Characterization of the Response of Dendritic Cells and Regulatory T Cells to Tumor Antigens in Patients with Renal Cell Carcinoma

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Background: This study characterized dendritic cells (DCs), regulatory T cells (Tregs) and the immune responses to tumor antigens in renal cell carcinoma (RCC) patients.

Methods: Thirty patients with RCC and five healthy donors were studied. DCs were generated from the adherent cells among peripheral blood mononuclear cells (PBMCs), then cultured in medium containing granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 for 7 days. The phenotypes of the DCs and Tregs were analyzed by flow cytometry. A mixed lymphocyte reaction (MLR) was performed to assess the functioning of the DCs and Tregs. A cytotoxic assay was performed to measure the antigen presentation ability of the DCs from the RCC patients (RCC-DCs). These DCs were pre-treated with TNF-α (TNF-DCs) or tumor lysate (TuLy-DCs) on the 3rd day of DC culture.

Results: The RCC-DCs expressed significantly less CD40 ($p = 0.03$) and CD80 ($p = 0.007$) upon TNF-α cultivation than the DCs from healthy donors. The peripheral Tregs during stage I disease were significantly less ($p = 0.032$) than during stages II-IV. The RCC-DCs were as efficient as DCs from healthy donors ($p = 0.83$) when stimulating the proliferation of allogeneic T cells; however, these RCC-DCs were less efficient when stimulating autologous T cells than allogeneic T cells ($p = 0.023$). Tregs inhibited autologous T cell proliferation rather than allogeneic T cell proliferation in response to TuLy-DCs stimulation. Prostaglandin E_2 did not increase the ability of immature DCs to stimulate T cell proliferation.

Conclusions: Patients with RCC have less potent anti-tumor immune responses.

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Key words: dendritic cells, regulatory T cells, peripheral blood, renal cell carcinoma

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The prognosis of renal cell carcinoma (RCC) is generally very poor. RCC is refractory to either chemotherapy or radiotherapy,[1] and the effects of immunotherapy have been marginal.[2-5] An alternative treatment for patients with RCC is required. Selection of a biological therapy depends on various findings ranging from intact immune surveillance and tumor sensitivity to immune-based destruction. In the development of immunity to tumors, $CD8^+$ cytotoxic T lymphocytes (CTLs) are very important.[6,7] In patients with cancer, CTLs are either not induced or only weakly induced because tumor antigens cannot be presented by the antigen presenting cells (APCs) or because the tumor-reactive T cells are tolerated by the tumor.[4,8,9]

Dendritic cells (DCs) are the most potent APCs. DCs are characterized by several important features: (1) the ability to stimulate a primary T-cell response, (2) cell motility and migration, (3) phagocytotic activity, (4) differential expression of certain DC-associated activation molecules, and (5) cytochemical reactions that are different from those of monocytes and macrophages.[10] Under the stimulation of danger signal such as prostaglandin, lipopolysaccharide, DCs will undergo maturation. The maturity of DCs is critical to the presentation of antigen to cytotoxic T lymphocytes (CTLs). Various DCs markers, including CD40, CD80, CD83, CD86, are highly expressed on mature DCs.

Regulatory T cells (Tregs) have been found to suppress immunity, mainly through the suppression of DCs. In general, CD4 and CD25 are most common markers for Treg,[11] although there are other novel markers, such as FoxP3 and glucocorticoid-induced TNF receptor (GITR).[12-14] $CD4^+CD25^+$ T cells include several subgroups; however, a significant portion of these are Tregs. There is a significant increased frequency of Tregs in RCC patients compared to normal donors.[15] In this study, we tested $CD4^+CD25^+$ Tregs, DCs and CTLs in patients with RCC.

METHODS

Patients and peripheral blood

Thirty patients with RCC and five healthy donors were studied after giving us informed consent. The RCC patients were found to be at different stages at presentation. Surgical specimens were processed for primary culture and the establishing of cell lines. Peri-operative peripheral blood was obtained for the characterization and the functional assay of DCs. The age of the 30 RCC patients ranged from 34 to 79 years. The median age was 59 years. There were 11 males and 19 females and thus the male to female ratio was 1:1.73. Of the 30 cases, there were 20 cases (67%) of the clear cell type, 3 cases (10%) of the papillary type, 4 cases (13%) of the chromophobe type and 3 cases (10%) of other types. Of the 30 cases with complete staging information, the incidence of stages I, II, III and IV was 12 (40%), 5 (16.7%), 1 (3.3%) and 12 (40%), respectively. This study was approved by the Institutional Review Board.

