Immunotherapy of Metastatic Renal Cell Carcinoma with Tumor Lysate-pulsed Autologous Dendritic Cells[1]

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ABSTRACT

Purpose: **We wanted to evaluate feasibility and safety of dendritic cell-based immunotherapy in patients with metastatic renal cell carcinoma (RCC).**

Experimental Design: **Patients with metastatic RCC ($n = 35$) received vaccinations (i.v. or i.d.) of $CD83^+$ autologous monocyte-derived dendritic cells (moDCs). MoDCs were loaded with lysate of cultured autologous or allogeneic permanent tumor cells (A-498) as well as keyhole limpet hemocyanin as control and helper antigen. Maturation of moDCs was induced by a combination of tumor necrosis factor α, interleukin 1β, interleukin 6, and prostaglandin E_2.**

Results: **Treatment was associated with transient flu-like symptoms. In 2 of 27 evaluable patients, any evidence of disease disappeared (complete response). In both cases, metastatic tissue had been the source of tumor antigen. One patient had an objective partial response. Seven patients had stable disease, the remaining 17 patients had progressive disease. In 11 of 11 patients evaluated, moDCs induced strong immune responses against keyhole limpet hemocyanin. In 5 of 6 patients tested, enhanced immune responses against oncofetal antigen (immature laminin receptor; OFA/LRP) could also be detected. The strongest responses against OFA/LRP were detectable in 2 patients with complete response and partial response, respectively. At the time of submission, mean follow up is 32 months and 8 patients are currently alive.**

Conclusions: **Our data indicate that moDC-based vaccination is well tolerated and has immunological as well as clinical effects in patients with metastatic RCC. OFA/LRP might be an attractive candidate antigen for DC-based immunotherapy of RCC.**

INTRODUCTION

RCC[4] (1) is resistant to conventional therapies such as radiation, hormone, and chemotherapy. At diagnosis, 20–30% of patients with RCC have metastatic disease and a substantial proportion of the patients with organ-confined tumors at nephrectomy will develop metachronous metastatic disease. Patients with metastatic disease have a poor prognosis with a mean survival time of ~1 year. Only 10% of these patients will be alive 2 years after diagnosis.

Frequent infiltration of RCC tissue with cells of the immune system (2–4) as well as rare spontaneous regressions and clinical responses to cytokine therapy resulted in the view that RCC is immunogenic and in the establishment of immunotherapy for RCC (5, 6). Cytokine therapies with IL-2 and IFN-α as well as adoptive immunotherapy with tumor-infiltrating lymphocytes or lymphokine-activated killer cells have been performed with moderate success and sometimes with considerable side effects.

More recent attempts included antitumor vaccination with, for instance, gene-modified tumor cells (7). In clinical trials using RCC cells engineered to produce GM-CSF (8), the accumulation of DCs at the injection site pointed to a crucial role of these cells in antitumor immune responses.

DCs play a central role in the immune system (9). They represent the most potent antigen-presenting cells, which induce T and support B lymphocyte responses. DCs can be generated *in vitro* in a two-step culture system (10–12). In the first step, GM-CSF and IL-4 promote the differentiation of monocytes into immature DCs with high antigen-capturing capacity (11). In the second step, proinflammatory factors induce the terminal maturation of $CD83^+$, immunostimulatory DCs. MoDCs are increasingly used in clinical settings to induce or enhance tumor immunity (13–21).

In this study, we have used autologous $CD83^+$, immunostimulatory moDCs to treat 35 patients with metastatic RCC. MoDCs were loaded with tumor cell lysate and with KLH. KLH is a highly immunogenic protein that serves as a control and helper antigen. KLH has recently been shown to augment DC-

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[4] The abbreviations used are: RCC, renal cell carcinoma; IL, interleukin; GM-CSF, granulocyte/macrophage colony-stimulating factor; DC, dendritic cell; moDC, monocyte-derived DC; KLH, keyhole limpet hemocyanin; PBMC, peripheral blood mononuclear cell; DTH, delayed-type hypersensitivity; NKC, normal kidney cell; PR, partial response; CR, complete response; OFA, oncofetal antigen; LRP, laminin receptor precursor.

induced antitumor immune responses (22, 23). MoDCs were administered either i.v. or i.d. Follow up is 32 months at the time of submission.

