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Impact of a Recombinant Fowlpox Vaccine on the Efficacy of Adoptive Cell Therapy With Tumor Infiltrating Lymphocytes in a Patient With Metastatic Melanoma

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Summary:

A patient with metastatic melanoma who had progressive disease after prior surgical resections, high dose interleukin-2, and anti-cytotoxic T lymphocyte antigen-4 antibody received sequential treatments with autologous tumor infiltrating lymphocytes that recognized the gp100 melanocyte differentiation antigen. Although no clinical response was seen when cells were administered alone, an objective clinical response to therapy was seen with tumor infiltrating lymphocytes administered together with a highly immunogenic fowlpox vaccine expressing a gp100: 209-217 (210M) epitope. Persistence of the transferred antigen-specific lymphocytes in the peripheral blood was observed only after adoptive cell therapy plus administration of vaccine. Cell proliferation in vitro was further stimulated by additional vaccine and interleukin-2. The patient has an ongoing partial response at 10 months after the last treatment.

Keywords

recombinant fowlpox vaccine; gp100: 209-217 (210M); metastatic melanoma; adoptive cell therapy; tumor infiltrating lymphocytes

BACKGROUND

Adoptive cell therapy (ACT) using autologous tumor infiltrating lymphocytes (TIL) has produced encouraging results in patients with metastatic melanoma. Published data from the Surgery Branch, National Cancer Institute demonstrated a 56% response rate in 93 patients with metastatic melanoma treated with autologous TIL after prior lymphodepleting preconditioning. Of the 93 patients reported in the study 10 had complete responses and 42 had partial responses.¹

Murine models based on the pmel transgenic mouse expressing a T-cell receptor that recognizes the gp100 melanocyte differentiation antigen on the B16 melanoma have demonstrated that vaccination against altered peptide ligands is an essential component for

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successful ACT.^{2,3} When used as sole treatment, cancer vaccines have limited or sporadic efficacy in the treatment of metastatic cancer.⁴ However, vaccines in combination with ACT have not been extensively investigated in human clinical trials.

This case report provides immunologic and clinical evidence from a patient with metastatic melanoma who received sequential treatments with the same autologous tumor-specific TIL. The results from various treatments strongly suggest that the persistence of antigen-specific T cells and the clinical response are significantly improved by the coadministration of a vaccine expressing an antigen recognized by the transferred T cells.

PATIENT HISTORY BEFORE ACT

The patient is a 56-year-old white male, human leukocyte antigen (HLA)-A0201 and A26, who was diagnosed with a primary facial melanoma. He subsequently underwent wide local excision followed by modified radical neck dissection. One of the 68 nodes harvested was positive for micrometastatic disease. He was then enrolled on an adjuvant vaccine trial of Bacille Calmette-Guérin and polyvalent allogenic irradiated whole cell lysate.⁵ Ten months later he developed 2 local recurrences, which were resected and followed with 2 cycles of adjuvant interleukin-2 (IL-2). He was disease free for 3 years and then developed right cervical lymphadenopathy. The nodes were resected and he was treated with adjuvant interferon- α for 1 year. He subsequently developed preauricular, supraclavicular, and retrosternocleidomastoid lymphadenopathy and was referred to the Surgery Branch, National Cancer Institute for treatment.

At the National Cancer Institute he was enrolled on a dose escalation trial of anti-cytotoxic T lymphocyte antigen-4 antibody with peptide vaccination.⁶⁻⁸ After one dose of anti-cytotoxic T lymphocyte antigen-4 antibody he developed an extensive grade 3 immune mediated dermatitis requiring topical therapy for 3 months. This excluded him from being retreated with additional doses of anti-cytotoxic T lymphocyte antigen-4 antibody. He developed progressive disease and was taken off study.

ACT WITHOUT VACCINE

Two cervical lymph nodes were resected for the generation of TIL. The techniques used to grow and test TIL have been described in detail.⁹ Briefly, the tumors were cut into individual fragments and placed in single wells of 24-well plates with media with IL-2. The lymphocytes were grown for 3 weeks until there were sufficient numbers for testing for tumor antigen reactivity. Some of the lymphocytes were then cryopreserved or further expanded for treatment. Reactive lymphocytes were placed in a rapid expansion protocol with soluble OKT3 (anti-CD3) antibody (Ortho Biotech, Bridgewater, NJ) and IL-2 in the presence of irradiated peripheral blood mononuclear cells.¹⁰ The rapid expansion protocol was performed over a 14-day period; greater than 1000 fold expansion was obtained from this method.

In the first protocol (Table 1) the patient received 31×10^9 TIL cells that were infused intravenously after a myeloablative regimen consisting of cyclophosphamide (60 mg/kg) for 2 days followed by fludarabine (25 mg/m²) for 5 days. On the day prior to cell infusion 200

cGy whole body irradiation was administered (Table 1). The infusion of TIL was followed by administration of intravenous recombinant IL-2 at 720,000 IU/kg every 8 hours. The patient tolerated a total of 11 doses of IL-2. Greater than 2×10^6 CD34⁺ granulocyte macrophage colony-stimulating factor mobilized stem cells were administered on day 2.

