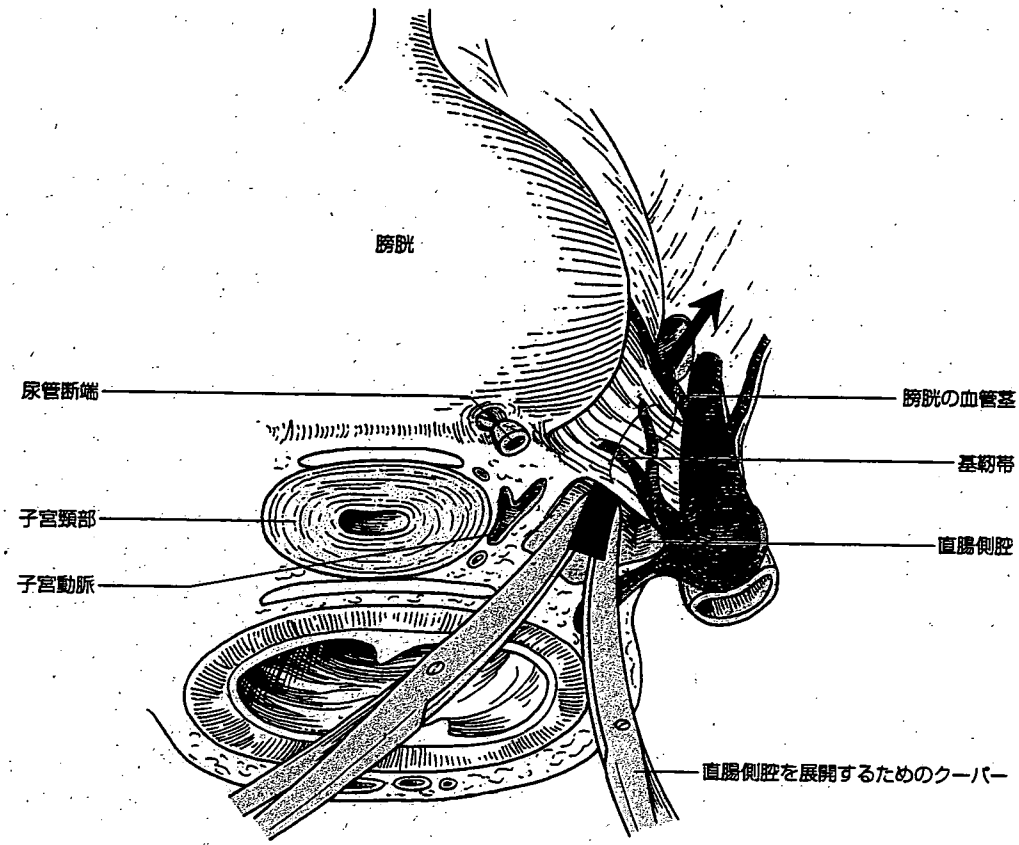
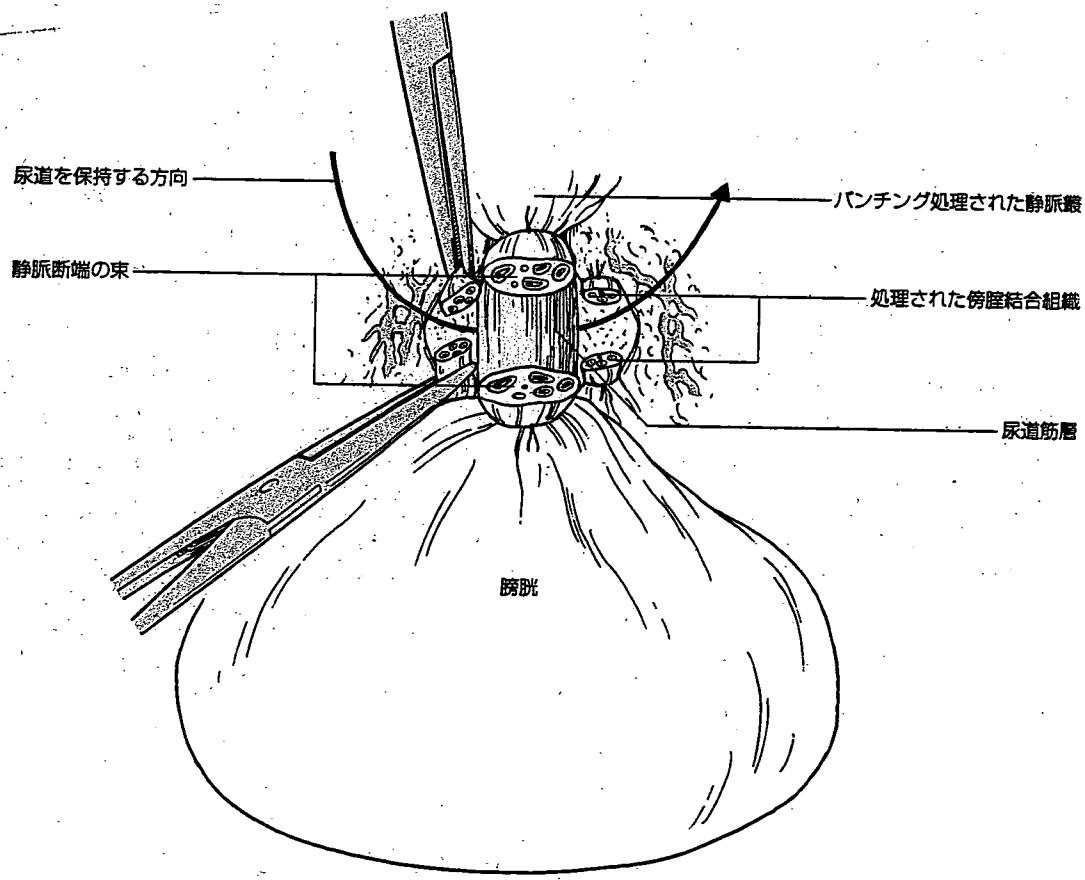


[7-77] 直腸側腔の展開法



[7-78] 尿道の処理法

より確実にするためである。この操作により傍腟組織の処理を確実に行うことが可能となる。

尿道周囲にはまさに前立腺の周囲のように静脈叢が取り巻いている。特に男性のSantorini静脈叢のように尿道前面で尿生殖隔膜に近いところでは，静脈は静脈洞になっているのではと思われるくらい，一度出血をきたすと運針では止血がなかなか困難な状況に追い込まれることがある。われわれはこの部位を処理するためには，まず膀胱頸部と尿生殖隔膜との中間あたりで尿道の深さを理解して尿道前面をパンチング鉗子で把持。これを収束結紮させ，前面の組織をまさにSantorini静脈叢を切断するように切開し，尿道筋層を確認。そのまま尿道外側とおぼしきラインを設定し，外側で腟壁に向かいメッツェンバウムなどで尿道外側を剥離，この段階で傍腟組織などから出血があるが，尿道外側の組織を鉗子で把持，これを収束結紮することで尿道のみとし，後の腟壁の切開のゴールとして理解できる状態にしている。[7-78]に概要を示した。

出血に対する対応として2-0程度の糸で運針してみて，さらに針穴からの止血が不十分の場合は4-0や3-0程度のむしろ細い糸で針穴周囲を運針する。この段階である程度の止血がなされていれば，それ以上止血を追求しないほうがよい。さらに大きく運針して止血を得ようとすると周囲の静脈が裂け，どんどん出血が多くなるという悪循環となる。これがときに起こる大量の出血の原因であると考えている。

（文献はp217を参照）

Review

Reconstruction of the urinary tract after cystectomy for transitional cell carcinoma of the bladder

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(Communicated by Takashi SUGIMURA, M. J. A., Sept. 12, 2003)

Abstract: Transitional cell carcinoma (TCC) in the urinary tract is characterized by the development of multiple tumors in time and space. When cystectomy is performed, urinary tract is reconstructed by various options including a neobladder using patient's own intestine anastomosed to the urethra. This procedure assures normal voiding from the urethra even after cystectomy. Use of the urethra for preserving urethral voiding and function of a neobladder are reviewed from viewpoints of carcinogenesis and quality of life after cystectomy. Incidence of subsequent urethral cancer arising after cystectomy is relatively high, however, if high risk patients are appropriately excluded, a neobladder can be constructed safely from the oncologic standpoint and patient's quality of life.

Key words: Bladder cancer; renal pelvic cancer; ureteral cancer; urethral cancer; urinary diversion; neobladder.

Introduction. Mucosal surface of the urinary tract, i.e., renal pelvis, ureter, bladder and the greater part of the proximal urethra, is covered with urothelium or transitional cell epithelium being composed with three to seven cell layers thick.[1] Typical structure is composed with basal cells, intermediate cells and superficial cells. Superficial cells have the binucleated, flat, large characteristic shape being called umbrella cells.[1] Luminal surface of the umbrella cells is covered with asymmetric unit plasma membrane which is effective to protect the tissue from high osmotic pressure of the urine.[2]

More than 90% of cancers arising in the urinary tract are transitional cell carcinomas (TCC), the rest are squamous cell carcinomas and adenocarcinomas. Multiple tumor development in the entire urinary tract in time and space is a well-known biological phenomenon of TCC, particularly in the bladder.[3] Ureteral and urethral involvement of TCC needs serious consideration when cystectomy is necessary and urinary reconstruction is indicated. This phenomenon is explained as a result of "field cancerization"[4)-7)] in which the entire urothelium from the renal pelvis to the urethra is susceptible to carcinogens flowing down in the urine. On the other hand, TCC cells can be implanted to other sites of the urothelium,[8] so called "implantation". These two mechanisms make it difficult to determine whether a recurrent tumor represents an inadequately treated initial one, or implantation of cancer cells, or the effects of multifocal carcinogenesis. Molecular analysis of multiple cancers in the bladder or multiple cancers developed in the upper urinary tract and the bladder tells us as one possibility that those multiple cancers are monoclonal origin indicating the implantation of cancer cells from the original tumors.[9)-12)] It is likely that all of these mechanisms are relevant. In fact, Akaza *et al.*[13] reported the biphasic pattern of recurrences of the bladder cancers after transurethral resection (TUR), which may indicate the combination of early implantation and late new growth.