PATIENTS AND METHODS

Patients. All patients with the primary tumor in place at diagnosis underwent nephrectomy. Patients with histologically confirmed RCC and bidimensionally measurable metastatic disease were included. Exclusion criteria were other immunotherapies or chemotherapy within 4 weeks before first DC vaccination, pregnancy, lactation, other malignancies within the last 5 years, use of immunosuppressive treatments such as prednisone, azathioprine, or cyclosporine A within 4 weeks before vaccination, history of autoimmune disease, presence of acute or chronic infections, HIV or viral hepatitis, or a Karnofsky Index <60. A positive skin test to recall antigens was not required. Patients were enrolled after having signed the informed consent form, which had been approved by the institutional review board. Before the first vaccination, an evaluation, including clinical history, physical examination, hematological and biochemical parameters, computed tomography scan of brain, chest, and abdomen as well as bone scan, was performed.

Preparation of Tumor Antigen. Lysates from cultured RCC cells were used as a source of tumor antigen. Lysates were either prepared from autologous RCC or from a permanent RCC cell line (A-498; Refs. 24, 25). A-498 was used when no tissue from the primary tumor was available, and no metastatic tissue was accessible. In three cases (patient nos. 11, 21, 33), metastatic tissue was used. Tissue was minced, digested with 1 mg/ml of type 1 collagenase (EC 3.4.24.3; Sigma, St. Louis, MO) and 40 units/ml of type 1 desoxyribonuclease (DNase, EC 3.1.21.1; Sigma), washed extensively, and the resulting cell suspension was seeded in 75-cm^2 culture flasks (Costar, Corning, Inc., NY) at a cell density of $3–5 \times 10^4$ cells/ml of RPMI 1640 supplemented with 10% fetal bovine serum (Hyclone, Logan, Utah). On day 2 or 3, nonadherent cells were removed and fresh medium was added. Subconfluent cells were adapted to fetal bovine serum-free medium for the last 48 h and then harvested. Cell lysates were generated by repeated freeze-thaw cycles (liquid nitrogen, 37°C water bath), irradiated with 100 Gy, and stored at −80°C. Protein concentrations of the lysates were determined using the Bio-Rad Protein Assay (Bio-Rad Laboratories, München, Germany).

Generation of Mature Antigen-loaded MoDCs. PBMCs were isolated from 150 ml of heparinized venous blood of the patient by standard density gradient centrifugation using Lymphoprep (Life Technologies, Inc., Lofer, Austria). PBMCs (2×10^6/ml) were seeded in 6-well plates (Costar), and after 2 h, the nonadherent cells were removed. Adherent monocytes were cultured in RPMI 1640 (Biowhittaker, Verviers, Belgium) supplemented with 2% heat-inactivated human serum, 50 IU/ml penicillin, 50 μg/ml streptomycin, 2 mM L-glutamine, 10 mM HEPES, 0.1 mM nonessential amino acids, 1 mM pyruvate (all from Biowhittaker), and 5×10^{-5} M 2-mercaptoethanol in the presence of 1000 units/ml of each recombinant human GM-CSF (Leucomax; Novartis) and recombinant human IL-4 (Strathmann Biotech, Hannover, Germany) for 5 days. On day 5, moDCs were pulsed with autologous tumor lysate (10 μg/ml) or A-498 lysate (10 μg/ml) and KLH (10–25 μg/ml; Calbiochem-Novabiochem, San Diego, CA). After 1 h at 37°C, 1000 units/ml of recombinant human tumor necrosis factor α, IL-1 (5 ng/ml), IL-6 (10 ng/ml; all from R&D Systems, Minneapolis, MN), and 1 μM prostaglandin E_2 (Prostin E_2; Pharmacia & Upjohn, Vienna, Austria) were added to induce maturation (26, 27). After 24 h, moDCs were harvested, washed, and resuspended in lactated Ringer's solution containing 1% autologous serum. An aliquot of the cells was removed for phenotypic analysis and for sterility testing at the local Institute of Hygiene. Release criteria were typical DC morphology under phase contrast (veiled cells) and phenotype (>90% of the cells $CD83^+$).

Patient Treatment. Treatment started ~1 month after nephrectomy. Complete treatment consisted of at least three vaccinations in monthly intervals. In a first series of patients, moDCs were administered i.v. In a second series, the cells were injected intradermally (i.d.). Reevaluation was performed 1 month after each vaccination. Medical history as well as standard blood tests, coagulation tests, and urine analysis were performed at each visit. Standard DTH testing for recall antigens (Tetanus, Diphtheria, Streptococcus, Tuberculin, Candida albicans, Trichophyton-mentagrophytes, Proteus-mirabilis; Multitest Immignost, Biosyn, Fellbach, Germany) was performed in 27 patients before treatment started. The DTH response was considered positive when at least one antigen induced induration and redness of >2 mm in mean diameter. Additionally, DTH skin testing with KLH (5–25 μg/injection) was performed before and after vaccination in 4 patients. These patients were excluded from KLH-specific immune monitoring. In the remaining patients, DTH skin testing with KLH was not performed because skin injection of KLH itself might have influenced the immune responses induced by KLH-loaded moDCs.