RCC HH244 culture and cell lysate preparation

The RCC cell line, HH224,[16] a gift from Dr. Shanka K. Nayak (Hoag Cancer Center. New Port Beach, CA, U.S.A.) was maintained in Royal Park Memorial Institute (RPMI) medium containing 10% fetal bovine serum (FBS), 1% L-glutamine, 1% sodium pyruvate, and 1% penicillin-streptomycin. Tumor cell lysates were obtained by triple freezing (–80°C for 10 min) and thawing (20°C for 10 min) the single cell suspension of HH244 derived from light trypsinization of the adherent cells followed by centrifugation at 3000 rpm for 10 min; the supernatant was then collected. The protein concentration of the supernatant was quantified by the bicinchoninic acid (BCA) assay (Sigma, St. Louis, MO, U.S.A.).

Peripheral blood mononuclear cell (PBMC) preparation

PBMCs were prepared using Ficoll-Hypaque (Pharmacia, Uppsala, Sweden). Sixty ml of whole blood was drawn from each patient and donor to obtain PBMCs. Cells were cultured in RPMI complete medium (CM) containing 10% FBS to generate DCs or cryopreserved for future use. The PBMCs were also phenotyped using antibodies against CD3, CD4, CD8, and CD25. All antibodies were obtained from Becton, Dickinson and Company (BD) PharMingen. Labeled cells were analyzed by a FACSCalibur flow cytometer and CellQuest software (BD Biosciences, Mountain View, CA, U.S.A.). For each sample, a negative control with isotype-matched control antibody was used to determine positive and negative cell populations. The p values

were determined by the Student's *t*-test.

T cells preparation

PBMCs were prepared using Ficoll-Hypaque and cultured in RPMI CM containing 10% FBS for 2.5 hrs. Following this, floating cells were collected as effector T cells. The purity of the CD3 cells was about 80% (data not shown).

DC culture

In total, 2×10^7 PBMCs were cultured in a T25 flask containing 5 ml of RPMI CM for 2 hrs. Non-adherent cells were removed by gently swirling the flask. Adherent cells were cultured in 5 ml of RPMI CM containing 800 units/ml GM-CSF (Immunex, Seattle, WA, U.S.A.) and 500 units/ml of IL-4 (Biosource, Cararillo, CA, U.S.A.) for 7 days to obtain immature DCs (imDCs). Finally, 1×10^6 imDCs were further co-cultured with TNF-α (50 μg/ml; TNF-DCs), PGE_2 (1 μg/ml, Sigma-Aldrich Corp. St. Louis, MO, U.S.A.; PGE_2-DCs), TNF-α (50 μg/ml) plus PGE_2 (1 μg/ml) (TP-DCs), or 100 μg/ml of tumor lysate proteins (TuLy-DCs) for 72 hrs.

Flow cytometric analysis of DCs

The DCs were dispensed into test tubes (1×10^5 cells/tube) and centrifuged to form a pellet. Fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated antibodies (1 μg/test or per manufacturer instructions) were added and incubated at 4°C in darkness for 30 minutes. The tested antibodies included CD54, CD80, CD83, CD86, and HLA-DR. Mouse anti-human IgG was used as an isotype-matched control.

Isolation of Tregs from peripheral blood

Tregs were isolated using a $CD4^+CD25^+$ Tregs isolation kit (MACS; Miltenyi Biotec, CA, U.S.A.). The $CD4^+CD25^+$ Tregs were isolated in a two-step procedure. First, non-$CD4^+$ cells were indirectly magnetically tagged with a cocktail of biotin-conjugated antibodies and anti-biotin microbeads. The tagged cells were subsequently depleted by separation over a MACS® column. The $CD4^+CD25^+$ T cells were then directly tagged with CD25 microbeads and isolated by positive selection from the pre-enriched $CD4^+$ T cell fraction. The magnetically tagged $CD4^+CD25^+$ T cells were retained on the column and eluted after removing the column from the magnetic field. For maximum purity, the positively selected cell fraction containing the $CD4^+CD25^+$ T cells was separated again over a second column.

