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Dendritic Cell-Based Autologous Tumor Vaccines for Head and Neck Squamous Cell Carcinoma: Promise vs Reality

Theresa L. Whiteside, PhD^{1,2,3,6}, Robert L. Ferris, MD, PhD^{1,3,6}, Miroslaw Szczepanski, MD⁶, Mitchell Tublin, MD⁴, Joseph Kiss, MD^{5,6}, Rita Johnson, RN⁶, and Jonas T. Johnson, MD^{1,6}

¹Department of Otolaryngology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15217 USA

²Department of Pathology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15217 USA

³Department of Immunology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15217 USA

⁴Department of Radiology, University of Pittsburgh School of Medicine, Pittsburgh, PA 15217 USA

⁵Department of Medicine, University of Pittsburgh School of Medicine, Pittsburgh, PA 15217 USA

⁶University of Pittsburgh Cancer Institute, Pittsburgh, PA 15217 USA

Abstract

Background—An autologous vaccine of apoptotic tumor cells (ATC) & dendritic cells (DC) was administered to stage III/IV HNSCC patients to study safety and feasibility.

Methods—Autologous DC were generated from monocytes, loaded with ATC and delivered intranodally. Delayed-type hypersensitivity (DTH) and immunological endpoints were measured pre/post vaccination. Clinical follow-up was required.

Results—Tumors obtained from 30 patients yielded $2 \times 10^6 - 2 \times 10^8$ tumor cells. Only 19/30 (63%) were sterile. 10/30 patients (33%) had 1×10^7 sterile tumor cells required for vaccine production. 8/10 had positive recall DTH. 5/10 were leukapheresed to generate DC. 4/5 were vaccinated. ATC-reactive T cells were detected in 3/4 patients. All 4 survived > 5 years. The trial failed to enroll the projected 12 patients and was terminated.

Conclusions—This vaccine was safe and immunogenic but feasible only in HNSCC patients with positive pre-vaccine DTH and 1×10^7 sterile tumor cells. All vaccinated patients were long-term disease-free survivors. [Words, 150]

Keywords

dendritic cells; apoptotic tumor cells; HNSCC; vaccine; T-cell responses

INTRODUCTION

Squamous cell carcinomas of the head and neck (HNSCC) account for ~ 40,000 new cases per year in the US alone and 500,000 cases worldwide.^{1, 2} Patients with early stage disease are treated with surgery or radiation therapy (RT) and have good prognosis. Patients with stage III or IV disease usually undergo combined surgery and radiation/chemotherapy (CRT), but the rate of recurrence or second primary tumors is high (~40%), and 15–20% of patients develop distant metastases.^{3, 4} Although early detection as well as surgical procedures and CRT delivery have improved in the last two decades, the five-year survival did not, remaining at approximately 50%. In recent years, targeted therapies largely aimed at silencing the epidermal growth factor receptor (EGFR) using tyrosine kinase inhibitors or antibodies alone and in combination with RT have been widely used.⁵ However, these therapies have had little impact on survival⁶ and novel approaches are needed to prevent recurrence, which remains a major problem. Vaccines represent another form of targeted therapies designed to activate anti-tumor immune responses and induce immunologic memory. In HNSCC patients with no evident disease (NED) after primary therapy, vaccines offer a potential of preventing recurrence.

Anti-tumor vaccines have been broadly used for immunotherapy of different cancers (reviewed in ⁷). However, in HNSCC, vaccines have only been evaluated in a small number of clinical trials^{8–11}, and dendritic cell (DC)-based vaccines for HNSCC have been rare.¹² This paucity of DC-based clinical trials in HNSCC is related to the lack of well-defined immunogenic tumor-associated epitopes for vaccine production. This is in contrast to other human malignancies, e.g., melanoma or breast carcinoma, where numerous epitopes can be selected for immunization.^{13, 14} An obvious alternative strategy is to use whole tumor cells as a source of tumor-associated epitopes, which can be *ex vivo* delivered to autologous DC.