For i.v. application, moDCs were resuspended in 10 ml of lactated Ringer's solution containing 1% autologous serum and administered through a peripheral i.v. catheter. The first 3 patients had prior medication with indomethacin and diphenhydramine to prevent possible allergic reactions. Premedication was omitted in the remaining patients because no adverse effects were observed. For the i.d. administration, moDCs were resuspended in 500 μl of lactated Ringer's solution containing 1% autologous serum and injected i.d. into the thigh next to the inguinal lymph nodes, which were apparently tumor-free in all patients treated.

Antigen-specific Proliferation and Cytokine Production. PBMCs were harvested before (preimmune) and after vaccination (immune) and cryopreserved. PBMCs were thawed, washed, and stimulated in triplicates in flat-bottomed 96-well plates (10^6/ml) with either KLH (5 μg/ml), RCC lysate or NKC lysate (10 μg/ml), or with recombinant OFA/LRP (0.1 μg/ml). Aliquots (50 μl) of day 5 supernatants were removed and assessed for cytokine contents (IFN-γ, IL-4, IL-10). Cultures were pulsed during the last 16 h with 1μCi [^{3}H]thymidine (1 μCi/well = 37 kBq/well; ICN Biomedicals, Eschwege, Germany)/well. Cells were harvested on glass fiber filters using a Skatron cell harvester (Skatron Instruments, Lier, Norway) and analyzed in a liquid scintillation counter. Results are mean cpm of triplicate wells.

Table 1 Patient characteristics

Patient (no.)	Age/Sex	Localization of primary tumor	Localization of metastases
1	68/M	Right	Lung, skin
2	57/M	Right	Bone, liver
3	53/M	Left	Lung, skin
4	70/F	Left	Lung
5	52/M	Right	Lung, adrenal
6	29/F	Left	Adrenal, brain
7	60/F	Left	Bone
8	56/M	Left	Lung
9	56/F	Left	Lung, bone, soft tissue
10	57/M	Right	Bone
11	59/M	Left	Lymph node
12	57/F	Right	Lung, skin, soft tissue
13	59/M	Right	Lung, lymph node
14	51/M	Right	Lung
15	51/F	Left	Lung, bone
16	52/M	Right	Lung, bone
17	49/F	Left	Bone, skin
18	54/M	Right	Bone
19	57/F	Right	Lung, liver
20	63/F	Right	Lymph node, soft tissue
21	49/M	Left	Lung, skin, lymph node, brain
22	71/M	Right	Liver, lymph node
23	46/M	Left	Bone
24	55/M	Left	Lung, liver, bone
25	52/M	Right	Bone
26	63/M	Left	Lung
27	53/M	Right	Soft tissue
28	55/M	Right	Lung
29	57/M	Left	Lung
30	66/M	Right	Lung
31	62/F	Right	Liver
32	62/M	Right	Lung
33	61/M	Right	Lymph node
34	63/M	Left	Liver, lymph node, bone
35	56/M	Left	Soft tissue

Immunohistochemical Analysis of a Regressing Lung Metastasis. A fine-needle biopsy was taken from a regressing lung metastasis under ultrasound control. Tissue processing and immunohistochemistry were performed as described previously (28).

RESULTS

Patients and Immune Status. Between October 1997 and November 1999, 35 patients (mean age: 56.6 years; 25 men, 10 women) with metastatic RCC with predominantly clear cell histology were enrolled in the study. Patient characteristics are summarized in Table 1. Of the 35 patients initially enrolled, full treatment consisting of three vaccinations could be performed in 27 patients, whereas 8 patients progressed early and died before completion of therapy. Of the 27 patients who received all three vaccinations, 6 patients were lost to follow up. Fourteen of 21 patients were subjected to a standard DTH test for recall antigen responses before treatment start. Ten of them had a positive DTH response to at least one antigen. Importantly, none of the DTH-negative patients is still alive (Table 2).

DC Generation and Administration. Patients were treated with fresh (not cryopreserved), autologous moDCs homogeneously positive for CD83 (data not shown; Ref. 26). Generation of moDC cultures was successful in all patients, and all vaccine preparations (no. = 136) were found to be free of microbial contamination. Establishment of primary tumor cell cultures was successful in all cases where tumor tissue was available. The total moDC dose per patient and the number of vaccinations per patient are listed in Table 2. A mean dose of 8.7×10^6 moDCs ($1.3–38 \times 10^6$) was administered per vaccination. The mean number of vaccinations was 4.6 (3–13). In 17 patients, the mean dose of moDCs applied i.v. was 9.95×10^6 ($3.2–15.5 \times 10^6$), whereas in 10 patients, the mean dose of moDCs injected i.d. was 5.42×10^6 ($3.2–10.3 \times 10^6$).