Mixed lymphocyte reaction (MLR)

The MLR was performed using a standard method. Allogeneic effector T cells were obtained from several different donors. The data were therefore normalized by the control T cells alone in every set of experiments. Briefly, the MLR were prepared in triplicate in flat-bottom 96-well plates in CM containing 10% FBS. Irradiated (30 Gy) DCs were used as stimulators at concentrations from 2,000-60,000 cells/well, whereas the effector T cells were used at a concentration of 2×10^5 cells/well. Tregs were co-cultured with or without DC-pulsed T cells at a concentration of 2×10^3 cells/well to compare the inhibition of T cell proliferation. The cells were incubated for 5 days in a humidified atmosphere of 5% CO_2 in air at 37°C and pulsed with 1 μCi/well ^{3}H-thymidine (80 mCi nmol21; NET-027Z thymidine, methyl-3H; Dupont, NEN Research Products, Boston, MA, U.S.A.) for 8 hrs before harvesting. Evidence of DNA incorporation of ^{3}H-thymidine, as an index of cell proliferation, was estimated by harvesting the contents of each well onto filters using a Packard Harvester (Packard Instrument Company, Meriden, CT, U.S.A.). After adding 25 μl Microscint 20 (Packard Instrument Company) per well, the plates were counted using a Packard TopCount•NXT. Responses were reported as the mean counts per minute (CPM) of triplicate estimates. To normalize the variation in T cell proliferation, we divided the results of the CPM of each DC-stimulated T cell proliferation (DC-T) by the CPM of T cell proliferation in each condition of the experiments. Therefore, the results are presented as proliferation index (PI). The PI is defined as the CPM ratio of DC-T to T. Proliferation was compared between the DC-stimulated group and the unstimulated group by a paired *t*-test.

Activation of effector T cells

The T cells were activated by TuLy-DCs. The T cells activated by non-pulsed DCs and unstimulated T cells were used as controls. Two $\times 10^6$ T cells were cultured in each well of a 24-well plate with 2 ml of complete medium containing 20 units/ml of IL-2

(R & D Systems, Inc., U.S.A.). The doses of IL-2 were kept low to maintain T cell viability in both the control and experimental groups. Low doses were also considered unlikely to significantly change the proliferation in the experiment. Into each well, 2.5×10^5 DCs were added for 5 days of stimulation. The immune responses of the effector T cells were detected by cytolytic activity.

^{51}Cr release cytotoxicity assay

Since autologous or major histocompatibility complex (MHC)-matched allogeneic RCC cell lines could not be obtained, we therefore used an allogeneic RCC cell line (HH244) for the cytotoxicity assay. HH244 tumor cells were used as targets and incubated with 100 μCi $Na_2{}^{51}CrO_4$ (DuPont) for 2 hrs. Effector T cells were added at an effector: target ratio of 20:1 in triplicate wells of U-bottom plates for 4 hrs. The radioactive content in the supernatant was measured by a γ-counter. Results are reported as the percentage of cytolytic activity.

RESULTS

The percentage of Tregs in PBMC is higher in RCC stages II-IV patients than in normal donors and RCC stage I patients

Thirty RCC patients were studied. From 60 ml of whole blood obtained from each RCC patient, $6 \times 10^7 \pm 5 \times 10^6$ PBMCs and $8.3 \times 10^6 \pm 4 \times 10^5$ of DCs were harvested. There were no significant differences in the yields of PBMCs and DCs between the patients and healthy donors. Upon TNF-α and tumor lysate cultivation, the TNF-DCs and TuLy-DCs showed a typical veiled pattern.

The median percentage of Tregs in patients with stage I cancer (n = 12), stage II cancer (n = 5), stage III cancer (n = 1) and stage IV cancer (n = 12) were 1.9% (95% CI: 1.6~6.6%), 6.4% (95% CI: 2.0~9.5%), 12%, and 6.4% (95% CI: 3.9~9.5%), respectively. The percentage of Tregs in all 30 RCC patients averaged 5.8 ± 4.4% (range, 0.23-15.07%). The percentage of Tregs in healthy donors (n = 5) averaged 2.3 ± 1.5% (range, 0.6-4.7%) (data not shown). There were no differences between the group of patients individually; however, when we compared stage I patients with stage II-IV as a whole, stage I RCC patients had significantly (p = 0.032) fewer $CD4^+CD25^+$ Tregs (Fig. 1).

