Pulmonary Sarcoid–like Granulomatosis after Multiple Vaccinations of a Long-term Surviving Patient with Metastatic Melanoma

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Abstract

Autoimmune side effects are frequent in patients with cancer treated with immune checkpoint–targeting antibodies, but are rare with cancer vaccines. Here, we present a case report on a patient with metastatic melanoma who developed pulmonary sarcoid–like granulomatosis following repetitive vaccinations with peptides and CpG. Despite multiple metastases, including one lesion in the brain, the patient is alive and well more than 13 years after the diagnosis of metastatic disease. The strongly activated tumor-specific CD8$^+$ T cells showed robust long-term memory and effector functions. It is possible that long-term survival and adverse autoimmune events may become more common for vaccines inducing robust antitumor immune responses as were present in this patient. Cancer Immunol Res; 2(12); 1148–53. ©2014 AACR.

Introduction

Metastatic melanomas are aggressive tumors, accounting for approximately 80% of skin cancer–related deaths. The prognosis is particularly dismal for patients with brain metastases as they face a median overall survival of only 4 months (1). Moreover, failure of treatment is frequent despite considerable recent progress.

Immunotherapy, aiming at the induction of tumor-specific immune responses, has emerged as a promising clinical weapon against cancer. During the past decade, several novel immunologic approaches, such as adoptive cell therapy and immune checkpoint blockade (targeting CTLA-4 or PD-1/PD-L1), have shown substantial clinical benefits in patients with advanced disease (2). However, because of their capacity to break immune tolerance, their toxicity profiles are usually high, resulting in autoimmune adverse events (AE; refs. 3, 4).

Significant progress has also been achieved with therapeutic cancer vaccines. Vaccines have the potential benefits of being highly selective, stable, and relatively easy to produce (5, 6). Furthermore, their low-toxicity profiles suggest that they can be attractive options for patients with cancer. Until recently, however, the majority of cancer vaccines have shown only limited immunogenicity and clinical responses (7, 8). In contrast, new-generation vaccines can give rise to strong responses of cancer-specific T cells (9). This is the case with the use of potent adjuvants, such as the Toll-like receptor ligand CpG (10). It is likely that further progress in vaccination will lead to strong and long-term immune responses with a broad spectrum of tumor antigen specificities (11).

Here, we present a case report on a patient with melanoma, who has one metastatic lesion in the brain and multiple peripheral metastases, and who experienced complete remission and long-term survival after treatment with vaccines. During the past 13 years, the patient progressively developed strong T-cell responses following repetitive vaccinations with Melan-A, NY-ESO-1, and MAGE-A10 peptides. The vaccine regimen comprised multiple peptides of melanoma-associated antigens augmented with CpG as adjuvant and was administered in a total of 49 injections. The peptide vaccines were the only systemic antitumor treatments that the patient received. The patient developed an autoimmune disease in the form of pulmonary sarcoid–like granulomatosis without clinical complications. To our knowledge, this is the first description of an autoimmune pulmonary AE related to a cancer vaccine.

Case Report

A 42-year-old man was diagnosed in 2001 with a left paralumbar ulcerated superficial spreading melanoma (Breslow 2.5 mm, Clark III, pT3pN1bM0). A wide local excision of the primary lesion and of the right inguinal sentinel lymph node was performed. He underwent right iliac obturator and inguinal radical lymph node dissection (none of the 10 iliac obturator lymph nodes or the four right inguinal lymph nodes were metastatic). He then continued with interferon-based immunotherapy for 4 years.

In 2005, 4 years after diagnosis and treatment, the patient developed a pulmonary AE related to a cancer vaccine. Here, we present a case report on a patient with metastatic melanoma who developed pulmonary sarcoid–like granulomatosis following repetitive vaccinations with peptides and CpG. Despite multiple metastases, including one lesion in the brain, the patient is alive and well more than 13 years after the diagnosis of metastatic disease. The strongly activated tumor-specific CD8$^+$ T cells showed robust long-term memory and effector functions. It is possible that long-term survival and adverse autoimmune events may become more common for vaccines inducing robust antitumor immune responses as were present in this patient. Cancer Immunol Res; 2(12); 1148–53. ©2014 AACR.

