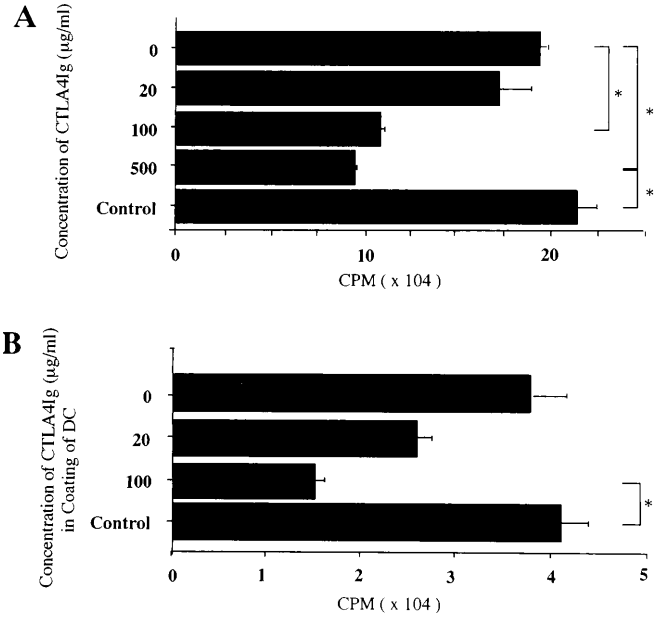

Fig. 3 **A** Effect of CTLA4Ig on *LEW*-to-*WKAH* mixed lymphocyte reaction (MLR). Purified CTLA4Ig at various concentrations were added to the medium at the beginning of MLR, remaining mixed throughout the reaction. **B** Purified CTLA4Ig at various concentrations were added to *DC* suspension for 60 min, then washed four times with phosphate buffered saline, followed by MLR

were given skin allografts. Skin donors were WKAH as syngeneic control, LEW as experimental allogeneic, and ACI as third-party control. All recipient rats accepted LEW allografts for the 50-day observation period. All third party skin allografts were rejected within 15 days. In a naive WKAH recipient, LEW skin was vigorously rejected. These results support that donor-specific tolerance was induced in rats in Group VI.

Histologic findings of allografts from long-term survivors

Biopsy of renal grafts was done from long-term survivors (> 100 days) in Group VI. Structures of glomeruli and renal tubules were well preserved, with arteries and arterioles being fairly well intact. Collections of small numbers of inflammatory cells, mostly lymphocytes, were present in the interstitium.

Discussion

Dendritic cells prepared in this study, expressed MHC class II, ICAM-1, OX-62, and, most importantly, CD80/86 antigens. They strongly stimulated allogeneic, and even autologous, MLR. As detected by flow cytometry, CTLA4Ig binds onto the surface of DCs, through the interaction with CD80/86 costimulatory molecules. The striking allostimulatory activity of DCs injected preoperatively was also shown in the form of accelerated graft rejection. The CTLA4Ig suppressed the DC-induced MLR, when added to culture media, or when pulsed onto DCs in a dose-dependent manner. However, preoperative injection of DCs pulsed with CTLA4Ig did not prolong rejection (Group IV), which indicates an unaltered allostimulatory activity. This could be due to detachment of, or internalization of a significant proportion of CTLA4Ig in vivo, or to newly synthesized CD80/86 molecules that may be present on the surface of DCs in vivo. Steurer et al. showed that ex vivo coating of islet cell allograft with CTLA4/Fc induces graft acceptance [15]. This may relate to the cells used in the two studies; islet cells and DCs have a strikingly different capability for activating T cells. This is possibly due to the differential density and the nature of costimulatory molecules. The present data indicated that concomitant inoculation of DCs and CTLA4Ig should be chosen instead of DCs pulsed with CTLA4Ig.

The most important observation in this study is that the strong allostimulatory action of preoperatively injected DCs is abrogated by the preoperative i.p. administration of CTLA4Ig. This procedure even led to long-term survivors. The suppression is specifically seen only in the combination of preoperative DCs and CTLA4Ig (Group VI), but not in preoperative DCs (Groups III, V and VII), or preoperative CTLA4Ig

Table 1 Renal allograft survival with preoperative administration of donor dendritic cells (DCs) in combination with CTLA4Ig[a]

Group	Treatment	Graft survival (days)	Mean survival (days)
I	None	6, 7, 8, 8, 10, 10	8.2 ± 1.6
II	Pre-transplant CTLA4Ig	6, 6, 8, 8, 8, 9	7.5 ± 1.2
III	DCs	3, 3, 3, 4, 5, 5, 6, 6	5.0 ± 2.2*
IV	DCs coated with CTLA4Ig	3, 3, 5, 5, 5, 6, 7, 7, 7, 8	5.6 ± 1.7
V	DCs coated with hIgG	3, 3, 4, 4, 4, 6	4.0 ± 1.1
VI	DCs and pre-transplant CTLA4Ig	4, 4, 4, 10, 15, 16, 21, 30, >60, >100, >100, >100	>38.7 ± 40.0**
VII	DCs and pre-transplant hIgG	3, 3, 3, 4, 5, 5, 5, 5	5.3 ± 4.1