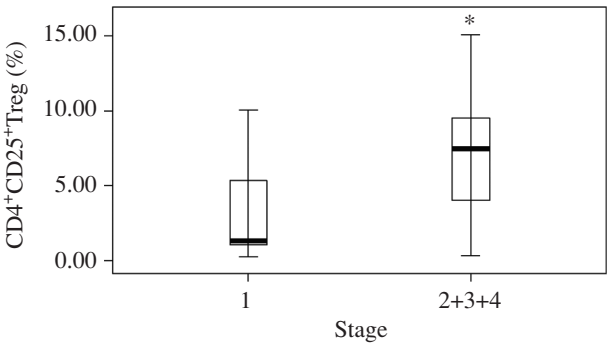

Fig. 1 A small number of $CD4^+CD25^+$ Tregs were detected in the peripheral blood of stage I patients with RCC. The percentage of $CD4^+CD25^+$ Tregs in stage I patients was significantly fewer (p = 0.032) than in stage II-IV patients with RCC. The maximal data is shown as the upper bar, and the minimal data is shown as the lower bar. Data is presented as mean ± standard deviation (95% confidence interval, rectangle).

The DC phenotype is less mature in RCC patients

The imDCs showed little or no expression of CD40, CD80, CD83, CD54, or CD86. When co-cultured with TNF-α, the TNF-DC phenotypes of the RCC patients were as follows: CD40, 55 ± 31%; CD80, 76 ± 15%; CD83, 43 ± 18%; CD54, 95 ± 3%; CD86, 93 ± 5%; and HLA-DR, 97 ± 1%. The DC phenotypes of the healthy donors were as follows: CD40, 83 ± 11%; CD80, 91 ± 2%; CD83, 60 ± 16%; CD54, 95 ± 3%; CD86, 93 ± 5%; and HLA-DR, 96 ± 4%. The RCC expression of CD40 (p = 0.03) and CD80 (p = 0.007) were thus significantly decreased in comparison with healthy donors. A representative case is shown in Fig. 2. The DCs from 25 RCC patients did not express significantly less CD83 (p = 0.09).

T cell proliferation by TNF-DCs and TuLy-DCs

Healthy donors did not differ significantly from RCC patients. Allogeneic MLRs revealed the same T cell stimulatory function in TNF-DCs from 9 RCC patients (14438 ± 4619 CPM) and 4 healthy donors (15143 ± 6766 CPM; p = 0.83). In 5 of 7 patients with RCC, the TuLy-DCs were less efficient in stimulating proliferation of autologous T cells than allogeneic T cells (p = 0.023).