Toxicity. Only flu-like symptoms with fever (Table 2), chill, and headache were noticed but did not require additional treatment or prolonged hospitalization. One patient (no. 3), who later turned out to have an objective PR, developed circadian fever with evening body temperature of 39°C, which lasted for 3 months. All patients could be discharged the day after vaccination. No patient developed systemic toxicity, allergic reactions, or clinical manifestation of autoimmune disease. Rheumatoid factor and antinuclear antibody were not measured routinely but remained negative in the few patients tested. After the second i.d. application, mild erythema and induration were observed in the majority of the patients lasting for 2–3 days. These DTH-like responses occurring upon repetitive vaccination, however, should not be considered a side effect but rather as an indication for the development of cellular immunity.

Clinical Outcome. After three vaccinations, 2 of 27 evaluable patients had no more evidence of their disease (CR). One patient had an objective PR with regression of all metastases (60–70% overall regression; Ref. 15). Seven patients were stable. The remaining 17 patients had progressive disease. Six patients were lost to follow up. Thus, long-term follow-up data are available for 21 patients. Mean follow up of these patients is 32 months (23–47 months). Currently, 8 patients are alive with a mean survival time of 28 months (23–42 months). Clinical outcome is shown in Table 2.

Of the 2 patients with CR, one patient (no. 33) had multiple enlarged neck and retroperitoneal lymph nodes. Neck dissection was performed to remove the largest lymph nodes. Pathological examination confirmed metastatic lesions of RCC. During vaccination, lymph nodes remained stable and completely regressed after three vaccinations (Fig. 1). The patient has been disease-free for 13 months. The second patient with a CR (no. 11) had enlarged mediastinal lymph nodes, some of which were removed for pathological examination. After the third vaccination, the remaining enlarged mediastinal lymph nodes regressed. The response lasted for 25 months before the patient experienced bronchial tumor recurrence. The patient received radiation therapy and additional vaccinations. Importantly, both cases with CR metastatic tissue had been used for the generation of tumor lysate. An objective PR was observed in 1 patient (no. 3) with five lung metastases, which all regressed after the second vaccination with an overall regression of ~70%. A biopsy of a regressing lung metastasis was taken for immunohistochemical analysis. Specific staining for CD3 revealed substantial infiltration with T cells (Fig. 2) indicative of an ongoing immune response. The infiltrate consisted of both $CD4^+$ and $CD8^+$ cells (data not shown). The patient remained stable for 15 months

Table 2 Patient treatment and clinical outcome/survival for patients who received full treatment consisting of three vaccinations

Patient (no.)	Previous therapy	DTH	Tumor antigen	DC dose (mio)	No. of vaccinations	Route	Fever	Outcome/time to progression	Survival (mo)[a]	Alive
2	No	Positive	Autologous	29.1	3	i.v.	No	PD[b]	1 + 4	No
3	No	Positive	Autologous	171.7	13	i.v.	39°C	PR/15	1 + 21.5	No
4	No	Positive	Autologous	70.4	8	i.v.	39°C	SD/38	1 + >42	Yes
5	IL-2/IFN-α	Positive	Autologous	61.9	12	i.v.	40°C	SD/21	25 + 25	No
7	No	Negative	A-498 cell line	18.7	3	i.v.	40°C	PD	1 + 10.4	No
9	No	Positive	A-498 cell line	52.4	6	i.v.	No	SD/5	1 + 13.5	No
11	No	Positive	Autologous	76	7	i.v.	38°C	CR/25	1 + >32.9	Yes
12	IFN-α	Positive	A-498 cell line	46.6	3	i.v.	No	PD	8 + 4	No
13	IFN-α	Negative	A-498 cell line	49	4	i.v.	No	PD	45 + 8.8	No
14	IL-2/IFN-α	Positive	A-498 cell line	39	3	i.v.	No	PD	Lost of follow up	?
15	IFN-α	Positive	A-498 cell line	70.9	6	i.v.	40°C	PD	16 + 5	No
16	IL-2/IFN-α/chemo	Positive	A-498 cell line	30.9	4	i.v.	No	SD/28	25 + >30	Yes
18	No	Negative	A-498 cell line	52	6	i.v.	No	PD	1 + 8	No
19	IL-2/IFN-α/chemo	Negative	A-498 cell line	32	3	i.v.	No	PD	Lost of follow up	?
20	IL-2/IFN-α	Positive	A-498 cell line	33	3	i.v.	38°C	PD	Lost of follow up	?
22	IL-2/IFN-α	n.d.	A-498 cell line	31	3	i.d.	No	PD	12 + 7	No
23	IFN-α	n.d.	A-498 cell line	47	4	i.v.	No	PD	Lost of follow up	?
24	IL-2/IFN-α/chemo	Positive	A-498 cell line	29	3	i.v.	No	PD	8 + 5	No
25	IL-2/chemo	Negative	A-498 cell line	12.5	3	i.d.	38°C	PD	53 + 5	No
27	IL-2/IFN-α	Positive	A-498 cell line	9.7	3	i.d.	No	PD	Lost of follow up	?
28	IL-2/IFN-α/chemo	Positive	A-498 cell line	9.7	3	i.v.	No	PD	Lost of follow up	?
29	No	n.d.	A-498 cell line	8.3	3	i.d.	No	SD/26	2 + >26	Yes
31	IL-2/IFN-α	n.d.	A-498 cell line	42.8	6	i.d.	No	SD/10	51 + >24	Yes
32	No	n.d.	A-498 cell line	11.1	3	i.d.	No	SD	2 + >23.5	Yes
33	IL-2/IFN-α	n.d.	Autologous	11	3	i.d.	No	CR	3 + >23.3	Yes
34	No	n.d.	Autologous	13.9	3	i.d.	No	PD	2 + 5	No
35	No	n.d.	Autologous	7.65	3	i.d.	No	PD	1 + >23	Yes