Urinary reconstruction after cystectomy has been conducted historically by uretero-sigmoidostomy, ileal conduit, cutaneous continent reservoir requiring self-catheterization and an orthotopic neobladder anastomosed to the urethra. An orthotopic neobladder assures normal voiding from the urethra. Each procedure has relation to cancer development in the reconstructed

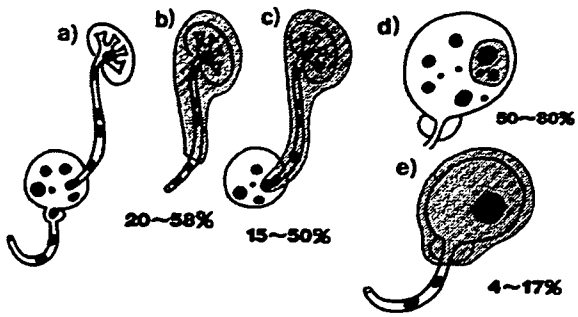

Fig. 1. Schematically illustrated multiple development of cancers in the urinary tract. The extreme left a) indicates a case having renal pelvic, ureteral, bladder and urethral cancers simultaneously. b) When ordinary nephrectomy is performed for the renal pelvic and/or ureteral cancer, subsequent ureteral cancer in the remaining ureter is 20-58%. c) Even though total nephroureterectomy is performed, subsequent bladder cancer occurs in 15-50%. d) When superficial bladder cancers are resected by transurethral procedure, subsequent bladder cancers arising in the normal appearing bladder mucosa are 50-80%. e) After complete removal of the bladder and prostate, incidence of subsequent urethral cancer is 4-17% (Ref.3)).

urinary tract.

In this review, urinary reconstruction will be discussed in relation to cystectomy from the standpoints of multiple, tumor development in the entire urinary tract and the function of a neobladder.

Carcinogenesis in the urinary tract. *Characteristic patterns of transitional cell carcinoma (TCC).* TCC can be classified as papillary carcinoma, nodular carcinoma and carcinoma *in situ* (CIS) according to their gross and microscopic configuration. Papillary carcinomas usually develop in multiple forms and frequently recur elsewhere, however, these tumors usually remain superficial confining to the mucosal layer, and the prognosis of patients, even treated conservatively, is generally fair. On the other hand, nodular carcinomas are usually deeply invasive when first observed, and the clinical outcome, even after cystectomy, is poor. CIS is a flat lesion with or without reddy velvet-like appearance of the mucosal surface, and although initially CIS is confined to the mucosal layer, CIS easily starts to invade to the submucosal or deeper muscular layer. TCCs are a mixture of these three basic patterns.[15]

Multiple development of TCC in the urinary tract. Multiple development of TCC in the entire urinary tract has been well documented. For example, renal pelvic, ureteral, bladder and urethral cancers are sometimes observed in a single case (Fig. 1a). When ordinary nephrectomy is performed for the renal pelvic and/or ureteral cancer, TCC arising in the remnant ureter, i.e. about one third of the lower part of the ureter, is reported in 20-58% (Fig. 1b). Consequently, the established state of the art operation for TCC of the renal pelvis and/or ureter is total nephroureterectomy indicating removal of the kidney, total ureter with resection of the small part of the bladder. Even such an operation is performed, however, a 15 to 50% incidence of subsequent TCC in the bladder is reported (Fig. 1c). When superficial papillary TCC of the bladder is treated by transurethral resection (TUR), the subsequent development of tumors having a similar nature in the normal-appearing bladder mucosa is reported to be 50-80% (Fig. 1d). After cystoprostatectomy (removal of the bladder and prostate) for bladder cancer in men, a 4-17% incidence of cancer in the remaining urethra is reported[16] (Fig. 1e). In female patients, involvement of the urethra in relation to bladder cancer is reported to be 1.4-36%.[17]

Table I. Pathological findings of the subsequently and concurrently resected urethras

	No. Cases
Subsequently resected urethra	
Coexistence of papillary and *in situ* ca	3
Ca *in situ*	5
Papillary ca	3
Invasion to the corpus spongiosum and cavernosum	7
Concurrently resected urethra	
No cancerous tissue	17
Small foci of ca in the corpus spongiosum	1
Small area of dysplasia	1

These data should be taken into consideration when we perform nephroureterectomy (removal of the kidney and ureter) or cystectomy and for the follow-up plans of the upper and lower urinary tract and the contralateral urinary tract assuming them as a single unit from the renal pelvis to the urethra.

Selection of urinary reconstruction in relation to carcinogenesis. When a patient is indicated for cystectomy as the treatment of invasive bladder cancer, basically three different options of selecting 1 of 3 types of urinary reconstruction are provided to the patients and families as information: (1) an ileal conduit, (2) a cutaneous continent reservoir requiring self-catheterization, or (3) an orthotopic neobladder anastomosed to the urethra to ensure urethral voiding. Presently, type (2) is not commonly used because patients do not choose this type of reconstruction mainly because type (2) seems to be intermediate of types (1) and (3). From the carcino-

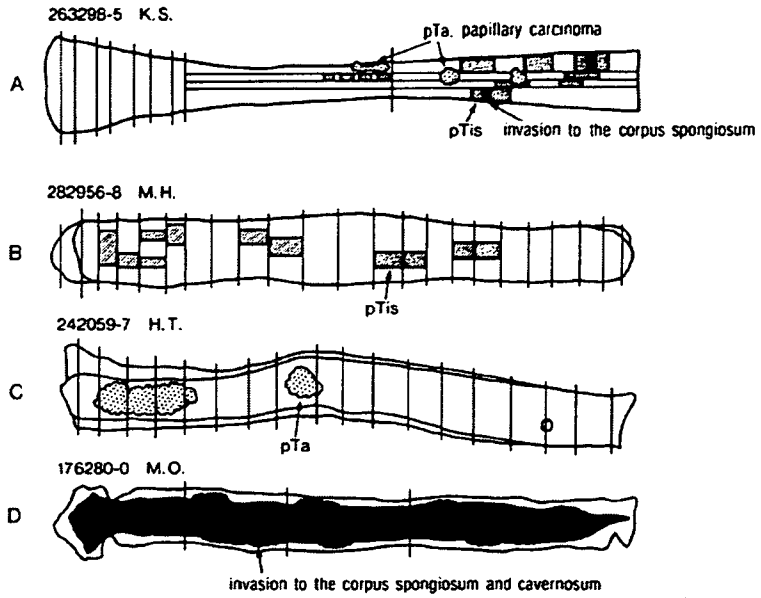

Fig. 2. Typical pathological findings in the resected urethra. Prostatic side is at left and meatus of urethra is at right side. Each specimen was examined by vertical or horizontal sections as indicated (Ref. 18). pTis indicates carcinoma *in situ* on the surface of the urethral mucosa. pTa indicates papillary superficial cancer without submucosal invasion.

geneic standpoint, an orthotopic neobladder anastomosed to the urethra is the biggest issue to be discussed. In male bladder cancer patients, urethral recurrence of TCC after cystoprostatectomy is reported to be 4-17% (Fig. 1e). In our series of patients analyzed by Tobisu *et al.*,[18] of 169 male patients who underwent cystectomy for bladder cancer, 18 (10.6%) demonstrated subsequent urethral cancer within 5 years after cystectomy. Risk factors for subsequent urethral cancer were analyzed in terms of the grade, stage, number, size, location and gross pattern of bladder cancers in the cystectomized specimens. Significant risk factors in bladder cancer relevant to the later development of cancer in the retained urethra were papillary cancers, multiple cancers, and tumors in the bladder neck, prostatic urethra and prostatic gland. On the other hand, 19 patients with concomitant CIS and/or multiple tumors in the bladder compatible to the above-mentioned risk factors underwent simultaneous prophylactic urethrectomy with cystectomy in the same observed period. Of them, 17 (89%) of 19 had no pathological lesion in the resected urethra (Table I). As is indicated in Fig. 2, pathological findings observed in the urethra subsequently resected after cystectomy were versatile but the urethra simultaneously resected with cystectomy exhibited almost no pathological lesions. Possible reason to explain this extreme difference may be that the urine stream is preserved in the latter cases until simultaneous removal of the bladder, prostate and urethra (cystoprostatourethrectomy). To support this hypothesis, urethral cancer development is not commonly observed and is not a serious problem for patients who undergo repeated TUR for multiple, frequent recurrences in the bladder. This hypothesis together with above mentioned risk factors for urethral cancer development in relation to bladder cancer supports our idea to reconstruct the urinary tract by anastomozing the neobladder to the urethra. Regarding urethral cancer after cystectomy, male patients are carefully analyzed, however, the incidence and characteristics of urethral involvement in female patients with bladder are not well documented. One reason for the very few amount of data may the principle of routine urethrectomy together with cystectomy is well established in female patients because the female urethra is short and easy to remove with the bladder. A 1.4% incidence of urethral involvement was observed during follow-up cystoscopy in 293 female patients with bladder cancer.[19] A few studies of bladder cancer were reported urethral involvement in cystourethrectomy specimens.[20,21] We reviewed 47 consecutive step-sectioned cys-