In the experiments involving allogeneic MLR,

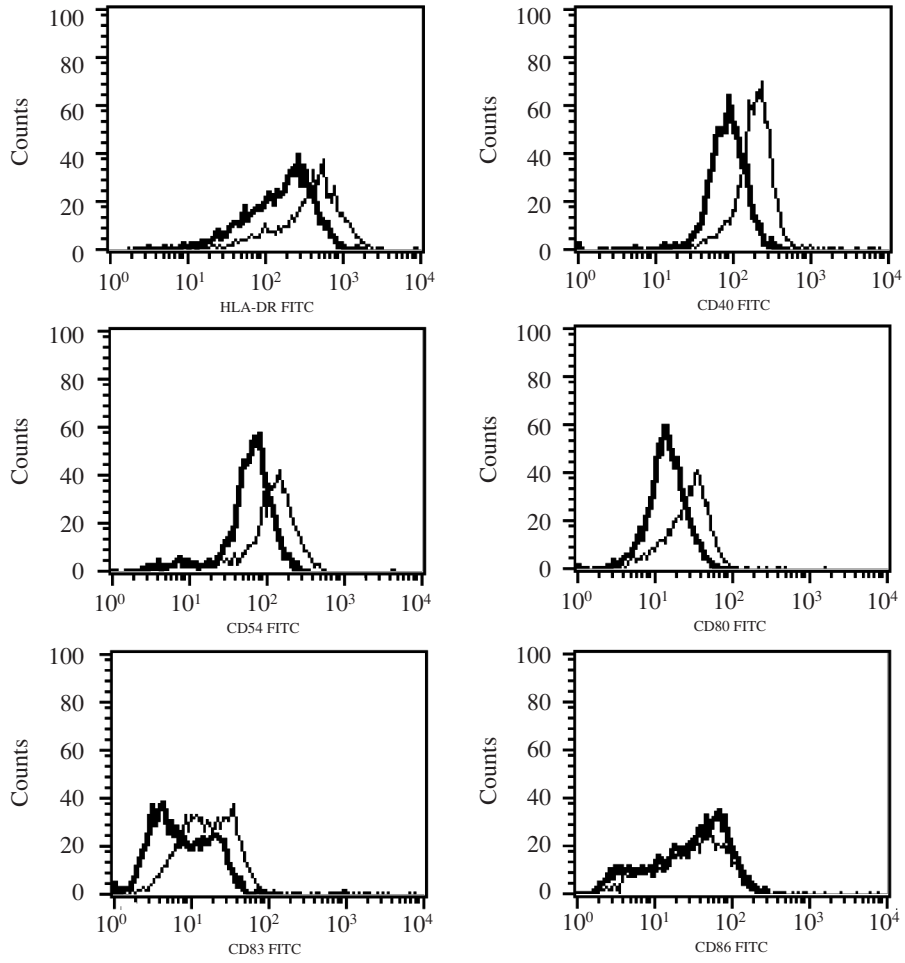

Fig. 2 Representative histograms of HLA-DR, CD40, CD54, CD80, CD83 and CD86 in TNF-DCs obtained from a stage II RCC patient (bold line) and a healthy donor (thin line).

the mean PI of the TNF-DCs (21.37), TuLy-DCs (20.24) and TP-DCs (18.41) were significantly higher than that of the imDCs (13.44) and PGE_2-DCs (13.45), indicating that TuLy-DCs were equally competent to stimulated T cell proliferation as TNF-DCs. Furthermore, PGE_2 had no synergistic or additive effect on TNF-α. In 15 RCC patients, the TNF-DCs had a higher median PI than the imDCs ($p = 0.008$) and PGE_2-DCs ($p = 0.0038$). The TP-DCs revealed a higher median PI than imDCs ($p = 0.026$) and PGE_2-DCs ($p = 0.01$). The TP-DCs did not show a higher median PI than TNF-DCs ($p = 0.9237$). Finally, the PGE_2-DCs did not show a higher median PI than imDCs ($p = 0.8398$) alone, which indicates that there was no additive stimulatory effect of PGE_2 on TNF-α (Fig. 3). Thus, the experimental results revealed no enhancement of stimulatory the function of imDCs by PGE_2.

Tregs inhibited autologous rather than allogeneic T cell proliferation

In 5 of 7 RCC patients, TNF-DCs were able to stimulate both autologous and allogeneic T cells proliferation. However, Tregs was able to inhibit autologous T cell proliferation (Fig. 4A) rather than allogeneic T cell proliferation in response to TuLy-DCs stimulation (Fig. 4B). Immature DCs are thus more potent at stimulating allogeneic rather than autologous T cells. TuLy-DCs were also more potent at stimulating allogeneic rather than autologous T cells.

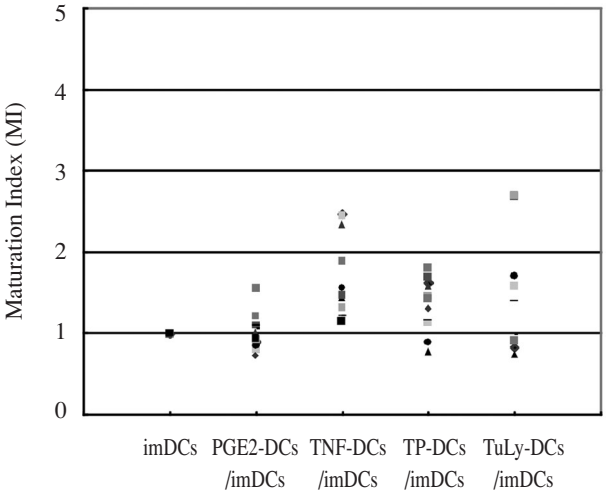

Fig. 3 The ability to stimulate allogeneic T cell proliferation by DCs obtained from medium under various conditions, including the presence of prostaglandin E2 (PGE_2-DCs), TNF-α (TNF-DCs), TNF-α plus prostaglandin E2 (TP-DCs) and tumor lysate (TuLy-DCs). The results of each condition were compared with DCs cultured in complete medium alone (imDCs). The ability to stimulate T cell proliferation was assayed by the mixed lymphocyte reaction (MLR). The maturation index (MI) was defined as the ratio of the ability to stimulate allogeneic T cells proliferation by various DCs compared to imDCs in the MLR experiments. The result demonstrated TNF-DCs, TuLy-DCs and TP-DCs were significantly more able to stimulate T cell proliferation than imDCs and PGE_2-DCs.