[a] Survival: months until therapy + months after therapy.
[b] PD, progressive disease; SD, stable disease; chemo, chemotherapy.

before he developed brain lesions and died 3 months later. Two of 7 patients, who presented with stable disease after the third vaccination are still stable (23.5 and 26 months, respectively). The other 5 patients progressed after a mean time of 20.4 months (5–38 months). Among patients with stable disease, 5 are currently alive.

Immune Responses Against the Control Antigen. DTH responses against KLH were assessed in 4 patients. All 4 patients showed positive DTH responses upon skin injection of KLH after but not before vaccination. In 1 patient, a biopsy was taken from the DTH site and immunohistochemical analysis revealed abundant $CD3^+$, $CD4^+$, and $CD8^+$ cells (28).

In 11 patients, PBMCs harvested before and after the first vaccination were tested for KLH-induced T-cell proliferation. All patients tested showed KLH-specific proliferation. Mean cpm prevaccination was 1778 (range, 74–19553). Mean cpm after vaccination was 63409 (range, 10495–210658). In all these patients, a single vaccination was sufficient to induce a measurable response (Fig. 3). The change in proliferation index (fold increase) differed among patients and ranged from 6 to 904 (mean increase, 35.6-fold). KLH-induced proliferation was associated with strong IFN-γ but not IL-4 production (28), consistent with a Th1-type immune response. Only 1 patient (no. 15) showed strong KLH-specific proliferation before the first vaccination (data not shown). Clinical history revealed that this patient had previous immunotherapy with s.c. KLH injections.

Immune Responses to a Tumor-associated Antigen. We have previously shown that RCC expresses OFA (29), which is identical with LRP (30). OFA/LRP is a tumor-associated antigen that has been shown to elicit IFN-γ-producing effector T cells as well as IL-10-producing regulatory T cells (30). To assess potential immune responses against tumor-associated antigens, preimmune and immune PBMCs from 6 patients (nos. 3, 27, 28, 29, 31, 33) were stimulated with recombinant OFA/LRP, and proliferative and cytokine (IFN-γ, IL-10) responses were assessed. Five of 6 patients (all but no. 29) developed OFA/LRP-specific proliferative responses defined by an at least 2-fold increase (33-fold, 2.5-fold, 2.5-fold, 3-fold, and 2-fold). The patient (no. 3) with the 33-fold increase in the OFA/LRP-specific proliferative response (Fig. 4) was unique in that he also showed a strong proliferative response against tumor lysate (RCC), NKC lysate, and lysate of the A-498 cell line (Fig. 4) and clinically presented with an objective PR (Table 2; Ref. 15). Importantly, this patient neither developed positivity for rheumatoid factor or antinuclear antibodies nor clinical signs of autoimmunity. Three of 6 patients tested (nos. 3, 31, 33) showed >2-fold increases in OFA/LRP-specific IFN-γ production (4.5-fold, 3.5-fold, and 40-fold). Most intriguingly, the 40-fold increase in IFN-γ production (increase from 10 to 418 pg/ml) was observed in a patient (no. 33) who had a CR (Fig. 1 and Table 2). Only 1 patient (no. 28) showed enhanced IL-10 production (4.5-fold increase).