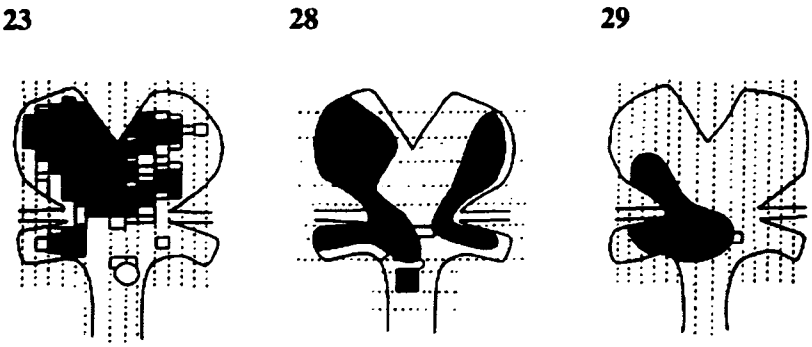

Fig. 3. Urethral involvement in 47 cystourethrectomy specimens in female bladder cancer (Ref.17)). Bladders are sagittally opened and lower part of the diagram indicates the urethra.

tourethrectomy specimens of bladder cancer in female patients to determine the incidence and characteristics of bladder cancer with the involvement of the urethra.[17] Of the 47 cases, 10 (23%) were papillary, 9 (21%) papillonodular that is intermediate between papillary and nodular carcinoma and 18 (42%) nodular carcinoma, and 6 (14%) primary or secondary CIS. There were 23 cases (54%) of invasive carcinoma of more than stage pT1 and 27 (63%) were grade 3 lesions. Urethral cancer was observed in only 3 cases (Fig. 3): 1 stage pT4, grade 3 papillonodular carcinoma developed widely in the bladder and, overriding the bladder neck and proximal urethra, stage pTa, grade 2 papillary cancer, was detected, while in 2 with nodular invasive lesions of the bladder including bladder neck, urethral cancer was detected either as a direct invasive extension via urethral CIS or as an intralymphatic spread without urethral mucosal change. These findings indicate the necessity for prophylactic urethrectomy in cases of papillary or papillonodular carcinoma encroaching on the bladder neck, and nodular invasive carcinoma infiltrating the bladder neck and trigone. Based on those analyses, by only removing the bladder, we successfully treated the first female bladder cancer patient by a neobladder anastomozing to the retained urethra.[22] Later, a large series of orthotopic neobladder for female bladder cancer patients including our series of patients were reported.[23]

Ureteral involvement in association with bladder cancer such as in a manner of spread of CIS is well documented. Consequently, it is a routine to examine the proximal end of the ureters by frozen section during cystectomy whether there is any CIS or cancerous lesions in the cut end of the ureters. Margin-free ureters must be used for ileal conduit or various forms of neobladder. In addition to this, when urinary tract is reconstructed after cystectomy via ileal conduit or neobladder, the incidence of appearing subsequent cancers in the remaining renal pelvis and/or ureter is reported to be 2-4%.[24] Bilateral involvement of the renal pelvis and ureter (synchronous or metachronous) occurs in 2-5%[25] of sporadic cases. Although the possibility is low, we have to be careful and always bear in mind this possibility.

Reconstruction of the urinary tract after cystectomy. Reconstruction of the urinary tract, particularly after cystectomy, must be planned from the two points, i.e., carcinogenic nature of remaining urinary tract, and postoperative function of the urinary tract and quality of life (QOL) of patients.

History. In 1852, Simon[26] performed the first continent urinary diversion in a patient with ectopic bladder using ureterorectal anastomosis. In 1911, Coffey[27] reported a physiologic implantation of the ureters to the sigmoid colon and in 1913, Lamoine[28] reported the first use of the true rectal bladder i.e., implanting both ureters to the rectum using the rectum as a bladder. Since 1913 ureterosigmoidostomy, implanting both ureters to the sigmoid colon, has been utilized as a major means of continent urinary diversion. With increased clinical experience, problems associated with ureterosigmoidostomy, such as recurrent pyelonephritis due to ureteral reflux, hyperchloremic acidosis, and the possibility of later colonic cancer development,[29] have become apparent. Development of mainly adenocarcinoma, occasionally TCC has been reported near the site of anastomosis between the ureters and sigmoid colon. Leadbetter[30] reported 45 cases arising cancer after ureterosigmoidostomy during 50 years. Physical irritation by fecal stream is thought to be one reason and the risk of developing this sort of cancer in patients who underwent ureterosigmoidostomy is 500 times higher than nor-

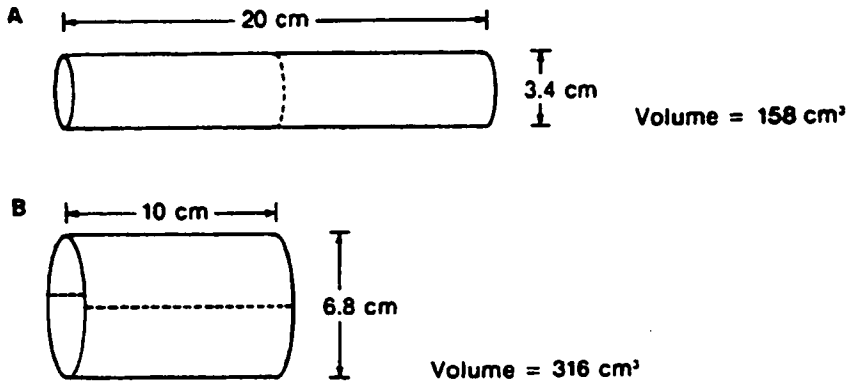

Fig. 4. Comparison of calculated capacities of (A) an intact 20 cm tube having 3.4 cm diameter and of (B) same segment opened lengthwise and folded upon itself (Ref. 38)).

mal controls.[31] This risk is evaluated as 5% for 6-50 years after ureterosigmoidostomy. Hydronephrosis appearing in patients who underwent ureterosigmoidostomy must be carefully checked bearing in mind the possibility of carcinoma near the ureteral anastomoses. In 1950, Bricker[32] first reported reconstruction of the urinary tract using an ileal conduit. This technique is widely accepted as the major procedure for urinary tract reconstruction. In 1951,[33] Couvelaire reported the first clinical use of bladder substitution, a kind of neobladder from the present meaning, through an anastomosis of the isolated ileum to the urethra. In 1985, Camey[34] used an isolated U-shaped ileum anastomosis to the urethra as a continent urinary diversion in more than 150 patients. Unfortunately, nocturnal incontinence due to increased pressure of the ileal segment resulting from ileal peristalsis occured. For a long time this type of procedure was used sporadically. In 1982, Kock *et al.*[35] reported on their pioneering use of a detubularized ileal segment i.e., opening the lumen of ileal tube and to use it as a ileal plate, a continent reservoir. With this breakthrough, an almost explosive interest in continent urinary reconstruction using cutaneous and urethrally anastomosed forms occurred throughout the world.

Theoretical considerations. The neobladder procedure involved postcystectomy construction using a segment of the patient's own intestine to form a new almost natural-like bladder. Ideally this neobladder must achieve high compliance, i.e., low pressure in neobladder, continence, and nonrefluxing reservoirs that allow adequate capacity and preservation of upper urinary tract function. The purpose of bladder replacement with an internal reservoir is not to improve survival of patients after cystectomy for bladder cancer but to improve quality of life. The status of continence and upper urinary tract function are evaluated by a normal micturition pattern, 24-hour continence, serum creatinine levels, and intravenous pyelography. QOL and functional comparison among various procedures of urinary tract reconstruction[36),37] have been reported.

The basic principles of a neobladder, including configuration of reservoir, accommodation, viscoelasticity and contractility have been thoroughly reviewed by Hinman[38] from the standpoints of physics, mathematics, and hydraulics. The configuration, and studies of the volume of the reservoir (height × radius^2) showed that the detubularized, folded pouch had almost twice the volume of the original ileal segment (Fig. 4). Interestingly, accommodation, volume to mural tension and viscoelasticity or compliance depend on the physical characteristics of the reservoir wall, and contractility depends on the motor functions of the bowel.

The clinical success of a neobladder is principally related to its reservoir geometry. The selected bowel segments are opened (detubularized) along the antimesenteric border and refashioned into various shapes, such as a U, S, M or W resembling to the shape of alphabet. Different reservoir shapes produce different characteristics in length and location of selected bowel, radius, and volume of the reservoir.

Quality of life after reconstruction of the urinary tract. Ileal conduit is the time-honored procedure since 1950[32] and significant number of patients underwent this surgery all over the world. In principle, this surgical procedure needs to apply urine-collecting pouch to the stoma where the distal end of ileal conduit is anastomosed to the skin. This pouch must be changed to the new one every 5 to 10 days. Patients must discard the

urine from pouch when it is full, 5-6 times a day. If the urine extravasates to the space between the stoma and pouch, severe dermatitis around the stoma occurs. Unexpected urine leakage may sometimes occur from the stoma. Renal pelvic stones may arize. These are the main clinical issues associated with ileal conduit. With neobladder, when successfully constructed, patients can enjoy almost normal life by voiding the urine from the urethra even after cystectomy. However, this procedure is relatively new compared to ileal conduit, we have only 10 to 15 years observation period after construction. About 10-15% of patients suffer from incontinence, particularly while sleeping, and approximately 10-15% of patients cannot void necessitating intermittent self-catherization indicating to introduce a catheter from the urethra to the neobladder. As a long-term sequelae, stone formation in the neobladder is known and hyperchloremic acidosis by absorbing the electrolytes in the urine stored in the neobladder, or excretion of calcium resulting in osteoporosis, particularly elderly female patients are also known. Consequently, both procedures have characteristic merits and demerits, respectively.

In summary, urinary reconstruction after cystectomy should be considered from the carcinogenic standpoint and the function of reconstructed urine flow route.