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doi: 10.1158/2326-6066.CIR-14-0143

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metastatic). Subsequently, the patient received six vaccines composed of MAGE-A10 (GLYDGMEHL) and Melan-A (ELAGIGILTV) peptides with or without SB-AS2 adjuvant (a mixture of MPL and QS-21 in an oil-in-water emulsion) at 3-week intervals for a period of 3 months (ClinicalTrials.gov identifier NCT00112216). The adjuvant was used for every second vaccine (vaccine nos. 1, 3, and 5), injected intramuscularly (i.m.) according to the manufacturer’s instructions, whereas the vaccines (peptides) without adjuvant were injected intradermally (i.d.; vaccines no. 2, 4, and 6) to optimally target the skin and its vaccine site skin-draining lymph nodes.

The patient remained disease free until 2005, when he developed a para-pubic subcutaneous (s.c.) nodule, 1.3 cm in diameter, on the right side. The mass was resected with histologic confirmation of melanoma metastasis. The patient then received seven monthly s.c. vaccinations with MAGE-A10 (GLYDGMEHL), Melan-A (ELAGIGILTV), and NY-ESO-1 (SLLMWTQQA) peptides and montanide ISA-51 as adjuvant over a period of 7 months (NCT00112242).

In May 2006, cytopenia revealed a new subpubic subcutaneous metastasis of 8 mm in diameter, again on the right side. In addition, right and left pelvic dissection showed nine right pubic cutaneous metastases, four left inguinal metastatic lymph nodes, and four left iliac obturator metastatic lymph nodes. Subsequently, the patient received monthly vaccinations with the same peptides and montanide, this time including CpG 7909 as adjuvant (NCT00112242). After three monthly vaccinations, the patient developed very strong subcutaneous reactions at injection sites. Therefore, the next 33 vaccinations included only CpG as adjuvant and without montanide. The patient had no other clinically significant treatment-related toxicity and remained disease free for 1 year.

November 2007 was marked by a third relapse of metastatic disease with a positive biopsy for a right inguinal cutaneous metastatic nodule (3 mm in diameter). A CT scan in February 2008 showed a single cerebral left frontal metastasis in the precentral gyrus (16 mm in diameter; Fig. 1A). Stereotactic radiosurgery (20 Gy) was performed and led to a 34% reduction in tumor size (Fig. 1B). However, the lesion enlarged progressively (Fig. 1C). Brain metastasis resection was performed, and the pathology findings confirmed the melanocytic origin of the lesion. The cerebral MRI showed a reduction of the lesion size (Fig. 1B). Remarkably, the lesion has disappeared 5 years after the surgery (Fig. 1E).

Although the patient continued to receive vaccinations with peptides and CpG, a CT scan in June 2011 revealed the apparition of new infiltrative mediastinal lymphadenopathies (pretracheal and right paratracheal; Fig. 1I), bilateral hilar lymphadenopathies (Fig. 1J and K), multiple pulmonary micronodular opacities, and centrilobular ground-glass infiltrate (not shown) that were not present on the control CT scan in December 2005 (Fig. 1F, G, and H). In June 2012, imaging disclosed a new left axillary lymphadenopathy (23.8 × 19.33 mm) and a subcutaneous nodule (6 mm in diameter) in the left anterior thoracic wall. The PET-CT imaging showed hypermetabolic axillary, multiple mediastinal, and bilateral hilar lymphadenopathy and a subcutaneous nodule in the left anterior thoracic wall (Fig. 1O). The lesions were suspected to be multiple melanoma metastases. An endobronchial ultrasound with multiple cytopenia in the right paratracheal and subcarinal lymph nodes were, however, negative for tumor cells.