Several years ago, we initiated a pilot clinical trial for advanced HNSCC patients designed to test whether autologous DC-based whole tumor vaccines can induce anti-tumor immunity in HNSCC patients and benefit those patients who, after successful first-line therapy, are at risk of recurrence or second primary tumor development. This report illustrates how problems inherent to HNSCC impose a limit on vaccine production, making this vaccination strategy an unlikely candidate for a wider clinical application and suggesting an alternative therapeutic strategy.

MATERIALS AND METHODS

The protocol schema

This pilot/feasibility trial was designed to enroll 12 patients with advanced operable HNSCC. The study was approved by the Institutional Review Board (IRB) at the University of Pittsburgh and by the Food and Drug Administration (FDA) (IND #010318). All patients signed the informed consent form. Patients underwent surgical resections, and their tumors or tumor-involved lymph nodes were harvested into sterile medium containing antibiotics. Tissues were enzymatically dissociated to yield single cells. The tumor cell suspensions were sampled for 14-day sterility tests, aliquoted into sterile vials, cryopreserved using Cryomed and banked in liquid N₂ vapors. Following surgery, the patients received

conventional therapy and two months after its termination were able to receive the vaccine, provided they met the eligibility criteria and the vaccine could be produced and released. Two consecutive vaccines were administered 6 weeks apart. Prior to and following immunization, the patients' peripheral blood was obtained for immune monitoring. The patients were clinically followed to assess their disease and monitored for recurrence or second primaries.

Eligibility criteria

Patients with stage III or IV resectable disease and histologically-confirmed HNSCC whose tumors yielded a sufficient number (10^7) of sterile tumor cells were eligible to receive the vaccine. In addition, only patients who after primary therapy had a positive delayed-type hypersensitivity (DTH) response to at least one of three recall antigens were eligible. This requirement was intended to limit eligibility to patients able to mount an immune response. Patients had to be willing to undergo leukapheresis to obtain monocytes for DC generation. Patients could not receive the first vaccine until two months after termination of primary therapy. No other oncologic therapies could be administered concurrently with the vaccine.

Autologous tumor (AuTu) cells

To recover tumor cells, tissues resected during surgery were delivered to the laboratory for processing. A part of the tumor was submitted for histopathology. Tissues were minced into small fragments using sterile disposable scalpels and digested with a cocktail of enzymes as previously described.¹⁵ Suspensions were layered on differential Ficoll-Hypaque gradients and centrifuged to separate tumor cells from inflammatory cells as described.¹⁶ The recovered tumor cells were counted in the trypan blue dye and sampled for 14-day sterility testing (BacT Alert System). Tests for mycoplasma and endotoxin were also performed.¹⁷

Monocyte recovery and DC culture

Patients underwent leukapheresis at >2 months after the termination of conventional therapy. The blood product was delivered to the IMCPL (the cGMP facility for the UPCI) and peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque centrifugation. The mononuclear cells recovered from the leukapheresis product of each subject represented a unique batch of cells and were handled accordingly. Monocytes were separated by plastic adherence for 2 h at 37°C in the atmosphere of 5% CO₂ in air. Lymphocytes were removed by washing 4–5 × with pre-warmed growth medium, and plastic-adherent monocytes were supplemented with growth medium containing cytokines and cultured to generate DC.

Monocytes were cultured in T162 plastic flasks containing AIMV medium supplemented with 1,000 IU/mL of IL-4 (CellGenix, Freiburg, Germany) and 1,000 IU/mL of GM-CSF (Bayer, Bridgewater, NJ) for 6 days. The cultures were maintained in an atmosphere of 5% CO₂ in air at 37°C and were supplemented on day 3 with IL-4 and GM-CSF added to fresh culture medium. On day 5, each flask was sampled for sterility, mycoplasma and endotoxin levels. Immature DC (iDC) were harvested on day 6, and an aliquot of these cells was used for phenotyping by flow cytometry. The remaining DC were co-incubated with autologous apoptotic tumor cells (ATC).