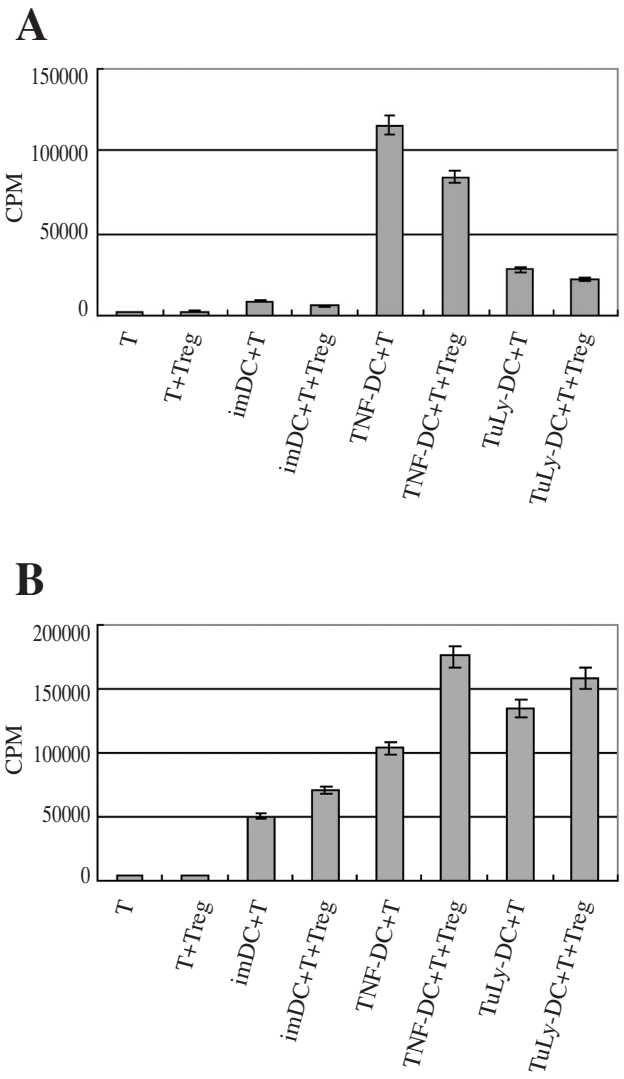

Fig. 4 The ability to stimulate autologous (A) and allogeneic (B) T cell proliferation with and without regulatory T cells (Tregs). T cell proliferation was assayed by the mixed lymphocyte reaction (MLR) and the results are presented as count per minutes (CPM). Several cells were added to stimulate T cell proliferation, including Tregs (T+Treg), immature DCs (T+imDC), imDCs and Tregs (imDC+T+Treg), TNF-α-stimulated DCs (TNF-DC+T), TNF-DCs and Tregs (TNF-TDC+T+Treg), tumor lysate-stimulated DCs (TuLy-DC+T) as well as TuLy-DCs and Tregs (TuLy-DC+T+Treg). Autologous T cell proliferation was significantly decreased in the presence of Tregs (imDC+T versus imDC+T+Treg, TNF-DC+T versus TNF-DC+T+Treg, TuLy-DC+T versus TuLy-DC+T+Treg). In contrast, allogeneic T cell proliferation was increased. The results demonstrated that the Tregs of RCC patients were able to inhibit autologous rather than allogeneic T cell proliferation.

Tregs were not able to inhibit allogeneic T cell proliferation by immature DC or TuLy-DC stimulation.

DCs presented antigens to generate effective CTLs in patients with RCC

RCC-DCs were able to generate both autologous and allogeneic cytotoxic T cells that had cytolytic effects on HH244. Seven RCC patients were studied. The baseline level of T cell cytotoxicity was low (0-20%). When T cells were stimulated by DCs pulsed with tumor lysate of HH244 (TuLy-DC), the T cell cytotoxicity against HH244 was increased significantly by 2-10 fold ($p < 0.001$) (Fig. 5). This *in vitro* study demonstrated that the DCs in these seven RCC patients were still able to generate cytotoxic T cells.