DISCUSSION

The purpose of this study was to evaluate the feasibility and safety of autologous antigen-loaded moDCs in patients with metastatic RCC. A total of 35 patients (Table 1) has been treated

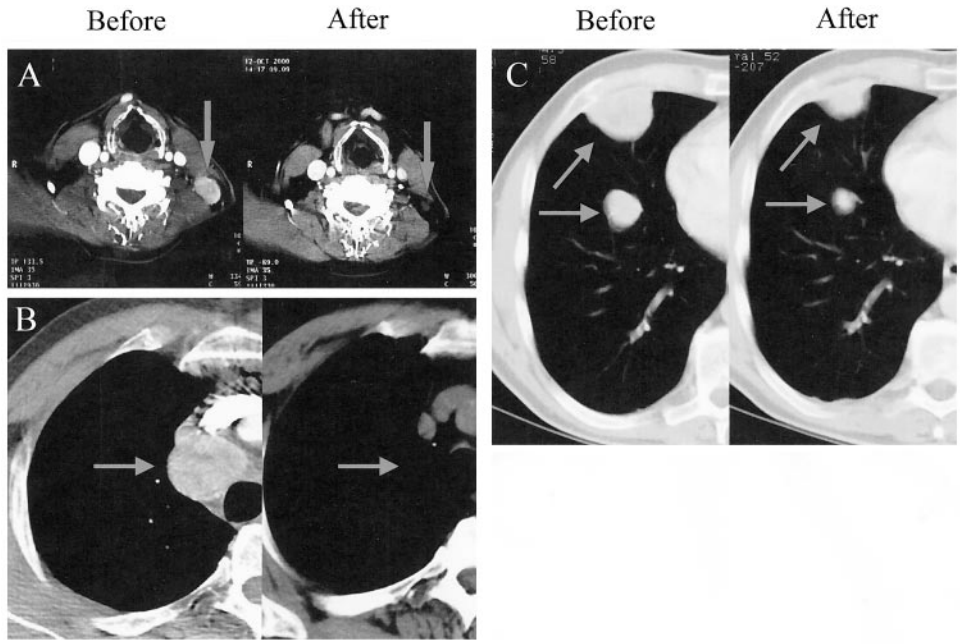

Fig. 1 Tumor regression after moDC vaccination. Computed tomography scans of patients no. 33 (*A*), no. 11 (*B*), and no. 3 (*C*) before and after moDC vaccination shows regression of lymph node and lung metastases, respectively.

with a mean follow-up period of 32 months at the time of submission. The study also included patients with previous immunotherapy or chemotherapy (Table 2). Likewise, no selection of immunocompetent patients was performed, for instance, on the basis of standard testing for DTH responses to recall antigens. Our results indicate that vaccination of these patients with autologous antigen-loaded moDCs is safe and can lead to enhanced immunity to the control antigen (KLH) as well as to the tumor-associated antigen OFA/LRP. It is also encouraging that among the 27 patients who completed therapy (at least three vaccinations), 2 CRs, 1 PR, and 7 stable diseases could be identified and that 8 patients are still alive after a mean follow up of 32 months. Intriguingly, the strongest immune responses against OFA/LRP after moDC vaccination were observed in 1 of 2 patients with CR (no. 33) and in the patient with PR (no. 3). Unfortunately, the second patient with CR (no. 11) was not available for monitoring of anti-OFA/LRP immune responses. OFA/LRP is a tumor-associated antigen expressed by many tumors, including lymphoma, carcinoma, and sarcoma (29, 30). It will be important to test whether OFA/LRP can be used as an antigen in broadly applicable DC-based vaccines for the immunotherapy of RCC and other tumors.

We had the opportunity to monitor immune responses against the control antigen in 11 patients. All patients developed KLH-specific immunity after vaccination with antigen-loaded moDCs. A single vaccination was sufficient to induce measurable responses (Fig. 3). Both i.v. and i.d. administration of moDCs resulted in KLH-specific immunity (Fig. 3 and Table 2). Several patients developed fever within few hours of moDC infusion (i.v.) in the absence of detectable viral or bacterial infection, suggesting that fever was part of the moDC-induced immune response most likely against the highly immunogenic control antigen KLH. This view was additionally supported by the fact that fever did not occur after the first but upon subsequent administrations of moDCs consistent with the development of KLH-specific immunological memory. Moreover, reducing the KLH concentration during moDC antigen loading also reduced the fever response of the patients (data not shown), indicating that it was antigen dependent. These observations also suggested that antigen-loaded moDCs, within a view hours of their administration, can induce T-cell activation and cytokine production followed by a central nervous system response that eventually leads to the up-regulation of body temperature. Taken together, these findings clearly indicate that moDCs can be used to immunize patients antigen-specifically and that patients with metastatic RCC are relatively immunocompetent.