Apoptosis of tumor cells

Vials of AuTu tumor cells were removed from liquid N₂ vapors, and cells were rapidly thawed and washed in sterile medium. Cells were counted in the trypan blue dye to determine recovery. Apoptosis of tumor cells was induced using UVB light. Briefly, tumor cells were irradiated using 1,500 $\mu\text{W}/\text{cm}^2$ UVB (bulb BLE-GT 302, Spectromics Corp., Westbury, NY) for 15 min.¹⁸ Previous experiments confirmed that HNSCC cells treated with UVB as described above undergo apoptosis (i.e., bind Annexin V) and fail to grow in culture¹⁸ or when injected subcutaneously into nude mice (data not shown).

Loading of iDC with ATC

iDC were resuspended in AIMV medium and co-incubated with ATC at the ratio of 5:1 for 18 h to allow for the ATC uptake by iDC. This was monitored by flow cytometry as previously described.¹⁸ Next, maturation of iDC was induced by the addition of IL-1 β , IL-6 and TNF- α , each at 10ng/mL, and PGE₂ at 1 $\mu\text{g}/\text{mL}$ to the co-culture. DC were incubated with these cytokines for 24h at 37°C, and mature DC (mDC) were tested for their phenotype by flow cytometry and for IL-12p70 production.

DC phenotyping

The phenotype of iDC and mDC was determined by flow cytometry following surface staining of cells with FITC- or PE-conjugated monoclonal antibodies (mAbs) as previously described.¹⁹ All mAbs were purchased from Becton Dickinson (San Jose, CA) or Coulter (Miami, FL) and included the following specificities: MHC-class I and class II, CD80, CD83, CD86, CD11c, CD40, CD54, CD14, CD3 and CD19. In addition, PE-labeled mAbs to CCR7 were purchased from R&D Systems (Minneapolis, MN). All mAbs were pre-titered on DC obtained from buffy coats of normal donors. Appropriate isotype control antibodies were included in all assays. At least 10,000 cells were acquired for analysis. The purity of at least 70% of DC was a release criterion for DC products generated for therapy.

DC potency

The ability of iDC or mDC to produce IL-12p70 following *ex vivo* stimulation with CD40L $\pm$ LPS was a measure of potency. The assays involved co-incubation with J558 cells transfected with the human CD40L gene as described by us.¹⁹ The assay results were recorded but were not used as a criterion for the product release.

Vaccine formulation and delivery

To prepare the vaccine for delivery, cells were adjusted to the final concentration of $2-5 \times 10^6$ mDC/mL, and the vaccine was divided into two equal parts. One aliquot was cryopreserved, while the other was formulated in 5% (v/v) human serum albumin (clinical grade) for immediate delivery. The cryopreserved portion of the vaccine was thawed just prior to 2nd vaccination, and the cells were washed $\times 3$ in phosphate buffered sterile saline and formulated for delivery. Thus, the first DC vaccine was made with freshly-harvested DC, while the second was prepared with cryopreserved/thawed DC. Viability and potency of cryopreserved, thawed and freshly harvested DC were found to be comparable. The vaccine was placed in a labeled, sterile 1mL tuberculin syringe and delivered by hand to the

radiology suite for immediate administration. Two consecutive immunizations given 6 weeks apart were delivered by intranodal injections under ultrasound guidance in the radiology suite. An aliquot (25%) of the vaccine (from 2 to 6×10^6 ATC-loaded DC) was also administered intra-dermally and tested for induration at 24–48 hours after vaccination.

Vaccine testing prior to release

For the vaccine release for therapy, results of Gram stain endotoxin and mycoplasma assays had to be negative. The tests were performed in the IMCPL as previously described.¹⁷ Fourteen-day sterility assays for aerobic and anaerobic microorganisms were performed using the BacT Alert System, but results were not available until after vaccine administration.

Immunologic Studies

Peripheral blood was obtained from patients immediately prior to first vaccination, on day 14, prior to second vaccination (week 6) and on week 12.