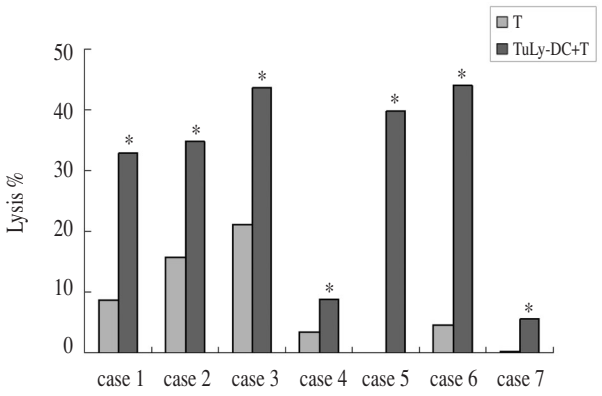

Fig. 5 The cytolytic activity of T cells with and without HH244 tumor lysate-pulsed DCs (TuLy-DC) in seven RCC patients. Cell cytotoxicity activity was performed by the ^{51}Chromiun release assay and the results are presented as the percentage of cell lysis. The cytolytic activity of T cells was significantly increased in the presence of TuLy-DCs in all seven RCC patients. *: $p < 0.05$ compared to T cell alone.

DISCUSSION

RCC accounts for approximately 3% of all adult cancers.[17] Approximately one-third of all RCC patients have metastatic disease at the time of diagnosis.[18] Chemotherapy and radiotherapy have limited value and, although relatively promising, immunotherapy is effective in only 20% of cases.[19] Hence, more effective treatment modalities and more follow-up studies are clearly needed.

Tumor cells might mimic "self," possessing insufficient tumor-associated antigens capable of T-lymphocyte recognition.[20] The downregulation of MHC molecules by those tumors will make the tumor unrecognizable by the immune system, which, together with inadequate numbers and decreased functioning of specific T lymphocytes, may impair the immune response.[20-24]

DCs are known to become dysfunctional in breast cancer, head and neck cancer, and hepatoma, but exhibit their normal stimulatory function in melanoma.[25-28] In the current study, DCs in RCC patients were defective, indicating that the tumors escaped immune surveillance by affecting the DCs. Several reports have shown a Th1 direction and CTLs generation in response to antigen stimulation.[29-36] After several hours of pulsation with tumor antigens, DCs are then able to introduce immunogenic antigens to T cells over several days.[33,34,37] The T cells are then primed and activated to give a specific immunological response. However, the results herein demonstrated a Th2 shift by the TuLy-pulsed DCs. Therefore, tumor lysates may not be the best source for inducing a Th1 response or CTLs generation.

Initial studies of DCs within urinary system tumors utilized immunostaining with antibodies against the S100 antigen. The S100 marker does not reflect the functional maturity of the DCs.[38] Up to the present, no marker specific to all cells of the DCs lineage has been identified. Activated DCs are more readily identifiable by their expression of CD83, p55, and CMRF-44; the expression of B7-1 (CD80) and B7-2 (CD86) is essential for effectively activating T lymphocytes.[39,40] A recent study described small, but significant subpopulations of DCs in human RCC.[41] Mature and functional DCs are characterized by CD83 expression, which presently is the most specific marker for mature DCs other than CMRF-44.[42] Studies of *in situ* DCs have yielded only descriptive evaluations rather than dynamic results.[43] Earlier studies described the correlation of presence of DCs in primary tumors with disease progression and patient survival.[44] The present study includes both descriptive and functional assays of DCs relative to tumor antigens.

To date, with RCC, various vaccines, such as those against host tumor cells and peptides, have yielded a 10-20% response rate. In DCs, tumor lysates have been used to generate cytotoxic T cells. As shown in the experimental results herein, the DCs that are present have a less mature phenotype and decreased T cell stimulatory function in response to TNF-α stimulation. In addition to TNF-α, PGE_2 did not further enhance stimulation of T cell proliferation by DCs. Luft *et al* demonstrated that PGE_2 regulates the migratory capacity of a specific DCs subset.[45] However, PGE_2 has been shown to downregulate DCs function as a consequence of increased IL-10 production.[46] Therefore, whether or not PGE_2 can be incorporated into DC vaccines for patients with metastatic RCC remains to be investigated. Our results indicate that PGE_2 has no synergistic or additive effects on TNF-α, but TuLy-DCs were equally competent to stimulated T cell proliferation as TNF-DCs. We suggest tumor lysate may be more potent

than PGE_2 as a means of stimulating the maturation of DC. In this context, the parameters used for assessing DC activity may be used to monitor treatments, particularly DC-based immunotherapy of RCC patients.