[a] CTLA4Ig 500 μg/ml was given intraperitoneally on days −9 (9 days before renal transplantation), −7 and −5 in Groups II and VI. Human IgG (hIgG) at 500 μg/ml was given intraperitoneally on days −9, −7 and −5 in Group VII
Dendritic cells of the donor strain were intravenously injected on day −9 in Groups III, IV, V, VI and VII. In addition, DCs inoculated in Groups IV and V were coated with either CTLA4Ig or hIgG
*$P < 0.01$ vs. Group I
**$P = 0.085$ vs. Group I, $P < 0.02$ vs. Group III

Table 2 Skin graft survival in long-term renal allograft survivors. Skin tissues from WKAH (syngeneic), LEW (allogeneic, experimental), and ACI (allogeneic, third-party) were transplanted to long-term survivors in Group VI ($n = 3$). Two pieces of skin tissues were transplanted from respective donor strains, resulting in six skin patches on the dorsum. Complete necrosis of graft was defined as rejection. Observation of the grafts stopped on day 50

Recipients of skin graft	Graft survival (days)		
	Skin graft from WKAH	Skin graft from LEW	Skin graft from ACI
Rat #1 in Group VI	>50, >50	>50, >50	12, 13
Rat #2 in Group VI	>50, >50	>50, >50	14, 14
Rat #3 in Group VI	>50, >50	>50, >50	13, 14
Naive WKAH rat	>50, >50	12, 16	13, 13

alone (Group II). Resultant unresponsiveness is donor-specific in that long-term survivors accepted skin grafts from the donor strain (LEW) but not from the third party strain (ACI). The mechanism of induction of donor-specific unresponsiveness, therefore, appears to be an incomplete alloantigen presentation by DCs to alloreactive T cells.

Four rats in Group VI failed to prolong survival, three of which exhibited accelerated graft rejection. Masking of CD80/86 on DCs by CTLA4Ig may not work, or preparation of DCs may fail. Allogeneic DCs inoculated in these rats might not be efficiently coated with CTLA4Ig in vivo; the dose of CTLA4Ig, 500 μg/ml i.p., could not be enough to reproduce complete in vivo masking. Conversely, we decline to postulate that preparation of DCs failed, because, provided that DCs inoculated in these rats were not viable, the grafts could not have been acceleratedly rejected. Instead, level of graft survival should be similar to that for Group II.

This study clearly demonstrated the critical importance of donor cells, especially donor DCs, in the induction of CTLA4Ig-mediated graft acceptance. The requirement for donor DCs in the effectiveness of preoperative inoculation of CTLA4Ig, strongly supports the concept that blockade of CD80/86 molecules on professional APC generates abortive alloantigen presentation, leading to donor-specific unresponsiveness.

Acknowledgements We are grateful to Ms. C. Sudo, Ms. M. Tateyama and Ms. Y. Furuno for technical assistance.

References

1. Burlingham WJ, Grailer A, Sondel PM, Sollinger HW (1988) Improved renal allograft survival following donor-specific transfusions. Transplantation 45:127
2. Baliga P, Chavin KD, Qin L, Woodward J, Lin J, Linsley PS, Bromberg JS (1994) CTLA4Ig prolongs allograft survival while suppressing cell-mediated immunity. Transplantation 58:1082
3. Havenith CEG, Breedijk AJ, Verdaasdonk MAM, Kamperdijk EWA, Beelen RHJ (1992) An improved and rapid method for the isolation of rat lymph node or spleen T lymphocytes for T cell proliferation assays. J Immunol Met 153:73
4. Klinkert WEF (1990) Lymphoid dendritic accessory cells of the rat. Immunol Rev 117:103
5. Knight SC, Farrant J, Bryant A, Edwards AJ, Burman S, Lever A, Clarke J, Webster AD (1986) Non-adherent, low density cells from human peripheral blood contain dendritic cells and monocytes, both with veiled morphology. Immunology 57:1986
6. Larsen CP, Morris PJ, Austyn JM (1990) Migration of dendritic leukocytes from cardiac allografts into host spleens: a novel pathway for initiation of rejection. J Exp Med 171:307
7. Lechler R. Batchelor JR (1982) Restoration of immunogenicity to passenger cell-depleted kidney allografts by the addition of donor strain dendritic cells. J Exp Med 155:31
8. Lee S (1967) An improved technique of renal transplantation in the rat. Surgery 61:771
9. Linsley PS, Wallace PM, Johnson J, Gibson MG, Greene JL, Ledbetter JA, Singh C, Tepper MA (1992) Immunosuppression in vivo by a soluble form of the CTLA-4 T cell activation molecule. Science 257:792
10. Nakagawa I, Murakami M, Ijima K, Chikuma S, Saito I, Kanegae Y, Ishikura H, Yoshiki T, Okamaoto H, Kitabatake A, Uede T (1998) Persistent and secondary adenovirus-mediated hepatic gene expression using adenovirus vector containing CTLA4IgG. Hum Gene Ther 9:1739