The phenotype by fluorescence-activated cell sorting analysis and the activity of the cells from the infusion product are shown in Table 2. A week after cell infusion, 2% of the CD8⁺ cells from the peripheral blood were positive for staining with the HLA-A2/gp100: 209-217 (gp209) tetramer (Fig. 1A). Computed axial tomography of the neck, chest, abdomen, and pelvis as well as magnetic imaging of the brain and photographs were used to document the sites of disease. The Response Evaluation Criteria In Solid Tumors was used to determine response.¹¹ At monthly follow-up evaluations there was a 20% reduction in the sum of the longest diameters of the 3 evaluable sites of disease, but the response did not meet criteria for a partial response (Fig. 1B).

The patient was then enrolled in a phase 1 trial with the intention of determining the impact of intra-arterial TIL infusion on clinical response. TIL from the initial bulk lymphocyte cultures were rapidly expanded for treatment and had similar phenotypic and functional characteristics as TIL used in his first treatment cycle (Table 2). The intra-arterial TIL infusion was followed by the infusion of 10 doses of intravenous IL-2. Owing to thrombocytopenia resulting from the first myeloablative regimen, no additional lymphodepletion was given before treatment 2.

A total of 26×10^9 cells were infused in the right external carotid, right thyrocervical, and right costocervical arteries under fluoroscopic guidance. However, after this treatment there was no antitumor response and no evidence of an increase in the number of circulating tetramer reactive T cells in the peripheral blood (Figs. 1A, B).

ACT WITH FOWLPOX GP100: 209-217 (210M) VACCINE

The patient then received the infusion of autologous TIL grown from cryopreserved lymphocytes that were same as those used to generate TIL from the prior treatments. However this third treatment included the infusion of a fowlpox vaccine encoding the gp100: 209-217 (210M) minigene (referred to as gp209-2M). This is a synthetic peptide in which methionine replaces threonine at position 2 in the native gp100: 209-217 peptide. The gp209-2M peptide has increased binding affinity to the HLA-A2 molecule.^{12,13}

Lymphodepletion with cyclophosphamide and fludarabine was performed on days -7 to -1. The first fowlpox vaccine was given intravenously a few hours before the 47×10^9 TIL and IL-2 was started a few hours later. He received 12 doses of IL-2. A second cycle of vaccine and IL-2 was administered 30 days later. There was no lymphodepletion or cell infusion during the second cycle (Table 1).

In contrast to the ineffectiveness of the prior treatments, the combination of TIL transfer plus vaccine had a marked impact on antigen-specific T cell engraftment and persistence. A week after infusion of the fowlpox gp209-2M vaccine and TIL cells, 7.0% of the CD8⁺ cells from the peripheral blood were gp209 tetramer positive up from 0.05% (Fig. 2A). A month later,

the frequency of antigen-specific cells had dropped to 0.73% at which time a second cycle of vaccine and IL-2 was administered without TIL transfer. After vaccine and IL-2 administration the level of the gp209 tetramer-positive cells in the peripheral blood rose to 11.3%.

Also in contrast to prior treatments without vaccine, the combination of TIL transfer plus vaccine resulted in a dramatic partial response at all tumor sites. Two sites of disease, the preauricular and supraclavicular lymph nodes had a complete response to treatment; and the largest tumor, a sternocleidomastoid lymph node metastasis had a 55% reduction in size (Figs. 1B, 2B). This response lasted for 58 weeks before evidence of progression of the sternocleidomastoid lymph node was noted (Fig. 1B).

In light of the significant response to ACT in combination with the fowlpox vaccine, the patient was retreated on this protocol. Bulk TIL that had been grown and cryopreserved were expanded for treatment and shown to have similar phenotypic and functional characteristics compared with the prior 3 TIL treatments (Table 1). The patient received 22.7×10^9 cells intravenously and administration of the fowlpox gp209-2M vaccine followed by 8 doses of IL-2. A month later, he returned for the second cycle of vaccine and IL-2 (Tables 1, 2).

The percentage of gp209 tetramer-positive cells in the CD8⁺ peripheral blood lymphocyte again increased after ACT with the vaccine reaching a maximum of 4%. In the second cycle of the protocol with only vaccine and IL-2, the percentage of the gp209 tetramer-positive cells in the peripheral blood increased to 2%. After this treatment there was a complete regression of the remaining 2 evaluable tumor sites and the objective response is currently ongoing at 10 months.

DISCUSSION

ACT has emerged as a promising modality for the treatment of patients with metastatic melanoma. ACT entails the infusion of autologous TIL which have been harvested from a metastatic lesion. Lymphocytes are selected for patient treatment based on their ability to expand in vitro and their ability to effect specific interferon- γ release against HLA-matched tumor targets or peptide-pulsed T2 cells in an overnight coculture assay.⁹ Despite recent reports of success with this therapeutic approach, most patients treated with tumor-reactive TIL relapse with progressive melanoma. Clinical strategies for improving this therapy deserve investigation.