As was stated earlier, the variety of cancerous changes observed in the 18 patients with urethral recurrence is in sharp contrast to the simultaneously resected urethras of 19 patients with almost no cancerous changes (Table I, Fig. 2). The shedding of cancer cells from malignant urethral tissue by urine flow appears to be an important mechanism when considering the very low incidence of urethral recurrence in the large numbers of patients who undergo repeated transurethral resections for multiple papillary bladder cancers (unpublished). Shed bladder cancer cells spilled in the urethra during cystectomy procedure are left intact in the remnant urethra because there is no urine flow after cystectomy. They are harvested in the urethra during the months or years after cystectomy. This mechanism may explain the difference shown in Table I. In addition, recent molecular evidence[9)-12)] indicates that the implantation of cancer cells may provide an explanation for the multiple development of TCC in the urinary tract.

For a support of this hypothesis, we are seeking molecular evidence to prove the same molecular changes in the bladder cancer and the urethral cancer. However, we are so far unsuccessful to obtain appropriate specimens to analyze. Should this be the case, subsequent urethral cancer development may not be a hindrance to neobladder construction when patients at high risk for urethral cancer are excluded.

Conclusion. Urinary reconstruction after cystectomy for bladder cancer has a long history. Modern surgical technique revolutionalized the procedure of reconstructions using patient's own intestine for a neobladder. Theoretical consideration must be added to construct the neobladder from the standpoints of the function of neobladder, selection of the bowel, shape and size of the reservoir in terms of length and radius. At the same time, when neobladder is anastomosed to the urethra to assure voiding from the urethra after cystectomy, carcinogenic risk factors of developing subsequent cancers in the urethra both in male and female patients must be seriously considered. In this respect, contribution from Japan was great as was reviewed in this article. QOL and cure of the disease are two most important factors when cystectomy is indicated for a patient with bladder cancer. Highest QOL and/or function of the neobladder and the lowest risk of the subsequent carcinogenesis must be compromised reasonably for each patient.

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厚生労働科学研究費補助金

医療機器開発推進研究事業：

身体機能解析・補助・代替機器開発研究

新たな手術用ロボット装置の開発に関する研究

平成１５～１９年度　総合研究報告書

主任研究者　垣添　忠生

平成２０（２００８）年4月１０日

２／３冊

doi:10.1016/j.ymthe.2004.07.001

Phase I Study of Autologous Tumor Vaccines Transduced with the GM-CSF Gene in Four Patients with Stage IV Renal Cell Cancer in Japan: Clinical and Immunological Findings

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Available online 19 August 2004

We produced lethally irradiated retrovirally GM-CSF-transduced autologous renal tumor cell vaccines (GVAX) from six Japanese patients with stage IV renal cell cancer (RCC). Four patients received GVAX ranging from 1.4×10^8 to 3.7×10^8 cells on 6–17 occasions. Throughout a total of 48 vaccinations, there were no severe adverse events. After vaccination, DTH skin tests became positive to autologous RCC (auto-RCC) in all patients. The vaccination sites showed significant infiltration by $CD4^+$ T cells, eosinophils, and HLA-DR-positive cells. The kinetic analyses of cellular immune responses using peripheral blood lymphocytes revealed an enhanced proliferative response against auto-RCC in four patients, and cytotoxicity against auto-RCC was augmented in three patients. T cell receptor β-chain analysis revealed oligoclonal expansion of T cells in the peripheral blood, skin biopsy specimens from DTH sites, and tumors. Western blot analysis demonstrated the induction of a humoral immune response against auto-RCC. Two of the four patients are currently alive 58 and 40 months after the initial vaccination with low-dose interleukin-2. Our results suggest that GVAX substantially enhanced the antitumor cellular and humoral

1525-0016/$30.00

doi:10.1016/j.ymthe.2004.07.001

immune responses, which might have contributed to the relatively long survival times of our patients in the present study.

Key Words: GM-CSF, renal cell cancer, $CD4^+$ T cell, $CD8^+$ T cell, T cell repertoire

INTRODUCTION

Each year, approximately 3000 people die of renal cell cancer (RCC) in Japan [1]. Conventional treatments, such as surgery, chemotherapy, radiotherapy, and cytokine therapies, have not been established for stage IV RCC. Approximately 25% of RCC patients have metastatic disease at the time of diagnosis, and RCC sufferers have a reported 2-year survival rate of less than 20% [2]. As RCC is considered an immunogenic tumor, various types of antitumor immunotherapy have been reported that use cytokines, such as interleukin-2 (IL-2) and interferon-α; cell therapy with LAK; or nonmyeloablative stem cell transplantation. As all of these therapies have their limitations, the introduction of more specific antitumor immunotherapy with less toxicity is required [2–8].

Granulocyte–macrophage colony-stimulating factor (GM-CSF)-secreting cancer cell vaccines, which are generated from cancer cells by *ex vivo* gene transfer, have been shown to elicit tumoricidal antitumor immune responses in a variety of animal models and in human clinical trials [9–11]. Irradiated GM-CSF-secreting cancer cell vaccines are thought to induce antitumor immune responses by recruiting antigen-presenting cells, such as dendritic cells (DCs), to the site of immunization. DCs, which are the most potent immunostimulatory antigen-presenting cells, are known to activate antigen-specific $CD4^+$ and $CD8^+$ T cells, by priming them with oligopeptides that are processed from the lethally irradiated dying cancer cells. The antitumor immune reaction induced by GM-CSF-transduced tumor cells has been reviewed previously [11].

Since the initial clinical report on the use of a GM-CSF gene-transduced tumor vaccine [10], there have been a number of clinical studies applying this technology to the treatment of melanoma, renal cell carcinoma, prostate cancer, pancreatic cancer, and non-small-cell lung cancer. All of these clinical studies were performed without any severe adverse events [12–22]. In a clinical study examining RCC, Simons *et al.* reported a randomized, double-blind dose-escalation study with equivalent doses of autologous, irradiated RCC vaccine cells, with or without *ex vivo* human GM-CSF gene transfer. GM-CSF gene-transduced vaccines were equivalent in toxicity to nontransduced vaccines up to the feasible limits of autologous tumor vaccine yield. There was no dose-limiting toxicity, no evidence of autoimmune disease, and no replication-competent retrovirus encountered in 18 patients receiving full follow-up care. This phase I study demonstrated the feasibility, safety, and bioactivity of autologous GM-CSF gene-transduced tumor vaccines for RCC patients. An objective partial response was observed in one of the three patients who received 1.2×10^8 GM-CSF gene-transduced cells and showed the largest delayed-type hypersensitivity (DTH) conversion [13,14]. However, the optimum number of GM-CSF-transduced autologous renal tumor cell vaccine (GVAX) cells for use in vaccination and boosting and the optimum frequency of cell administration remain to be determined.

To determine more precisely whether GM-CSF-secreting RCC vaccines can be used safely to induce antitumor immunity in advanced RCC patients, we conducted a clinical trial of this treatment strategy. Our clinical protocol consisted of tumor resection by nephrectomy, the establishment of primary RCC cultures, and *ex vivo* gene transfer, which was carried out in our own cell-processing facility [23]. The minimum dosage of the vaccine cells was set according to the previous report on RCC by Simons *et al.* [14], and the booster schedule was based on a previous report on non-small-cell lung cancer by Soiffer *et al.* [16]. This was the first clinical trial of human gene therapy for cancer patients approved by the Japanese government and performed in Japan. The results of the present study indicate that this novel RCC immunotherapeutic regimen, which features vaccination with GM-CSF-secreting, irradiated autologous RCC tumor cells, is feasible, safe, and capable of eliciting systemic immune responses against RCC tumor cells. Furthermore, these patients, some of whom also received systemic low-dose IL-2 therapy, have been followed up on an outpatient basis.

RESULTS

Case Presentations

Forty patients suffering from either primary RCC with or without metastases or postoperative relapsed RCC were evaluated at our hospital between July 1998 and March 2001. Of these, 6 preoperative patients with stage IV RCC (UICC classification 1997) with metastatic lesions were allowed to participate in the present clinical study by our ethics committee, based on clinical condition and eligibility criteria listed under Patients and Methods. As

TABLE 1: Patient characteristics and clinical response to GVAX

	Patient			
	1	2	3	4
Age (years)/sex	60/male	71/male	57/female	50/male
Tumor site				
Primary	Right RCC	Right RCC	Left RCC	Left RCC
Metastases	Lung, liver	Sacral bone	Liver, lung	Lung
Previous therapy	None	Sacral irradiation	None	None
GM-CSF production[a] (ng/10^6 cells/24 h)	49	98	51	116
No. of GVAX treatments	10	17	15	6
Vaccinated total cell number	2.2×10^8	3.7×10^8	3.2×10^8	1.4×10^8
Adverse events				
Systemic	Low-grade fever	Low-grade fever	None	None
Local	Erythema, pruritis	Erythema, pruritis	Erythema, pruritis	Erythema, pruritis, blister
Eosinophil number[b] (/μl; mean ± SD)	718 ± 76	437 ± 306	226 ± 283	390 ± 150
Clinical response	PD	SD	PD	PD, MR
Survival (months from first vaccination)	7.5[c]	>62	45[c]	>44

PD, progressive disease; SD, stable disease; MR, mixed response.
[a] GM-CSF production rate from each autologous GM-CSF-transduced RCC.
[b] Eosinophil number was measured 48 h after vaccination.
[c] Patient passed away.

2 patients, a 48-year-old Japanese man having right RCC with multiple lung metastases and a 58-year-old Japanese man having right RCC with metastases to the right clavicle, bilateral lung, and liver, were excluded from this study because their GM-CSF-transduced RCC cells did not produce enough GM-CSF to satisfy the eligibility criteria as described under Autologous Vaccine Yield and Gene Transfer, 4 patients received GVAX.