11. Pearson TC, Alexander DZ, Winn KJ, Linsley PS, Lowry RP, Larsen CP (1994) Transplantation tolerance induced by CTLA4-Ig. Transplantation 57:1701
12. Salvatierra O, Vincenti F, Amend W, Potter D, Iwaki Y, Opelz G, Terasaki P, Duca R, Cochrum K, Hanes D, Stoney RJ, Feduska NJ (1980) Deliberate donor-specific transfusions prior to living related renal transplantation: a new approach. Ann Surg 192:543
13. Schwartz RH (1992) Costimulation of T lymphocytes: the role of CD 28, CTLA-4, and B7/BB1 in interleukin-2 production and immunotherapy. Cell 71:1065
14. Severson CD, Burg DL, Lafrenz DE, Feldbush TL (1987) An alternative method of panning for rat B lymphocytes. Immunol Let 15:291
15. Steurer W. Nickerson PW, Steele AW, Steiger J, Zheng XX, Strom TB (1995) Ex vivo coating of islet cell allografts with murine CTLA4/Fc promotes graft tolerance. J Immunol 155:1165
16. Sun H, Subbotin V, Chen C, Aitouche A, Valdivia LA, Sayegh MH, Linsley PS, Fung JJ, Starzl TE, Rao AS (1997) Prevention of chronic rejection in mouse aortic allografts by combined treatment with CTLA4-Ig and anti-CD40 ligand monoclonal antibody. Transplantation 64:1838
17. Tamatani T, Miyasaka M (1990) Identification of monoclonal antibodies reactive with the rat homolog of ICAM-1, and evidence for a differential involvement of ICAM-1 in the adherence of resting versus activated lymphocytes to high endothelial cells. Int Immunol 2:165

ANNOUNCEMENTS

2000

2000 Duke Urologic Assembly
14–19 March 2000, Cancun, Mexico

Subject: Contemporary Issues in Urology
Information: Linda Mace, Assembly Coordinator, Box 3707, Duke Medical Center, Durham, NC 27710, USA; Tel.: 919-684-2033, Fax 919-684-4611

WHO Consensus Conference:
Public Health and Clinical Significance of Premalignant Alterations in the Genitourinary Tract
June 8–9 2000, Stochholm, Sweden

Information: Prof. Lennart Andersson, WHO Collaborating Center for Urologic Tumors, Karolinska Hospital, SE-171 76 Stockholm, Sweden; Fax +46-8-32 61 13, E-mail: Lennart.Andersson@kirurgi.ki.se

Urology 2000:
State of art-looking at the future
14–15 June 2000, Lipari, Eolian Islands

Topics: Vaginal and abdominal prolapse repairs, rationale for urologic cancer lymphadenectomy, future perspectives in reconstructive surgery using tissue engineering, bladder overactivity, the ageing male: a challenge for urologist, how to manage male incontinence, erectile dysfunction: present and future, continent urinary diversion, prostate cancer, gene therapy for urological cancer, endourology, laparascopy, reconstructive urology, paediatric urology, urodynamics and BPH, urodynamics and female incontinence, superficial bladder cancer, invasive bladder cancer, kidney cancer.
Information: Prof. Carlo Aragona, Chairman and director of the Course, Unit Operativa di Urologia, Azienda Ospedallera Papardo, Contrada Papardo, 98158 Messina, Italy; Tel: + +39 090 3992491, + +39 090 3992281, Fax: + +39 090 3992284, e-mail: carlo.aragona@mail.net.it or to Organising Secretariat, Progetto GEA s.r.l., Serenella La Cavera, Via F.P. Di Blasi, 1, 90144 Palermo, Italy; Tel: + +39 091 6262660, + +39 091 6264517, Fax: + +39 091 303937, e-mail: progettogea@manol.com

28th Munich Endourological Symposium with Nursing Staff Seminar
5–6 October 2000, Munich, Germany

Chairman: Prof. Dr. R. Hartung,
Information: Dr. R. Paul, Department of Urology, Technische Universität München, Klinikum rechts der Isar, Ismaninger Str. 22, 81675 München, Tel: 089/41402507, Fax: 089/41402585, e-mail: mriu@lrz.tu-muenchen.de, Internet: http://www.med.tu-muenchen.de/uro/endo.html