This case offered a unique opportunity to observe the results of multiple ACT treatments in the same patient that differed by the inclusion of a patient vaccine. ACT treatments that included gp209-2M vaccine resulted in improved antitumor response and persistence of tetramer-positive cells in the peripheral blood compared with treatments that did not include the vaccine. The fluorescence-activated cell sorting tetramer analyses reported here were performed with the native gp100: 209-217 tetramer, although identical results were obtained in the same experiments using a tetramer constructed with a modified gp100: 209-217 (210M) peptide that is expressed by the fowlpox vaccine.

The 4 treatments had the same starting bulk TIL that had been rapidly expanded for treatment. As measured by interferon- γ release, the infusion samples had similar activities. The percentage of CD8⁺, CD4⁺, and gp209 tetramer-positive cells in the infusion samples were also similar between the treatments. Thus differences in the results among the trials are unlikely to be due to variation between infusion products. It is possible that the additive effect of sequential lymphodepletions could have affected the results seen in this patient. Similarly, the activated state of TIL relative to reconstituting peripheral blood leukocyte may have contributed to their disproportionate expansion upon IL-2 administration. However, during the second cycle of treatments number 3 and 4 there was a substantial increase in the gp209 tetramer-positive cells relative to all other CD8 cells (endogenous and other administered TIL). This increase occurred with administration of the vaccine and IL-2 alone, despite the fact that there was no additional lymphodepletion in these second cycle, strongly suggesting an antigen-specific rather than a nonspecific mechanism.

Studies of animal models and the results presented in this report support the need for additional clinical trials to test the efficacy of coadministration of TIL cells with vaccines expressing altered peptide ligands for ACT for metastatic melanoma. Pox virus-based vaccines seem to represent an ideal vaccine to combine with cell transfer therapies.

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FIGURE 1.

Clinical and immunologic impact of all treatments. A, Percentage of the gp209 tetramer-positive cells (percent of the total CD8 population) in the peripheral blood varies depending on the treatment administered. B, The longest diameters of the evaluable sites of disease is graphed versus time. LN indicates lymph node; Rx, treatment.

FIGURE 2.

Clinical and immunologic impact of the third treatment. A, Persistence of antigen-reactive cells in the peripheral blood lymphocyte (PBL). B, Computed axial tomography of the evaluable lesions before treatment 3 and the maximum response (arrows indicate the sites of evaluable disease). Cy indicates cyclophosphamide; Flu, fludarabine; Fp, fowlpox; TIL, tumor infiltrating lymphocytes.

TABLE 1.
Schedule of the 4 Treatments Administered on the 3 Protocols of Adoptive Cell Therapy

Day of Treatment	-7	-6	-5	-4	-3	-2	-1	0	1	2	3	//	30	31	32	33
Rx1	Cy	Cy	Flu	Flu	Flu	Flu	Flu									
							TBI	TIL (IV)								
								IL-2	IL-2	IL-2						
Rx2										CD34 ⁺						
								TIL (IA)								
								IL-2	IL-2	IL-2						
Rx3 and Rx4	Cy	Cy	Flu	Flu	Flu	Flu	Flu	Fpgp100					Fpgp100			
								TIL (IV)								
								IL-2	IL-2	IL-2			IL-2	IL-2	IL-2	

Cy indicates cyclophosphamide; CD34⁺, hematopoietic stem cells; Flu, fludarabine; Fpgp100, recombinant fowlpox vaccine expressing gp209-2M; IA, intra-arterial; IL-2, interleukin-2; IV, intravenous; Rx, treatment; TBI, total body irradiation; TIL, tumor infiltrating lymphocytes.

TABLE 2.

Characteristics of Treatments*

Infusion	Cell Number (×10 ⁶)	IL-2 (Doses)	Phenotype by FACS (%)				Melanoma Cell Lines				T2 Cells/Peptide (μM)				
			CD8 ⁺				HLA-A2 ⁻	HLA-A2 ⁺	MART	gp100	gp209-2M				
			CD8 ⁺	CD4 ⁺	gp209 ⁺	MART-I ⁺	888	938	526	624	(1.0)	(1.0)	(1.0)	(0.1)	(0.01)
			Interferon-γ (pg/mL)												
Rx1	31.0	11	65	25	6.3	0.09	5	75	1090	2210	81	8720	2540	12180	1280
Rx2	26.0	10	77	13	2.2	0.92	146	130	7230	13120	49	8500	670	556	482
Rx3	47.0	12, 3	84	6	7.4	0.41	29	110	1760	3260	39	6900	6110	4810	2420
Rx4	22.7	8, 7	85	7	6.5	0.31	57	579	3470	9180	20	17000	10970	8130	5710

* Activity measured by interferon-γ release by overnight coculture with 1e5 targets to 1e5 effectors and FACS analysis.
Values in bold are twice background and greater than 200 pg/mL.
FACS indicates fluorescence-activated cell sorting; gp209-2M, gp100; 209-217 (210M) peptide; HLA, human leukocyte antigen; MART, MART-1 27-35 peptide; Rx, treatment.