After terminating vaccination in April 2012, the PET-CT performed in September 2012 (Fig. 1P) indicated an increased size and radiotracer uptake of the left axillary lymphadenopathy and the subcutaneous thoracic nodule, and an increased radiotracer uptake of bilateral hilar and mediastinal lymphadenopathies. Axillary lymph node dissection and resection of the subcutaneous nodule confirmed a fourth relapse of the disease with one left metastatic axillary lymph node and one metastasis in the left anterior thoracic wall. Biopsies of the right paratracheal and pretracheal lymph node from a mediastinoscopy were not positive for tumor cells but showed numerous well-formed giant-cell granulomas. These were characterized by macrophages (CD68⁺; Fig. 2A) surrounded by an inflammatory ring with predominant CD4⁺ (Fig. 2B) over CD8⁺ T lymphocytes (Fig. 2C), large numbers of activated lymphocytes (CD45RO⁺; Fig. 2D), and few regulatory T cells (FoxP3⁺ and CD25⁺; Fig. 2E and F). Ziehl–Neelsen stain was negative and no malignant cells, mast cells, or eosinophils were observed. These histopathologic features, along with the presence of multiple micronodular opacities, bilateral hilar and right paratracheal lymphadenopathies, and ground-glass infiltrate in the CT scan, were characteristics for sarcoidosis. However, the patient did not develop any sarcoidosis symptoms, and the clinical examination was normal. The control CT scan done 10 months later showed a stabilization of the mediastinal lymphadenopathies with no new lesion (Fig 1L, 1M, and 1N). Until today, he remains relapse free and enjoys a good quality of life.

**Tumor antigen expression and immune response**

Homogenously strong positive expression of Melan-A and MAGE-A10 was confirmed by reverse transcription PCR assay (RT-PCR) or IHC on various samples (Fig. 1: lymph nodes 1, 2, and 3 and subcutaneous melanoma metastases 1, 2, and 3). The cerebral metastasis was too necrotic to be analyzed by either IHC or RT-PCR. NY-ESO-1 was not expressed.

Peripheral blood mononuclear cells (PBMC) collected at different time points before and after vaccinations were analyzed directly ex vivo by flow cytometry. The percentages of CD3⁺, CD4⁺, and CD8⁺ cells were stable (Fig. 3B). In contrast, the frequency of Melan-A⁺-, MAGE-A10⁺-, and NY-ESO-1–specific T cells changed substantially according to the antigens used in the vaccination. The SB-AS-2 adjuvant administered in conjunction with Melan-A and MAGE-A10 peptides had a moderate impact on the frequencies of MAGE-A10–specific T cells (maximum of 0.04%; Fig. 3H) and Melan-A–specific T cells (maximum of 0.02%; Fig. 3D), whereas the NY-ESO-1–specific T cells remained unchanged (Fig. 3F). The subsequent vaccines with montanide induced a moderate increase, reaching 0.04% for Melan-A–specific, 0.05% for NY-ESO-1–specific, and 0.03% for MAGE-A10–specific CD8⁺ T cells (Fig 3D, F, H). However, the addition of CpG to the vaccine formulation resulted in strong expansions of Melan-A–specific T cells (maximum of 9.94%; Fig. 3A, left and D), NY-ESO-1–specific T cells (maximum of 1.58%; Fig. 3A, middle and F), and MAGE-A10–specific peptides and CpG, a CT scan in June 2011 revealed the apparent of new infiltrative mediastinal lymphadenopathies (pretracheal and right paratracheal; Fig. 1I), bilateral hilar lymphadenopathies (Fig. 1J and K), multiple pulmonary micronodular opacities, and centrilobular ground-glass infiltrate (not shown) that were not present on the control CT scan in December 2005 (Fig. 1F, G, and H). In June 2012, imaging disclosed a new left axillary lymphadenopathy (23.8 × 19.33 mm) and a subcutaneous nodule (6 mm in diameter) in the left anterior thoracic wall. The PET-CT imaging showed hypermetabolic axillary, multiple mediastinal, and bilateral hilar lymphadenopathy and a subcutaneous nodule in the left anterior thoracic wall (Fig. 1O). The lesions were suspected to be multiple melanoma metastases. An endobronchial ultrasound with multiple cytopenia in the right paratracheal and subcarinal lymph nodes were, however, negative for tumor cells.