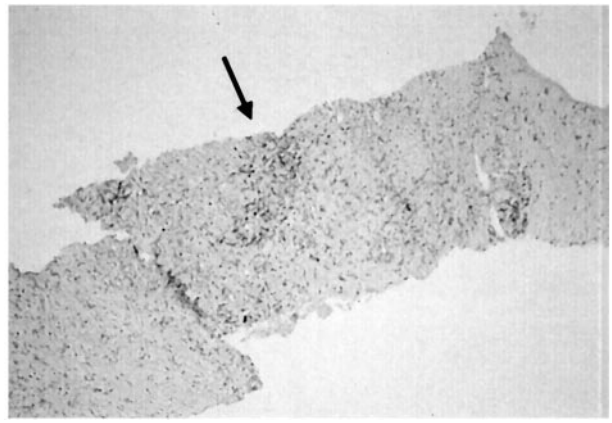

Fig. 2 Immunohistochemical analysis of a regressing lung metastasis revealed T-cell infiltration. A fine-needle biopsy was taken from a regressing lung metastasis from patient no. 3 and analyzed immunohistochemically for T-cell infiltration by staining for CD3. *Arrow*, nest of T cells within the tumor area.

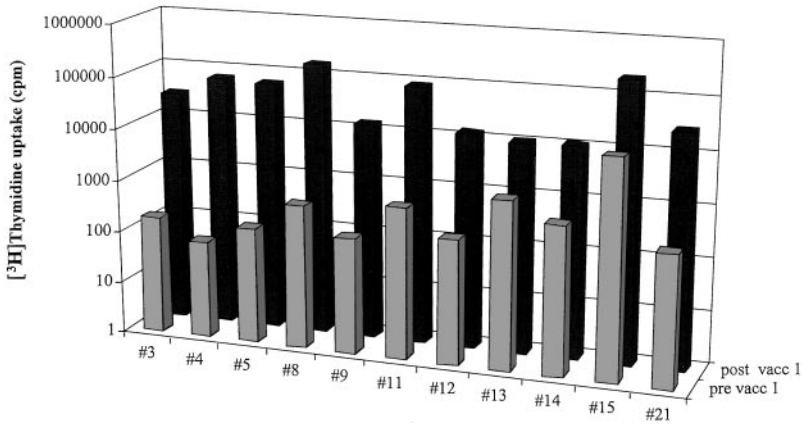

Fig. 3 KLH-specific immunity after a single vaccination with antigen-loaded moDCs. KLH-dependent proliferative responses pre- and post-vaccination 1 as determined by [^{3}H]thymidine incorporation (cpm). Mean values of triplicate measurements were determined (y-axis: log scale).

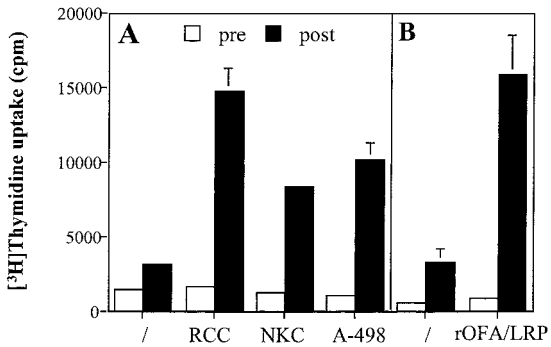

Fig. 4 Responses against tumor lysate and OFA/LRP in a patient with a PR. Proliferative responses against cell lysates or recombinant OFA/LRP as determined by [^{3}H]thymidine incorporation (mean cpm values ± SD) in patient no. 3 after three administrations of moDCs. Tumor regression occurred after vaccination 2. Lysates were prepared from autologous RCC or NKCs as well as from a permanent RCC cell line (A-498).

However, evidence that immunocompetence may be limited in these patients has also been obtained. In a standard skin test for DTH toward microbial recall antigens, 5 of 19 patients tested failed to respond to one of the seven test antigens. Importantly, none of these DTH-negative patients is still alive. Moreover, time to death was half as long for patients with a negative DTH test. Thus, pretreatments to improve the immune status of these patients are badly needed to pave the way for more successful DC-based antitumor vaccination.