The first patient (Case 1), a 60-year-old Japanese man, was diagnosed in August 1998 with RCC of the right kidney with multiple lung and liver metastases. His largest metastatic tumor, which was located in the right hilar region, was calculated volumetrically as 135 ml by computed tomography (CT) scan. The vaccine preparation used, his clinical course, and the autopsy findings have been reported previously [24]. Furthermore, he received a total of 2.2×10^8 GVAX cells over 10 subcutaneous injections. The adverse events he experienced during vaccination are summarized in Table 1. He received gamma knife irradiation for his brain metastases and was initiated with low-dose (700,000–140,000 IU) recombinant IL-2 (rIL-2; Imunace, 350,000 IU/vial; Shionogi, Osaka, Japan), which was administered intravenously according to the patient's request. One week after the start of the rIL-2 treatment, the patient's right hilar mass lesion became smaller and decreased by 30% of the total volume within 1 month (Fig. 1A). Unfortunately, this patient died of multiple RCC metastases on July 8, 1999, 10 months after nephrectomy and 7 months after the start of GVAX vaccination (Fig. 2A).

The second patient (Case 2), a 71-year-old Japanese man, was diagnosed in December 1998 with a sacral tumor that metastasized from RCC of the right kidney. He received a total dose of 30 Gy of irradiation of the sacral metastasis in February 1999 for severe pain, which was followed up with spinal anesthesia and oral morphine sulfate. The patient was nephrectomized on April 6, 1999, 43 days after the local irradiation, and pathology showed clear cell carcinoma. He received a total of 3.7×10^8 GVAX cells in 17 subcutaneous injections from June 3, 1999, to February 3, 2000. The adverse events he experienced during vaccination are summarized in Table 1. His pain at the sacral area disappeared completely after the 5th vaccination, and oral morphine sulfate was discontinued. He experienced mechanical ileus due to nephrectomy after the 13th vaccination, which resolved after a few days of iv fluid treatment. The ileus was not related to the vaccination, and no recurrence of the ileus was noted after 4 further vaccinations. During the course of vaccination, the growth rate of the sacral tumor was stable as assessed by CT scan. His clinical course with the change in tumor size is described in Fig. 2B. The serum level of the nonspecific tumor marker immunosuppressive acidic protein returned from double the normal level to normal after the 6th vaccination, and a thallium scan showed decreased uptake of thallium at the tumor site on completion of the vaccination protocol (data not shown). Eleven months after the start of vaccination, pathological examination of the biopsied sacral bone specimen showed no RCC. This patient had been doing well without any treatment, with a performance status of zero, until he experienced a dull pain in his right femoral area in late November of 2001, 29 months after the 1st vaccination. He was diagnosed as having a 1-cm lytic metastasis in the right femoral bone. He received local

doi:10.1016/j.ymthe.2004.07.001

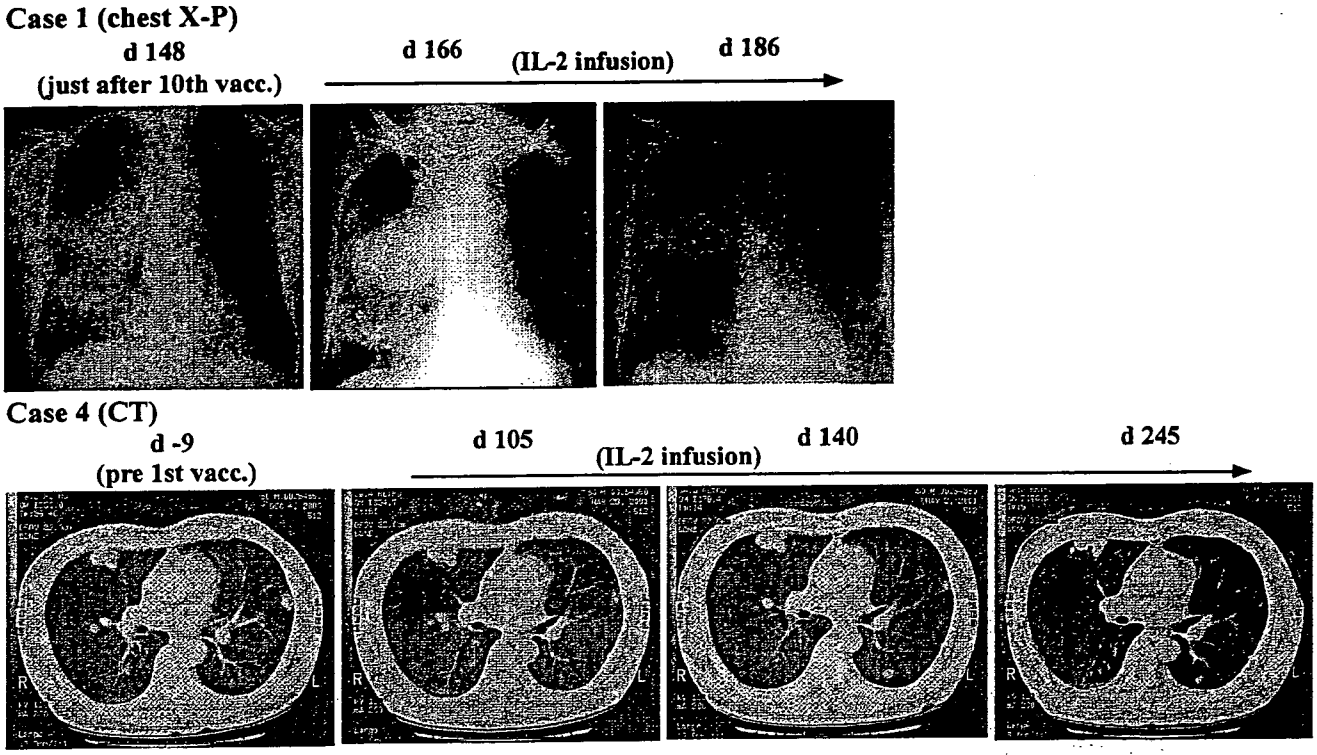


FIG. 1. Size change of the lung metastases in Cases 1 and 4 during the observation period after GVAX vaccination. (A) In Case 1, the size of the right hilar tumor, the largest metastatic lesion, became smaller after 1 week of low-dose IL-2 (d 166, day 166) and decreased by 30% after 1 month of low-dose IL-2 compared with the tumor just after the 10th vaccination. Chest X-ray films are presented. (B) In Case 4, the size of the left lung metastasis became smaller during vaccination as shown on two films for comparison between prevaccination and 105 days after the 1st vaccination. After the start of low-dose IL-2 between days 105 and 245, both the metastatic lesions in the left lung and those in the right lung became smaller. CT scan films are presented.

irradiation at a dose of 30 Gy to his femoral metastasis followed by daily low-dose rIL-2 (700,000–140,000 IU). His performance status at present, 58 months after the start of vaccination and with low-dose rIL-2 (350,000 IU) treatment, is zero.

The third patient (Case 3), a 57-year-old Japanese woman, was diagnosed in October of 1999 with RCC of the left kidney with multiple liver and lung metastases. She was nephrectomized on December 9, 1999, and the pathology showed clear cell carcinoma. She received a total of 3.2×10^8 GVAX cells in 15 subcutaneous injections, from February 22, 2000, to September 19, 2000. The adverse events she experienced during vaccination are summarized in Table 1. During the course of vaccination, the growth rate of the multiple liver tumors slowed, but the numbers and sizes of the masses did not decrease as assessed by CT scan (Fig. 2C). The sizes of the metastases in her right renal pelvis and lungs, observed on CT scan, were stable during vaccination. Her performance status was maintained at zero. After completion of the vaccination regimen, she requested systemic rIL-2 and interferon-α, but the cytokine treatments were discontinued due to the appearance of liver dysfunction, which resolved after discontinuation of cytokines. Thereafter, she received monthly LAK (lymphokine-activated killer cells) therapy, upon her request. However, her metastatic lesions gradually increased, and she ultimately died of multiple RCC metastases on November 3, 2003, 47 months after nephrectomy and 45 months after the start of GVAX vaccination.

The fourth patient (Case 4), a 50-year-old Japanese man, was diagnosed in July of 2000 with right RCC with multiple lung metastases. He was nephrectomized on September 20, 2000, and pathology showed clear cell carcinoma. He received a total of 1.4×10^8 GVAX cells in six subcutaneous injections from December 13, 2000, to February 20, 2001. The adverse events he experienced during vaccination are summarized in Table 1. During the course of vaccination, the growth rate of the largest lung tumor slowed, and several tumors disappeared or were reduced in size, i.e., a mixed response was obtained. However, the sum of all the masses was increased, as assessed by CT scan. After the sixth injection, he was found to have a metastatic brain lesion with a maximum diameter of 1 cm, and the vaccination was discontinued according to our eligibility criteria. He received gamma knife irradiation to his brain metastasis and low-dose rIL-2 (700,000–140,000 IU) was initiated, which was given