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Figure 1. Radiologic images and disease course. A to E, cerebral MRI. White circles, brain metastasis. F to N, thoracic CT scan. Red circles, right paratracheal lymphadenopathies (I and L), right (J and M), and left hilar lymphadenopathies (K and N). F, G, and H, no lymphadenopathies. O to P, whole body PET-CT scan. Black arrows, left axillary lymphadenopathy and left anterior chest wall nodule. Bottom, timeline showing therapy and disease status. Sentinel lymph node procedure (Sent. LN). Lymph node metastasis surgery 1 (LN1), right iliac obdurator and inguinal radical lymph node dissection. LN2, right and left pelvic lymph node dissection. LN3, left axillary lymph node dissection + mediastinoscopy + left anterior chest wall nodule resection. Subcutaneous melanoma metastasis resection 1 (Subcut. MM1), right parapubic subcutaneous nodule resection. Subcut. MM2, right subpubic subcutaneous nodule resection. Subcut. MM3, right inguinal subcutaneous nodule resection. Sarcoid-like granulomatosis (Sarc.-LG). SB-AS2: i.d. vaccines 1, 3, and 5 of Melan-A + MAGE-A10; i.m. vaccines 2, 4 and 6 of Melan-A + MAGE-A10 + SB-AS2. ISA-51: s.c. vaccines of Melan-A + MAGE-A10 + NY-ESO-1 + Montanide. ISA-51 + CpG, s.c. vaccines of Melan-A + MAGE-A10 + NY-ESO-1 + Montanide + CpG. CpG, s.c. vaccines of Melan-A + MAGE-A10 + NY-ESO-1 + CpG; MM, melanoma metastasis; NED, no evidence of disease. PD, progressive disease; vertical arrows, surgeries; irradiation symbol + vertical arrow, radiosurgery.
T cells (maximum of 0.08%; Fig. 3A, right and H). Despite these strong expansions of tumor antigen–specific T cells, the total leukocyte counts (absolute numbers) remained in the normal range, between 1.5 and 4 G/L (data not shown).

The three antigen-specific CD8+ T-cell populations showed different kinetics, particularly after the introduction of CpG as vaccine adjuvant (Fig. 3D, F, and H). Furthermore, for all three antigen specificities, we observed significant T-cell differentiation to effector memory cells (CD45RA−CCR7−CD28+) and terminally differentiated effector “EMRA” cells (CD45RA−CCR7−CD28−; Fig. 3E, G, and I).

Finally, we also evaluated the capacity of the tumor antigen–specific CD8+ T cells to produce IFNγ. The frequency of Melan-A–specific IFNγ-producing CD8+ T cells increased 352-fold after vaccination in conjunction with CpG. IFNγ+ NY-ESO-1– and MAGE-A10–specific CD8+ T cells increased a maximum of 79-fold and 33-fold, respectively, compared with the values before vaccination (Fig. 3C).

Discussion

Therapeutic cancer vaccines are known to be well tolerated and only rarely are associated with severe AEs (12, 13). In fact, very few trials have documented detrimental effects that would raise safety concerns (14). The most common AEs are local induration, pain, and erythema at injection sites. Yoshida and colleagues (15) investigated the severe AEs (grade ≥3) after therapeutic peptide vaccinations in >500 patients with advanced cancer. They found that 20.4% of patients presented at least one AE during the vaccine trial, with 5.9% of the AEs being vaccine related and consisting of skin reactions at the injection site, edemas of the head and neck regions, colitis, and rectal bleeding. Similar findings were made in another phase III trial with 440 patients (16). In a first randomized phase II trial conducted by Slingluff and colleagues (17) of vaccination using four melanoma peptides (either with granulocyte-macrophage colony-stimulating factor and montanide or pulsed on monocyte-derived dendritic cells) with systemic low-dose IL2 infusion, grade 3 toxicities occurred in 35% of patients as diarrhea, rash, and pain. In a second randomized phase II trial of two multipeptide vaccines (8), 38% of patients experienced grade 3 toxicities, and local vaccine reaction was the most frequent grade 3 toxicity (15%–32%). Notably, no lung toxicity was reported in these clinical trials.