Additional factors such as previous immunotherapy or chemotherapy, the source of tumor antigen, the moDC dosage, or the route of moDC administration may, in principle, affect the clinical outcome of this treatment form. However, in this study, no clear evidence in favor of one or against another approach was obtained. Patients with CR, PR, and stable disease included both patients with and without prior immunotherapy and/or chemotherapy. The route of DC administration has been suggested to affect quantity and quality of the DC-induced immune response (31). After i.v. administration, DCs redistributed to the liver, spleen, and bone marrow (32). In contrast, DCs injected i.d. migrated to regional lymph nodes (32). Recently, differences in the differentiation state of T-cell populations in spleen and lymph nodes have also been reported. Although lymph nodes contain naive and nonpolarized T cells, the spleen also contains Th1 and Th2 T cells in addition to naive and nonpolarized T cells (33). Despite all these differences, we observed KLH-specific immunity after i.v. and after i.d. administration (Fig. 2 and Table 2). Moreover, 1 CR and 1 PR occurred after i.v. infusion, and 1 CR was noticed after i.d. injection. Among the patients with SD, 4 patients had i.v. and 3 patients had i.d. administration. Future attempts may combine various routes of administration (i.v., i.d., intranodal) in an order that has yet to be determined.

It was interesting to note that in all 3 patients with tumor regression (nos. 3, 11, 33), autologous tumor cells had been the source of antigen. Moreover, in both cases with CR (nos. 11, 33), metastatic tissue had been used for the preparation of tumor lysate. Conversely, in numerous patients with progressive disease, the A-498 cell line had been used as a source of tumor antigen (Table 2). This observation suggested that autologous tumor tissue is required to induce clinical responses and that metastatic tumor tissue should be preferred when it is available. However, the only patient (no. 3, PR) who had measurable immune responses against tumor lysate *in vitro* (15) responded similarly to autologous tumor lysate, to NKC lysate, as well as to lysate of the A-498 cell line (Fig. 4*A*). The observation that the strongest responses against OFA/LRP (40-fold and 33-fold increase) were detected in 2 patients with CR (no. 33) or PR (no. 3), respectively, might suggest that the OFA/LRP contents in different cell lysates influence the clinical outcome. However, by using flow cytometry and Western blotting, we detected similar amounts of OFA/LRP in primary RCC (29) and in the permanent RCC line A-498.[5] Among the patients with stable disease, in 5 cases A-498 and in 2 cases autologous tumor had been the source of antigen. No correlation between moDC dosage and clinical outcome was observed.

In this study, mature, CD83$^+$ moDCs were used. Mature DCs are resistant to the immunosuppressive effects of IL-10

[5] C. Zelle-Rieser, R. Ramoner, A. L. Barsoum, G. Bartsch, J. H. Coggin, Jr., and M. Thurnher, manuscript in preparation.

(34), a cytokine that may be abundant in tumor patients (35). The half-life of antigen-MHC complexes is dramatically extended on mature DCs (36), which obviously increases the likelihood of inducing antigen-specific responses. Mature DCs express CCR7, which is required for the chemokine-dependent migration of DCs to secondary lymphoid tissues (37). Most importantly, recent work has indicated that immature moDCs stimulate the development of IL-10-producing regulatory T cells (38). Such regulatory T cells must be expected to counteract the development of tumor immunity and to promote tumor tolerance and progression.

Evidence to suggest that moDCs generated with GM-CSF and IL-4 have an incomplete functional repertoire and may therefore not be optimal for vaccination purposes has recently been obtained (39). Suppression of the arachidonic acid metabolism by IL-4, which is continuously present in the culture system, may be responsible for the observed handicaps of moDCs (reviewed in Ref. 39). In particular, the inability to produce leukotrienes (40) will negatively affect the migratory capacity of moDCs (41). In ^{111}indium-labeling experiments, we and others have observed that only a minority of the moDCs injected i.d. migrates to regional lymph nodes.[6] Failure of moDCs to produce leukotrienes may also result in a reduced ability to stimulate humoral immune responses (42). Antibodies may, however, also be required to control tumor growth and spread (43). In this regard, a recently described culture system is attractive, which uses GM-CSF in combination with type 1 IFN (instead of IL-4) to generate DCs from monocytes (44). Of interest, such DCs were markedly chemotactic in response to chemokines *in vitro* and demonstrated strong migratory behavior in SCID mice (45). In SCID mice reconstituted with human peripheral blood leukocytes, they were able to induce a potent primary human antibody response and IFN-γ production (45) indicative of a Th1-type immune response. These observations confirm that refinements of the DC culture system must be explored to identify optimal DCs for clinical vaccination purposes.

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