Although $CD4^+CD25^+$ are known to be the markers of Tregs in the mouse system, CD25 (IL-2α receptor) has been found to be expressed on $CD4^+$ non-regulatory T cells under certain condition including, various autoimmune diseases, allergy, chronic infection and established donor-specific transplantation tolerance.[47] In this study, we still chose $CD4^+CD25^+$ as the surface markers of regulatory T cells because all the subjects we studied did not have any such known co-morbidity. Their $CD4^+CD25^+$ T cells were sorted from PBMCs using the MACS magnetic bead cell sorting system. The purity of the $CD4^+CD25^+$ T cells was 85-95%, so these cells might contain both active T cells ($CD4^+CD25^{dim}$) and Tregs ($CD4^+CD25^{high\ or\ bright}$). More specific Treg markers such as FoxP3 and GITR were not available at the time we conducted this study. Clearly, the identification of $FoxP3^+CD4^+CD25^+$ T cells may be an even better way of characterizing the number of Tregs in RCC patients.

Our results indicated that Tregs cells from RCC patients were able to inhibit autologous T cell proliferation rather than allogeneic T cell. Such a difference has also been reported in lung cancer patients.[48] These results suggested that there may be MHC specific inhibition of Tregs in cancer patients.

Having established a number of defects associated with the T cell immune response to tumor antigens in RCC patients, important issues for further consideration are how to repair these defects. Proposed approaches to these issues include activating DCs function using lentivirus containing the GM-CSF gene and activating CD40 in antigen-loaded DCs in order to facilitate their migration into lymph nodes, which is where DCs are able to efficiently undergo T cell priming and expansion, followed by induction of peripheral dissemination of CTLs from the lymph node.[49,50] During this process, the CTLs retain strong anti-tumor capacity.

Comprehensive testing of every subject in this investigation was not feasible. More than 200 ml of whole blood would have been required from each subject. Furthermore, the *in vitro* conditioning experiments may not reflect the true *in vivo* status. The use of *in vitro* conditioning comparisons allowed us to simulate and elucidate the *in vivo* condition. The CTLs activity against the HH244 cell line is an important finding. However, the cytotoxic activity against HH244 cells may not be an anti-tumor response, but rather an anti-alloantigen-specific reaction. Further strategies are needed to enhance the autologous anti-tumor response in order to strengthen immunotherapy as a mean of treating RCC.

Acknowledgements

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腎細胞癌患者對腫瘤抗原之免疫反應

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背 景： 本計劃研究腎細胞癌患者的樹突狀細胞、調節型 T 細胞，以及對腫瘤抗原之免疫反應。

方 法： 共三十位腎細胞癌患者與五位健康受試者參與研究。樹突狀細胞從周邊血液分離出來，然後培養在顆粒球單核球株生長刺激因子 (GM-CSF, 800 units/ml) 和白介素-4 (IL-4, 500 units/ml) 7 天。樹突狀細胞及調節型 T 細胞的表型以流式細胞儀分析。利用混合淋巴球反應來研究樹突狀細胞及調節型 T 細胞的功能。以細胞毒殺試驗來評估腎細胞癌患者的樹突狀細胞表現抗原到 T 細胞的能力。這些樹突狀細胞在培養第三天時加入腫瘤壞死因子 (TNF-α) 或腫瘤溶解碎片作培養。

結 果： 腎細胞癌患者的樹突狀細胞於腫瘤壞死因子培養時，其表現 CD40 ($p = 0.03$) 與 CD80 ($p = 0.007$) 明顯比正常人差。第一期腎細胞癌患者周邊血的調節型 T 細胞功能，明顯比其他期數的功能差 ($p = 0.032$)。腎細胞癌患者的樹突狀細胞，在刺激異體 T 細胞增生能力與健康者有相同之效果 ($p = 0.83$)，但是在刺激自體 T 細胞增生能力，則較健康者明顯有較差之效果 ($p = 0.023$)。在七位腎細胞癌患者中，有五位的調節型 T 細胞在腫瘤溶解碎片作培養時，其抑制自體 T 細胞增生比異體 T 細胞增生明顯。當腎細胞癌患者的 T 細胞受到腫瘤溶解碎片培養過的樹突狀細胞刺激後，仍能夠在試管中生成毒殺型 T 細胞。前列腺素 E_2 並不會增加未成熟樹突狀細胞刺激 T 細胞增生的能力。

結 論： 腎細胞癌患者有較差的抗腫瘤免疫反應。

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關鍵詞： 樹突狀細胞，調節型 T 細胞，周邊血液，腎細胞癌

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