Immunofluorescence of tumor tissues

Sections of OCT-embedded tumor tissues were stained with labeled monoclonal antibodies specific for various immune cells as previously described.²⁰ H&E stained sections were also prepared.

ELISPOT assays

ELISPOT assays were performed in the IMCPL as previously described.²¹ Responder T cells were sensitized *in vitro* by co-incubation with ATC for 24h prior to ELISPOT to amplify the *in vitro* response. Spots were counted by computer-assisted image analysis (Zeiss ELISPOT 4.143, Jena, Germany), and the counts used to calculate the frequency of ATC-reactive T cells/ 10^5 PBMC. The background values (in control wells) were subtracted from experimental values.

Absolute numbers of lymphocyte subsets

A two-platform method was used. First, routine WBC counts and lymphocyte differentials were obtained. Next, the percentages of T-cell subsets were calculated using flow cytometry values to determine absolute counts for CD3+, CD4+ and CD8+ T cells and CD19+ B cells.

Annexin V binding to T cells

Flow cytometry was used to measure the percentages of CD8+ T cells binding Annexin in the peripheral circulation as previously described.²²

Treg percentages

PBMC were stained with anti-CD3, -CD4, -CD25 mAbs and were studied by flow cytometry gating on lymphocytes. The percentages of circulating CD3+CD4+CD25^{high} FOXP3+ T cells were determined as described.²³

RESULTS

Eligibility criteria and patients

The protocol stipulated that at least 10^7 AuTu cells were to be recovered from surgically-removed tissues to produce the DC-based vaccine. Further, the tumor cells had to be free of bacterial, fungal or mycoplasma contamination following processing and banking. Tumor (n=11), tumor-involved lymph nodes (LN) (n=18) or tumor and LN (n=8) obtained from 30 patients yielded from 2×10^6 to 2.3×10^8 tumor cells. Specimens ranged in weight from 0.3 g to 24 g, with a mean weight of 6.5 g. Only 19/30 (63%) tumor cell suspensions were sterile by the 14-day BacT Alert test. Of these, 10 (33%) had 10^7 cells available for producing the vaccine. Of the 10 patients who qualified for vaccination based on the number of available tumor cells, only 8 were positive to at least one of 3 DTH recall antigens and thus were eligible to receive the vaccine. One patient died and two refused to undergo leukapheresis, which was necessary for DC generation. Five patients were leukapheresed, and DC products were generated from all five. However, only 4/5 received the two vaccines, as the laboratory encountered problems with generating sufficient number of DC for the 5th patient. Overall, the number and sterility of recovered AuTu cells, both essential for the production of DC-based vaccines, proved to be the factors limiting therapy in this cohort of 30 patients with resectable stage III or IV HNSCC.

All 4 patients who received the vaccine presented with histologically-confirmed stage III or IV HNSCC (T1N1M0, T1N1M0, T0N2M0, T1N3M0) at diagnosis. Three of four had a smoking history and reported only moderate alcohol intake. None had previously received immunotherapy. Prior to vaccination, the patients underwent surgery and chemoradiotherapy (CRT) which had to terminate at least two months prior to vaccination to allow for the recovery of the lymphoid compartment after CRT. The mean interval between surgery and leukapheresis for vaccine production was 9 months, because patients often wished to rest after CRT before committing to adjuvant vaccination. Patients had to be NED to receive the vaccine.

Quality of DC generated for therapy

In all 5 eligible patients, sufficient numbers of monocytes were recovered from leukapheresis products, and the quality of generated iDC and mDC was comparable to that of the respective DC populations cultured from monocytes obtained from normal donors (Figure 1A). Loading with apoptotic tumor cells under conditions previously described¹⁸ yielded iDCs with internalized tumor-derived material (Figure 1B). Maturation of these iDC in the presence of a mix of cytokines up-regulates TAP1 and TAP2 expression resulting in efficient antigen processing and presentation to T cells.²⁴ The DC recovery ($3\text{--}6 \times 10^7$), viability (90–100%), purity (80–95%) as well, and potency (IL-12p70 production) the phenotype of DC fed with ATC were similar to those we routinely see with normal DC. The vaccine potency defined by IL-12p70 secretion by ATC-loaded mDC of the patients at a mean of 831 pg/mL/ 1×10^6 DC/24h higher than the norm established at the IMCPL for 15 normal donors (125–345 pg/mL).