Sarcoidosis is a multisystem granulomatous disorder of unknown etiology with both pulmonary and extrapulmonary manifestations. Many patients with sarcoidosis do not require therapy, and two thirds experience a remission within a decade after diagnosis with few or no consequences. The presence of granulomatous inflammation is thought to result from an exaggerated cell-mediated immune response to unidentified antigens (18). Although its exact immunopathogenesis is not clearly understood, this disorder is characterized by a T-helper 1 (Th1) cell response. The release of IFNγ and IL2 by Th1 cells promotes macrophage accumulation and activation, as well as aggregation with fibroblasts and T cells, resulting in the development of granulomatous inflammation.

Our patient presented radiologic and histologic alterations characteristic of sarcoidosis (19, 20), while he was treated with cancer vaccines. He developed and maintained particularly high frequencies of cancer antigen-specific T cells, reaching a maximum of almost 10% of his circulating CD8+ T cells, induced by multiple injections of highly immunogenic vaccines. Even though such strong T-cell responses are rarely seen after vaccination, we (10) and others have described this phenomenon previously for single-peptide vaccines. However, to our knowledge, such high T-cell frequencies were not yet described after vaccination with multiple peptides. Therefore, it seems possible that vaccination with multiple antigens and high CD8+ T-cell frequencies may be associated with autoimmunity.
This AE was classified as grade 1 according to Common Terminology Criteria for Adverse Events (version 4) lung toxicity. To our knowledge, cancer vaccine–induced granulomas have not been described previously in patients with melanoma. Interestingly, sarcoidosis was described in patients with melanoma treated with IFNα (21, 22) or

![Figure 3. T-cell responses. A, representative example dot plots from PBMCs, showing Melan-A–specific cells (left), of NY-ESO-1–specific cells (middle), and MAGE-A10–specific cells (right) among CD8+ T cells. B, percentages of CD3+, CD4+, and CD8+ T cells in PBMCs. C, direct ex vivo ELISPOT analysis of total PBMCs, IFNγ production after 24 hours incubation with specific peptides (Melan-A, NY-ESO-1, and MAGE-A10). Ex vivo tetramer analysis of Melan-A– (D), NY-ESO-1– (F), and MAGE-A10– (H)–specific CD8+ T cells after the different vaccinations. Percentages of CD28+ cells among the Melan-A–specific (E), NY-ESO-1–specific (G), and MAGE-A10–specific (I) CD8+ T cells.](image-url)
ipilimumab (23, 24). Sarcoïd-like reactions accompanying malignancy were reported in 0.42% of patients with melanoma (25), raising the alternative possibility that the patient’s sarcoidosis could have developed incidentally and independently of immunotherapy.

Cancer vaccines are usually safe and display a low toxicity. However, with the increasing potential of new-generation and powerful vaccines, clinicians may face unexpected AEs, especially in patients with strong immune responses, such as the one presented here.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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Acknowledgments
The authors thank the patient for his dedicated collaboration. They gratefully acknowledge L.J. Old, J. O’Donnell-Tormey, L. Harmer, and J. Skipper for support, and M. Swartz, R. Verdua, L. Pan, D. Rimoldi, and the members of their groups for collaboration and advice.

Grant Support
This work was supported by grants from the Swiss National Science Foundation (grant no. 310030_135553), the Swiss Cancer League (grant no. 02836-08-2011), Ludwig Cancer Research (United States), the Cancer Research Institute (United States), and the Cancer Vaccine Collaborative (United States).

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Received August 4, 2014; revised September 1, 2014; accepted September 14, 2014; published OnlineFirst October 2, 2014.

References
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