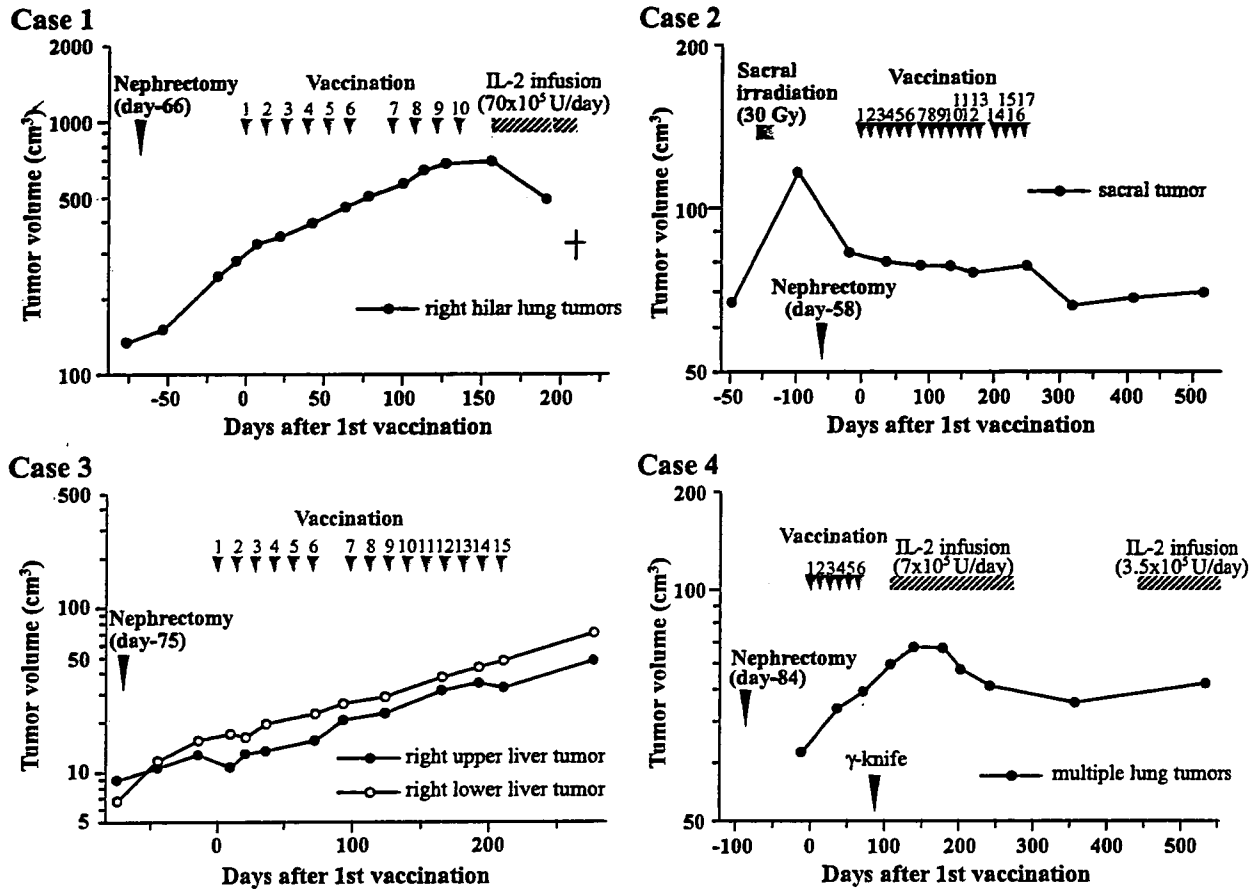


FIG. 2. Clinical summary of the four stage IV RCC patients (Cases 1 to 4) who received GVAX. The days of each vaccination are indicated by the short arrows, and the upper numbers signify the number of vaccinations. The long arrow indicates the time of nephrectomy of an RCC that involved the kidney. The tumor volume of each target metastatic lesion was measured periodically by CT scan or MRI. In Cases 1 and 4, low-dose IL-2 was administered after vaccination, upon request by the patients. The sacrum and brain were irradiated in Cases 2 and 4, respectively, to control sacral pain and brain edema.

subcutaneously, according to the patient's request. One month after the start of the rIL-2 treatment, the patient's total lung tumor volume was reduced and decreased to 30% of the peak volume over 3 months (Figs. 1B and 2D). His metastatic brain tumor was resected in January of 2002, and his performance status at present, 40 months after the start of vaccination and with low-dose rIL-2 (350,000 IU) treatment, is zero.

Autologous Vaccine Yield and Gene Transfer

In this trial, we generated the primary RCC cultures from large, advanced cancers with some areas of necrosis. The rate of successful vaccine cell expansion was 100% (6/6). In our preclinical models, the expression of paracrine GM-CSF by vaccine cells at levels higher than 40 ng/10^6 cells/24 h induced antitumor immunity, and thus, we excluded cases producing less than this level from the present study [13,14]. A single transduction with MFGS-GM-CSF generated GM-CSF secretion levels of >40 ng/10^6 cells/24 h in four of the six patients (66.6%) and their production levels are shown in Table 1. The level of GM-CSF secretion by nontransduced cells ranged from 0 to 19 ng/10^6 cells/24 h. We excluded two of the patients, who had GVAX production of only 20 and 12.4 ng/10^6 cells/24 h, respectively, from our study. Cells from these individuals incorporated fewer copies of the integrated GM-CSF cDNA, and the cell doubling times were approximately two times greater than those of the cells producing >40 ng/10^6 cells/24 h GM-CSF (data not shown). It is likely that the extended cell doubling times resulted in poor GM-CSF transduction efficiency in the two excluded cases.

Safety of Administration and Systemic Toxicities

All of the six patients' primary cultures met the vaccine cell yield specifications for at least six injections. Importantly, the tests for microbial contaminants gave negative results for all six GM-CSF-transduced products. All four patients, designated Cases 1, 2, 3, and 4, satisfied all of the eligibility criteria for this study and

doi:10.1016/j.ymthe.2004.07.001

received vaccinations (Table 1). No surgical complications were encountered that would preclude subsequent vaccination, although Case 1 had mechanical ileus 30 days after nephrectomy, which was before vaccination, and Case 2 had ileus 219 days after nephrectomy, between the 12th and the 13th vaccine injections. These symptoms were resolved by intravenous fluid replacement for several days. In the latter case, ileus was thought to be a late adverse event related to nephrectomy, but not vaccination because reinitiation of vaccination did not cause any other ileus symptoms. When vaccine yield and clinical status permitted, we performed multiple vaccinations for analysis of cumulative side effects. Cases 1, 2, 3, and 4 received GVAX at 66, 58, 75, and 84 days after nephrectomy, respectively. They received 48 fully evaluable, 14-day treatment cycles. Finally, Cases 1, 2, 3, and 4 received total cell doses of 2.2×10^8, 3.7×10^8, 3.2×10^8, and 1.4×10^8, respectively. During the vaccinations, we observed no hepatic, renal, pulmonary, cardiac, neurological, or gastrointestinal toxicities in any of the patients other than mechanical ileus in Case 2 as stated above. We observed significant increases in the numbers of peripheral blood eosinophils, but not other leukocytes, after immunization, as shown in Table 1, and the peak eosinophil level gradually increased in each case after repetitive vaccinations (data not shown). We did not observe the two most concerning toxicities, vaccine site-specific ulceration and development of acute autoimmune disease and, specifically, nephritis in uninephric patients, except in Case 4, who experienced blister formation at the vaccination site following the 6th vaccination (Table 1). We detected no RCR (replication-competent retrovirus) during the postvaccination follow-up period in any of the four patients who received the vaccine cells. The apparent lack of acute, systemic toxicity in this trial was paralleled by the lack of plasma elevation of GM-CSF in pharmacokinetic studies following treatment (data not shown). Follow-up observations for long-term toxicity, including autoimmune disease, have been under way on our two surviving patients, Cases 2 and 4, and no vaccine-related long-term toxicity has been noted to date.

Phenotype of Cells at the Sites of Vaccination

Although there was some individual variation, we noted significant infiltration by $CD4^+$ T cells and eosinophils by day 30 (after the third vaccination); Case 3 had modest eosinophilic but intense mononuclear cell infiltration throughout the course of the vaccination protocol. Thereafter, these cell infiltrations were reduced in Cases 1 and 4, but increased in Cases 2 and 3. We could detect $CD68^+$ macrophages and CD20cy-positive B cells as minor populations, but their levels were unaltered during the course of the vaccination protocol. The level of HLA-DR expression by infiltrating cells was initially low, but increased by day 30. Intradermal $S100^+$ dendritic cells were occasionally observed in most cases. These findings were comparable with previous reports [14–20].

Delayed-Type Hypersensitivity Reactions

DTH tests using Multitest CMI showed that Cases 1 and 3 had anergic scores and Cases 2 and 4 had normal scores, i.e., within the range seen for normal volunteers or patients with localized cancer (data not shown). As shown in Table 2, we did not observe significant DTH reactions (>10 mm) to unpassaged, irradiated autologous RCC cells in any of the four patients prior to treatment. Following vaccination, we observed significant DTH reactions in all patients and they were strongest after the sixth vaccination. We also observed DTH reactions to normal renal cells (NRCs), but these reactions were almost always smaller than those to RCC cells.

We examined pathological phenotypes and numerical analysis of the DTH reactions. In all four cases, significant DTH reactions against RCC were induced by days 24–28 (following the second vaccination), compared with the day 0 controls (prevaccination). $CD4^+$ T cells were more dominant than $CD8^+$ T cells at the sites of DTH reaction, followed by $CD68^+$ macrophages and a few B cells, which was a common feature of DTH in all cases. We also observed various degrees of eosinophilic infiltration with degranulation. There were no significant differences between RCC and NRC with regard to the phenotypes of the cells in the DTH reaction, although more intense cell infiltration was observed against RCC than against NRC (data not shown). Although we detected significant DTH reactions until day 133 in Case 1, we observed a certain degree of attenuation in other cases.