***In situ* analysis of tumor-infiltrating lymphocytes (TIL)**

As cryopreserved tumor tissues of all 5 eligible patients were available for *in situ* multi-color immunofluorescence, the TIL phenotype and localization were determined. While differences were observed in the degree of TIL infiltration, the CD4/CD8 ratios, the frequency of Treg as well as the frequency of CD11c+ DC, these tumors contained many inflammatory cells, as illustrated in Figure 2.

Vaccine delivery

Four patients received both vaccines. No problems were encountered and no vaccine-related toxicities were evident. The DTH sites were examined at 48 and 72h post intradermal challenge with the first vaccines and were recorded as negative (no induration). This response remained negative after the second vaccine, indicating an absence of *in vivo* detectable anti-tumor response.

Immunological responses

The vaccine did not alter absolute counts of lymphocytes. One in four patients had borderline low lymphocyte count (Table 1). Figure 3 shows the frequency of CD4+ and CD8+ T cells, CD19+ B cells or CD3-CD56+CD16+ NK cells in the peripheral circulation of the immunized patients. The frequency of Treg (CD4+CD25^{high}FOXP3+) in the patients' circulation prior to vaccination ranged from 0.5–1.0% and did not increase after vaccination, remaining within the normal range. However, the ratio of CD8+T/Treg was increased in 2/4 patients (Figure 4A). We observed a decrease in the percentages of circulating ANX V+ CD8+ T cells after vaccination in all four patients (Figure 4B).

ELISPOT assays following IVS with ATC were positive in 3/4 patients after the second vaccine (Table 2). In this study, 1/4 patients had detectable tumor-reactive T-cell precursors prior to vaccination. This patient also had a normal lymphocyte count (#3 in Table 1), a favorable CD8+T/Treg ratio of 5 (Figure 4A) and a relatively low frequency of ANXV +CD8+ T cells (Figure 4B). One patient did not respond, although her T cells were responsive to OKT3 Ab. She remains NED 12 years after vaccination. The ELISPOT results confirm that HNSCC patients are capable of response to the vaccine and that the generated responses can target the autologous tumor.

Clinical responses

The four vaccinated patients were regularly followed after vaccination with half-yearly clinical examinations. All remained NED for 5 years. One patient (the oldest treated at the age of 75 years) died 6 years after primary surgery of natural causes. Three others are alive and are NED at 10, 11 and 12 years after surgery, respectively, and are still being followed.

DISCUSSION

This pilot feasibility study of a DC-based vaccine for the stage III or IV operable HNSCC patients was well tolerated and no significant toxicities were observed. The study remained opened for 5 years but since enrollment was unsatisfactory, it was then terminated. This was entirely due to difficulties in meeting the eligibility criteria, which, when seen in retrospect,

were probably too stringent. Specifically, the requirement for DTH responses to 1/3 recall antigens was perhaps unrealistic for HNSCC patients with stage III/IV disease, as previously suggested.²⁵ The other limiting factors were related the inadequate number of tumor cells recovered and the lack of sterility of the tumor cell suspensions. Only 4/30 patients whose tumors were processed met the eligibility criteria (i.e., responded to recall antigens and had sufficient numbers of sterile tumor cells for vaccine production) and thus could be vaccinated. All 4 patients who received the vaccine had advanced disease with nodal metastases, and the vaccine was administered following CRT in the minimal-residual-disease setting. All 4 patients remained NED for at least 5 years after vaccination and 3/4 remain disease free 10–12 years after their initial surgery. However, this cannot be interpreted as vaccine-related survival because the prognosis of patients with T1N1-3M0 disease is generally favorable. All vaccinated patients had tumors that were richly infiltrated with lymphocytes, generated sufficient numbers of iDC from monocytes and showed evidence of increased post-vaccination frequency of circulating AuTu-reactive T cells. Peripheral absolute lymphocyte counts remained stable after vaccination, the CD8+T/Treg ratio increased or remained unchanged and percentages of AnnexinV+CD8+ T cells decreased, suggesting a partial immune recovery. The trial never accrued the projected 12 patients and despite the absence of toxicity, no technical difficulties with culture of DC and potentially beneficial outcome in 4 vaccinated patients, it was considered a failure.

Today, after several years of delivering anti-tumor vaccines to patients with many different tumor types, such vaccines are considered to be feasible, safe and potentially beneficial.²⁶ In HNSCC, where targeted drug therapies have not improved overall survival, anti-tumor vaccines could offer another targeted strategy as a potentially beneficial adjunct to conventional therapies. However, the selection of an optimal vaccine represents a considerable problem in HNSCC. Despite intensive search worldwide, few, if any, well-defined, immunogenic peptides are available for direct delivery or DC pulsing in HNSCC.^{26, 27} AuTu cells fed *ex vivo* to DC represent an alternative source of processed and presented peptides in HNSCC. Experience with other AuTu-based vaccines used for therapy of human solid tumors suggests that these vaccines are often capable of overcoming tolerance to self and of repairing the aberrant tumor-specific immunity, shifting the prevalent Th2 to Th1 responses and prolonging patient survival.^{28, 29} It has been well documented that HNSCC progression is associated with immune suppression.³⁰ Therefore, a vaccine given after reduction of the tumor burden and capable of reversing immune suppression could restore anti-tumor immune responses, induce immunologic memory and prevent recurrence. This early pilot clinical trial was initiated as a proof-of-principle that a vaccine consisting of AuTu cells fed to DC was safe and immunogenic in patients with stage III/IV resectable HNSCC. Unfortunately, most patients failed to meet the entry criteria, specifically DTH positivity to at least 1/3 recall antigens. The intent to enroll those patients with stage III/IV disease who were immunologically competent was rational but proved to be unrealistic. In view of older data in the literature²⁵ this is not surprising. The second problem arose because of the requirement for a stipulated number of sterile AuTu cells for vaccine production. Although tumor cells derived from HNSCC specimens were harvested into sterile media in the OR, they were frequently contaminated with endogenous microorganisms, most commonly with *S. viridans*. Such cells could not be co-incubated with

DC to generate the vaccine. Based on our experience, it appears that vaccination with AuTu for HNSCC has to be approached differently. For example, the use of metastatic lymph nodes instead of primary tumor tissues might improve sterility. Another alternative might be direct injections of AuTu lysates together with an adjuvant or a cytokine such as IL-2 to HNSCC patients, similar to vaccinations in patients with melanoma.²⁹ In fact, endogenous bacteria present in such lysates could potentially serve as an additional adjuvant.

Despite failing the feasibility criteria, this trial demonstrated that AuTu-derived antigens presented by DC to patients' immune cells were effective in inducing immune responses. The small number of vaccinated patients precludes any meaningful correlation between these immune responses and the vaccine clinical efficacy. The trial also provided an opportunity for assessment of the tumor milieu before therapy through the study of tumor-infiltrating immune cells in specimens designated for processing. *In situ* immunophenotyping analysis indicated that these tumors attracted immune cells. As current data link the type and density of immune infiltrates into tumors (i.e., the immune score) with improved prognosis³⁰, an opportunity for examination of the tumor microenvironment could be helpful in elucidation of its role in the vaccination outcome.