Immunophenotypic Analysis of Tumor-Infiltrating Lymphocytes (TILs)

We performed immunophenotypic analysis of TILs for Case 1. Immunohistochemical analysis revealed that $CD4^+$ T cells were the predominant infiltrating cell type in pretherapy primary tumors, followed by B cells (data not shown). Fig. 3 shows TILs in perivascular areas (Fig. 3A), around the foci of tumor cell apoptosis (Figs. 3B and D), and in an unremarkable area (Fig. 3D), within biopsied skin metastatic RCC specimens that were obtained 5 months after initiation of therapy. Regardless of the area under observation, the T cells in this specimen were $CD8^+$, and we detected virtually no B cells. In addition, we observed increased numbers of $CD68^+$ macrophages, especially around the apoptotic foci. In contrast to the results of the DTH test, we did not observe eosinophilic infiltration of the tumors (data not shown). Interestingly, immunophenotypic analysis of the infiltrating cells at the sites of surgically resected renal cancer and normal renal tissue, autopsied normal liver, lung, and kidney showed a predominance of

TABLE 2: Immunological findings in patients who received GVAX

	Patient 1	2	3	4
Response to DTH skin test (mm)[a]				
Prevaccination	7 × 7/6 × 4.5	0 × 0/4 × 2	3 × 6/2 × 6	0 × 0/2 × 2
Peak reaction	85 × 65/30 × 35 (6)[b]	15 × 15/10 × 10 (6)[b]	25 × 25/2 × 1 (9)[b]	17 × 11/22 × 18 (6)[b]
Lymphocyte proliferation (cpm)				
Prevaccination	5,513	5,385	1402	1550
Post third	11,637	16,486	2836	6084
Post sixth	15,845	40,578	2442	6445
Cytokine production (pg/ml)				
IFN-γ				
Prevaccination	2746	UD	50	102
Post third	4952	199	481	UD
Post sixth	3568	394	967	UD
IL-5				
Prevaccination	UD	UD	395	128
Post third	1124	UD	863	1331
Post sixth	2088	792	1850	3017
IL-10				
Prevaccination	170	UD	UD	UD
Post third	80	UD	43	254
Post sixth	235	155	130	297
Cytotoxicity assay (%)				
Prevaccination	62.8	0.2	16.0	13.0
Post third	51.4	17.0	52.9	21.0
Post sixth	45.3	27.3	36.8	27.5
TCR Vβ gene-segment repertoire analysis	PB: 9,14,15,17 TIL: 10,17,21 DTH: 10,17	PB: 1,7,10,11,21 DTH: 1	PB: 4,18 DTH and Vac: 4,7	PB: 21,23 Vac: 9 DTH: 21

UD, under the detection level; PB, peripheral blood; TIL, tumor infiltrating lymphocytes; DTH, delayed type hypersensitivity.

[a] DTH reactions were examined using cultured autologous RCC cells/normal renal cells as antigens.

[b] The numbers in parentheses show that peak DTH reactions were observed after sixth or ninth vaccination.

$CD4^+$ cells, whereas analysis at the sites of biopsied or autopsied tumor tissues obtained after vaccination or after vaccination followed by low-dose IL-2, respectively, showed a predominance of $CD8^+$ cells (data not shown).

Vaccination Enhances the Proliferative Responses and Cytokine Production Against Autologous Tumors

We assessed the cellular immune responses using the peripheral blood mononuclear cells (PBMC) of patients who received GVAX. PBMC proliferated well in response to autologous RCC cell stimulation at all times tested (Table 2). In Case 2, the proliferative response observed before vaccination was augmented after vaccination. In all cases, vaccination markedly enhanced the proliferative responses to autologous RCC in the presence of IL-2. Especially, in Cases 2 and 4, those with prolonged clinically stable disease, the proliferative responses against autologous tumor cells remained high until the end of the study (data not shown).

IFN-γ in cultures stimulated with autologous RCC was enhanced after the initial vaccinations in Cases 1, 2, and 3, but not in Case 4 (Table 2). Conversely, IL-5 and IL-10 production was enhanced after vaccination in all cases. The enhancement of IL-5 seemed to correlate with the eosinophilia observed after the sixth vaccination. We also measured IL-4 production, but the levels of this cytokine were all below the limits of detection (data not shown).

Vaccination Induces Cytotoxicity Against Autologous RCC, Allogeneic RCC, and Autologous NRC

Case 1 showed comparatively high cytotoxicity against autologous RCC before vaccination. This level was maintained until after the fifth vaccination, after which it decreased. This was consistent with the higher DTH responses against autologous RCC and NRC seen in this case (Table 2). In Cases 2, 3, and 4, vaccination increased and maintained cytotoxicity against autologous RCC (Table 2, Fig. 4). Moreover, the addition of F(ab′)2 anti-CD3 mAb efficiently inhibited cytotoxicity against autologous RCC, suggesting the involvement

doi:10.1016/j.ymthe.2004.07.001

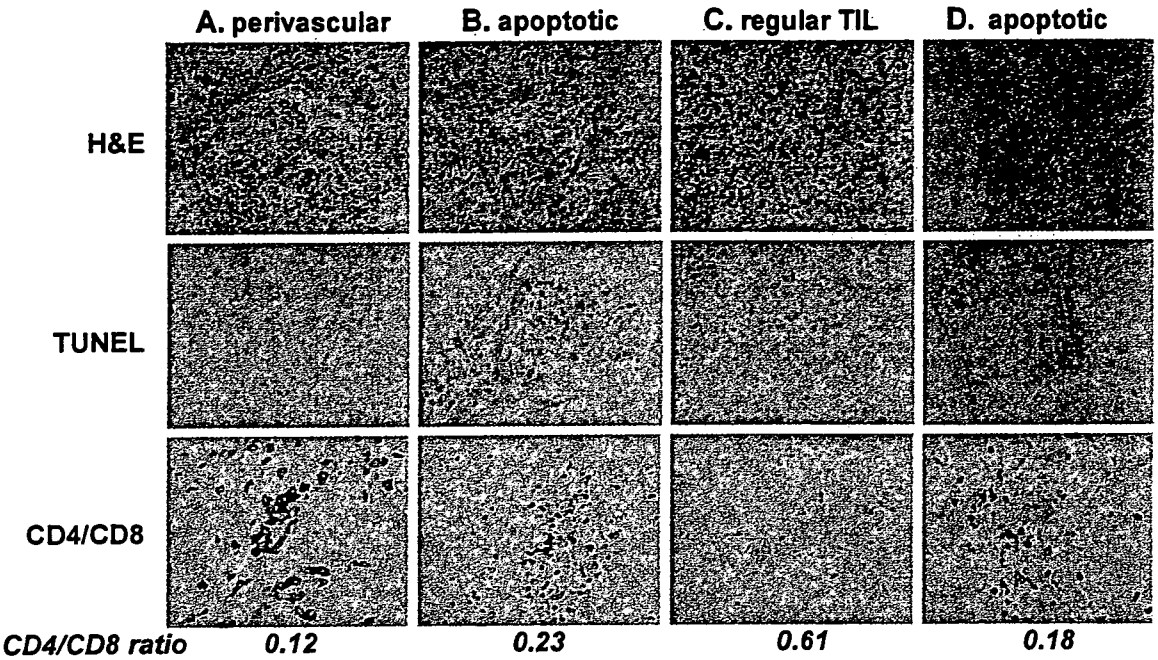
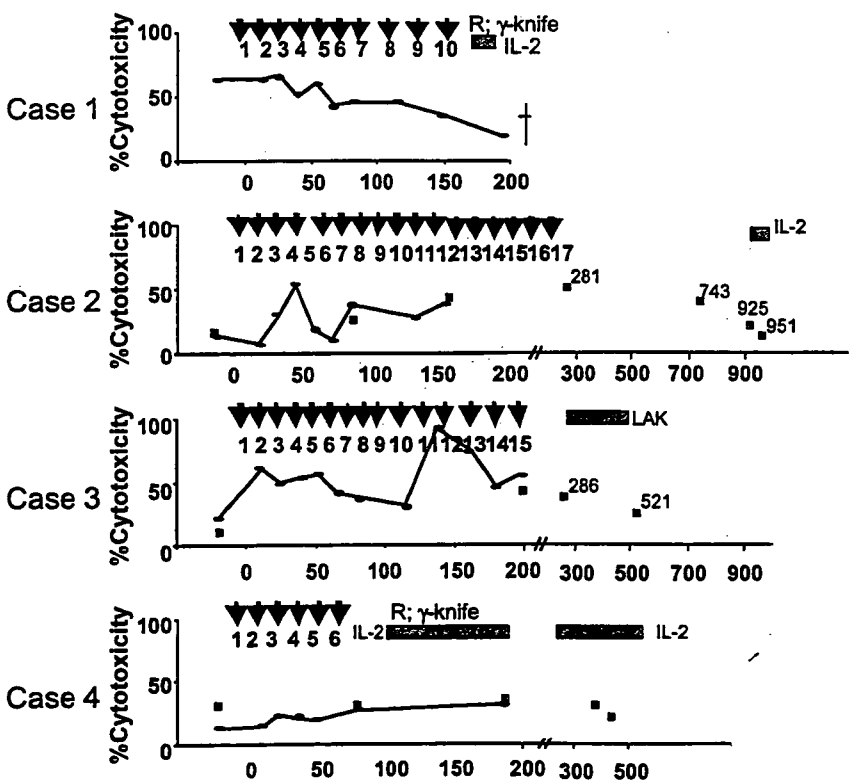


FIG. 3. Immunophenotypic analysis of tumor-infiltrating lymphocytes (TILs) in the RCC tumors of Case 1. TILs were observed in various areas, particularly (A) in the perivascular area, (B, D) around foci of tumor cell apoptosis, and (C) in unremarkable areas, within metastatic RCC that were obtained 7 months after the initiation of therapy. The T cell phenotype in this specimen had been converted to CD8 dominant, and few B cells were detected. To detect tumor apoptosis, the TUNEL method was applied as described under Patients and Methods.

FIG. 4. Cytotoxicity against autologous RCC in Cases 1 to 4. PBMC were cultured with irradiated GM-CSF-transduced autologous RCC in the presence of IL-2 for 7 days in 96-well microplates and ^{51}Cr-labeled target cells were added. After a 6-h incubation, cytotoxicity was measured as described under Patients and Methods. Asterisks in the panels for auto-RCC targets show the cytotoxicity, which was inhibited by more than 25% by the addition of F(ab')2 anti-CD3 mAb. Additional experimental values (squares) assessing the long-term results with additional treatment after the last vaccinations are also shown. IL-2, the rectangular bar represents the period of the administration of low-dose IL-2; R; γ-knife, gamma knife treatment was done for brain metastasis before the administration of IL-2.



of MHC-restricted T cell receptor (TCR)-mediated cytotoxicity.