In summary, this pilot trial illustrates the difficulties as well as opportunities in performing AuTu-based immunotherapy in patients with HNSCC. Because of the above discussed feasibility issues, it is unlikely that AuTu-pulsed DC will be used as vaccines in HNSCC. Nevertheless, the delivery of AuTu lysates relying on endogenous adjuvant-activated DC remains a possibility. At the very least, this pilot trial demonstrated that AuTu is a source of antigens that can provoke anti-tumor immune responses in patients with advanced HNSCC and that few patients who made such responses following vaccination have benefited from this therapy.

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Figure 1A

Figure 1B

Figure 1.

Phenotypic characteristics of DC generated for the vaccine (A) and evidence for internalization of apoptotic tumor by iDC (B). In A, representative flow cytometry data of one patient and one normal donor are presented for selected DC markers. The gate was set on DC: shaded area = isotype control; dotted line = iDC; solid line = mDC. In B, a confocal image of iDC with tumor fragments inside the cell is seen by confocal microscopy. Tumor cells were labeled with green fluorescent protein (GFP) prior to co-incubation with iDC as described in ref. 18. iDC were labeled with anti-HLA-DR-PE. Mag $\times$ 4,500

Figure 2 A, B

Figure 2 C, D

Figure 2.

Immune cells in the tumor-involved lymph node of one of the vaccinated patients. Sections of the OCS-embedded tissue were stained with H&E for light microscopy (**A**) or with antibodies specific for various immune cells (**B**, **C**, **D**) and examined in a fluorescent microscope. (**A**) Tumor cells (**TC**) are surrounded by a moderately dense infiltrate of immune cells. (**B**) Immunostaining for CD4⁺T-cells (green), CD8⁺T-cells (red) and DAPI (blue). (**C**) Immunostaining for CD3⁺T-cells (green), CD11c⁺ DC (red) and DAPI (blue). (**D**) Immunostaining for CD25⁺ cells (red), FOXP3⁺ cells (green) and DAPI (blue). CD25+FOXP3+ Treg cells are double-colored (red and green = yellow). Mag × 200 in **A**, **B**, and **C**. Mag × 400 in **D**. Inserts are enlargements of selected areas at Mag × 600.

Figure 3.

Frequency of CD3⁺CD4⁺ and CD3⁺CD8⁺Tcells, CD3⁻CD56⁺CD16⁺ NK cells and CD19⁺ B cells in the peripheral circulation of the four vaccinated patients prior to and after second vaccination. The gate was set on lymphocytes. The bars for patients #1 to #4 are averaged in order from left to right.

Figure 4A

Figure 4B

Figure 4.

The CD8+/Treg ratio (A) and the frequency of CD8+ANXV+ T cells (B) in the peripheral circulation of the four vaccinated patients prior to and post second vaccination. The gate was set on lymphocytes.

Table 1

Absolute lymphocyte counts for the patients who received the vaccine

Patient No.	Prior to Vaccination	Post 2 nd vaccine
	# lymphocytes/mm ³	
1	535	329
2	782	846
3	1,829	1,920
4	1,020	980

Table 2

Results of INF- γ ELISPOT assays performed with blood specimens obtained prior to and after vaccination ^a

Time of Blood Draw	Pt #1		Pt #2		Pt #3		Pt #4	
	OKT3	AuTu	OKT3	AuTu	OKT3	AuTu	OKT3	AuTu
Pre VAC	212	0	693	0	570	290	1056	0
Post 1 st VAC	625	0	824	0	449	20	693	0
Post 2 nd VAC	82	18	659	0	1100	156	1211	28
12 Wks Post VAC	410	18	844	9	1058	132	770	10

^aThe data are spot numbers responder cells. Prior to ELISPOT, patients' PBMC harvested before vaccination, after the 1st and 2nd vaccinations and on week 12 after vaccination were cultured with DC pulsed with autologous tumor (AuTu) lysate at the calculated 10:1 ratio for 24. Following this *in vitro* sensitization (IVS), ELISPOT assay was performed using IVS-sensitized PBMC as responders and AuTu cells as stimulators at the 1:1 or 5:1 ratios. ELISPOT responses to OKT3 were also measured to establish that the patient's T-cells were able to signal via the TcR.