TCR Vβ Clonotypic Analysis in DTH, Lung Metastasis, and RCC

In Case 1, we analyzed TCR Vβ gene usage in peripheral blood lymphocytes (2 days before vaccination and after the 6th and 9th vaccinations) and in tumor-infiltrated lymphocytes collected from tissue samples from the original surgically resected tumor, DTH skin biopsy (biopsied after the 4th vaccination), a metastatic skin lesion (biopsied after the 10th vaccination), and autopsied right hilar main lung metastases. We estimated the variation in the signal intensity by comparing the ratio of each Vβ signal observed in the regressed metastasized lung lesion with that observed in the nonregressed lung lesion or at the biopsied RCC or NRC DTH sites. The Vβ repertoire, which was overexpressed in the former sample, but not in the latter, was considered to indicate strong candidate T cell clones that were specifically induced by GVAX. In Case 1, we observed oligoclonal expansion of T cells with Vβ 9, 14, 15, and 17 repertoires in peripheral lymphocytes after the 9th vaccination (Fig. 5A). T cells with Vβ 10, 17, and 21 repertoires infiltrated the regressed tumor to a greater extent than the nonregressed tumor. These cells were also observed in the metastatic skin lesion (Fig. 5B). Interestingly, after vaccination, T cells with Vβ 10, 17, and 21 repertoires expanded clonally in the peripheral blood of Case 1, and the amplified TCR exactly matched those of the amplified fragments in a tumor-specific manner, namely T cells with Vβ 10 expanded dominantly in original tumor and lung metastasis, T cells with Vβ 17 in lung metastasis and much less in original tumor, and T cells with Vβ 21 in arm and lung metastasis and less in original tumor (Fig. 5C).

In Case 2, a T cell clone with Vβ 1 was increased in the pre- and postvaccination peripheral blood, nephrectomized tumor, biopsied sacral tumor, and DTH sites (Table 2). We saw oligoclonal expansion of T cells with Vβ 7, 10, 11, and 21 in peripheral lymphocytes after the vaccinations. Among them, T cell clones with Vβ 10, 11, and 21 expanded gradually after the first vaccination, and those with Vβ 7, 11, and 21 were also found in the nephrectomized original tumor.

In Case 3, the number of peripheral blood T cells with Vβ 18 was increased after the 6th, 9th, and 11th vaccinations and those with Vβ 4 were increased only after the 1st vaccination. Interestingly, T cells with Vβ 4 and 7 were increased at the RCC DTH sites after vaccination. These cells were also found at the vaccination site. T cells with Vβ 4 also infiltrated the metastasized liver tumor and nephrectomized original tumor.

In Case 4, peripheral lymphocytes contained increased numbers of T cells with Vβ 23 after the vaccinations. T cells with Vβ 9 were expanded after the sixth vaccination at the vaccinated site. T cells with Vβ 21 were expanded at the RCC DTH sites. The results of the T cell repertoire analysis are summarized in Table 2.

Western Blotting

To examine whether the therapeutic regimen induced antitumor antibody responses in patients, we compared the serum antibody reactivity against autologous tumor cell lysates before and after initiating the therapy by immunoblot analysis. Using posttherapy serum as probes, high-molecular-weight proteins of around 250 kDa generated clear signals, whereas the pretherapy serum showed no or only weak signals at the same position (Fig. 6A), suggesting that the GVAX induced an antibody response in Cases 1, 2, and 4, while the results were less clear in Case 1. The signals appeared in similar positions in all patients, suggesting the presence of common antigens. These high-molecular-weight antigens were present in both tumor lysates and NRC lysates (Fig. 6A). In addition, human lip-derived fibroblasts might have identical antigens, while H69 lung cancer cells did not (Fig. 6B). Furthermore, the changes in the magnitude of the antibody immunoreactivity over time were analyzed using serum from Case 2. The strongest signal was observed in serum obtained 67 days after the initial vaccination, between the 5th and the 6th vaccinations. This response was maintained from day 67 until day 281, just after the 17th vaccination, and the immunoreactivity remained until the last time point examined at day 950 (Fig. 6C).

Clinical Outcomes

According to the standard clinical criteria, Case 2 was in stable disease, Case 4 was in mixed response, and Cases 1 and 3 were in progressive disease during the course of GVAX treatment (Table 1). As described under Case Presentations, Case 1 and Case 3 died of multiple metastases 7.5 and 45 months, respectively, after the first vaccination. Case 2 and Case 4 are alive 62 and 44 months, respectively, after the first vaccination in a stable condition with a performance status of zero. Interestingly, Case 1 with progressive disease and Case 4 with mixed response showed 30% decreases in their main or total lung metastatic lesions, respectively, 1 to 3 months after the start of low-dose IL-2 treatment (Figs. 2A, B, and 4).

DISCUSSION

Although the production of the GVAX from all six patients was successful, in two cases the levels of GM-CSF produced were not high enough for cell injection. Our production rate of 67% was compatible with previous reports [14–16]. The poor transduction efficiencies in our two patients were probably due to slowed proliferation of these RCC cells, as reflected by the extended doubling times. To overcome the heterogeneous transduction efficiency with retroviral vectors,

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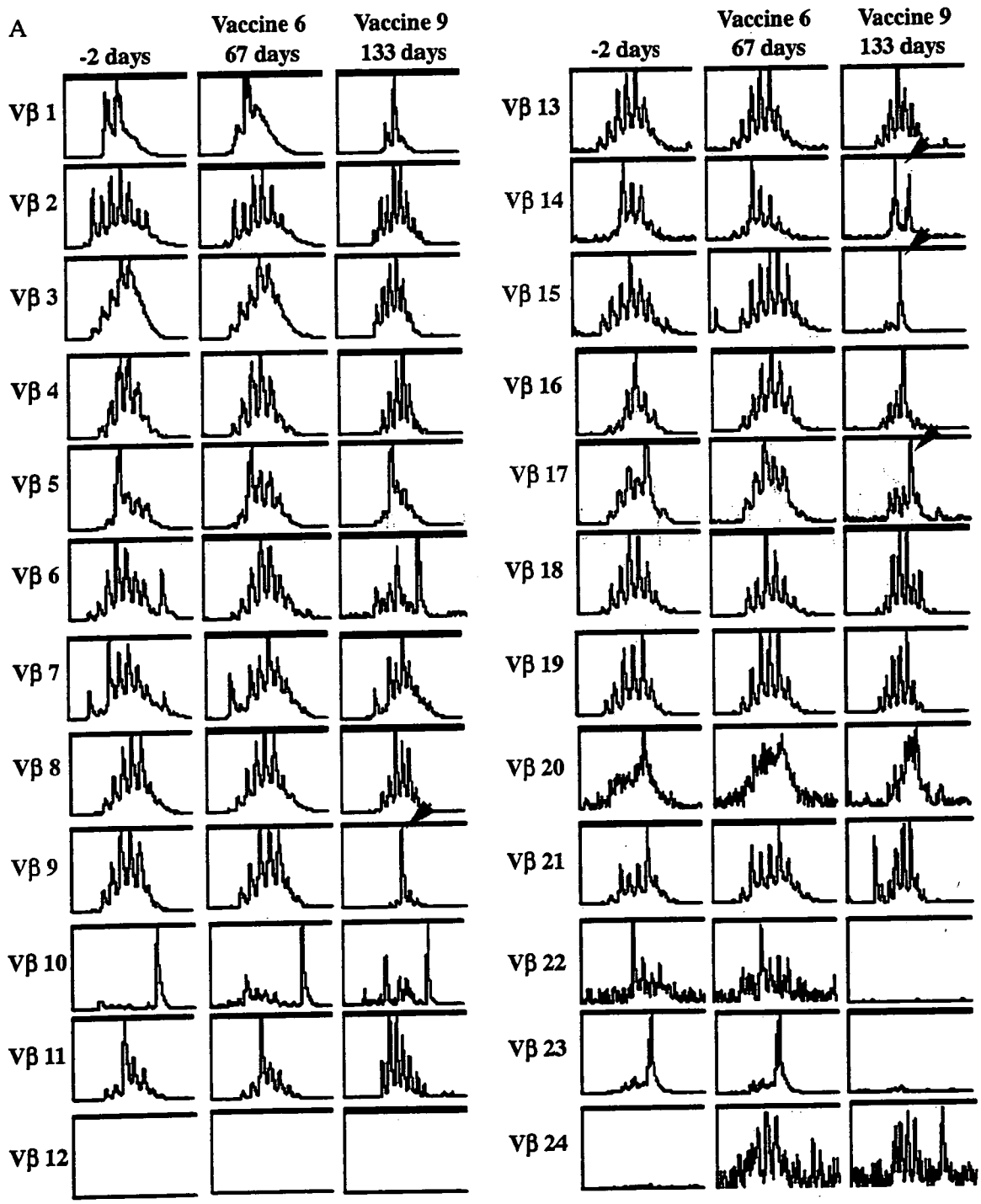

FIG. 5. TCR Vβ clonotypic analysis of the T cell infiltration in DTH, lung metastasis, and renal cell carcinoma in Case 1. (A) Oligoclonal expansion of T cell Vβ 9, 14, 15, and 17 repertoires was observed in peripheral lymphocytes after the ninth vaccination. (B) Larger numbers of T cells with Vβ 10, 17, and 21 repertoires infiltrated the regressed tumor than the nonregressed tumor. These cells were also observed in the skin metastasis designated Arm Meta. (C) After vaccination, T cells with Vβ 10, 17, and 21 repertoires were clonally expanded, and the amplified respective Vβ fragments exactly matched those of the amplified fragments in a tumor-specific manner.