Inoculation site from a cutaneous melanoma patient treated with an allogeneic therapeutic vaccine: a case report

Mariana Aris¹, Alicia Inés Bravo², Maria Marcela Barrio¹ and José Mordoh¹,3,4 *

INTRODUCTION

A 45-year-old Caucasian woman (patient #1) underwent resection on May 2012 of a spontaneously bleeding congenital nevus in her back. Histological analysis revealed an ulcerated nodular cutaneous melanoma (CM) with a Breslow thickness of 7.8 mm, epithelioid and spindle cell morphology, non-brisk immune infiltration, and satellitosis. Concurrent adenopathies were detected in her right axilla; axillary lymph node dissection revealed 4/23 metastatic nodes. After signing informed consent on August 23, 2012, this high-risk patient underwent the routine scanning procedure of the CASVAC-0401 study; on the basis of a normal CAT scan on June 28, 2012, and normal laboratory, the patient was classified as stage III and randomized to the vaccine arm. The patient only received one dose of vaccine (16 × 10⁶ lethally irradiated allogeneic CM cells, plus 10⁶ cfu of Bacillus Calmette Guerin (BCG) and 400 μg of recombinant human granulocyte macrophage-colony stimulating factor (rhGM-CSF) in four daily doses), since already at the first visit (September 03, 2012) she complained of lumbar and left rib cage pain. Presence of bone metastases were suspected and were confirmed by a PET scan (October 03, 2012), which revealed bone metastases at the ninth left rib, the right acetabulum as well as soft tissue metastases at the right infra-axillary region. The patient was therefore already at stage IV of her disease, she was discontinued from the study and continued appropriate treatment elsewhere. The bone metastases were irradiated, and she started treatment with Vemurafenib since her tumor had the BRAFV600E mutation. Seven weeks after the single vaccination, the patient decided to remove her subcutaneous (s.c.) vaccination nodule. The patient developed progressive disease, including brain metastases, and died on January 15, 2014.

We have developed a therapeutic vaccine consisting of a mixture of lethally-irradiated allogeneic cutaneous melanoma cell lines with BCG and GM-CSF as adjuvants. The CSF-470 vaccine is currently being assayed in a Phase II–III trial against medium-dose IFN-α2b. All vaccinated patients immunized intradermally developed large edematous erythema reactions, which then transformed into subcutaneous nodules active for several months. However, vaccine injection sites were not routinely biopsied. We describe the case of a female patient, previously classified as stage III, but who, due to the simultaneous discovery of bone metastases only received one vaccination was withdrawn from the study, and continued her treatment elsewhere. This patient developed a post-vaccination nodule which was surgically removed 7 weeks later, and allowed to analyze the reactivity and immune profiling of the inoculation site. An inflammatory reaction with zones of fibrosis, high irrigation, and brisk lymphoid infiltration, primarily composed of CD8+ cells proliferated; most of them contained intracellular MART-1 Ag, and some interacted with CD8+ lymphocytes. These observations suggest a potent, long-lasting local inflammatory response with recruitment of Ag-presenting cells that incorporate melanoma Ags, probably leading to Ag presentation to naïve T cells.

Keywords: cutaneous melanoma, allogeneic therapeutic cell vaccine, local injection reaction site, vaccination site biopsy immune profiling, local Ag presentation

Abbreviations: Ag/Ags, antigen/antigens; APC, Ag presenting cells; BCG, bacillus calmette guerin; CM, cutaneous melanoma; DC, dendritic cells; LN, lymph node; rhGM-CSF, recombinant human granulocyte macrophage-colony stimulating factor; s.c., subcutaneous; TLS, tertiary lymphoid structures.
vascularization, and brisk lymphoid infiltration could be distinguished (Figure 1A–III). Lymphocytes and polymorphonuclear cells were distributed around vessels (Figure 1B). Dense nested structures comprised of macrophages, histiocytes and polymorphonuclear cells, typically found in inflammatory processes including responses to BCG, were observed in the highly infiltrated zone (Figure 1C). BCG was rapidly cleared from the site, since no remaining bacilli were detected by Ziehl–Neelsen staining at the vaccination site (Figures 1D,E). Immune profiling analysis revealed brisk CD8+ and CD20+ lymphocyte infiltration, non-brisk CD4+, and scarce Foxp3+ T cells infiltration (Figures 2A–D,G). MART-1 antigen (Ag), derived from the vaccine, was found throughout the inoculation site (Figure 2E). CD11c+ cells were observed in dense nested structures (Figure 2F). Quantification of the immune infiltrate (number of cells/mm² tissue surface, mean ± SD, two determinations) (G) revealed that some CD11c+ APCs, both dispersed and nested, proliferated (Figures 3A,B,G), most had phagocytosed MART-1 Ag (Figures 3C,D,G), and some were surrounded by CD8+ lymphocytes (Figures 3E,F), suggesting local Ag presentation. The near absence of Foxp3+ lymphocytes also suggests that an immunogenic environment was created.

BACKGROUND
Cutaneous melanoma is a prototypic immunogenic tumor for which several immunotherapeutic approaches are currently under

FIGURE 1: Histological analysis of CSF-470 vaccination site biopsy from patient #1. Hematoxylin–Eosin stained sections were examined by optical microscopy (Olympus BX40, Tokyo, Japan); pictures were acquired with Olympus Digital Camera DP72 and analyzed with Image J software. (A) Low magnification image of the granulomatous nodule distinguished a fibrosis zone (I), a highly vascularized zone (II), and a brisk-infiltrated with inflammatory cells zone (III). (B) Zone (II), in detail, showing lymphocytes and polymorphonuclear cells. (C) Zone (III) showing dense nested structures with a polynuclear cell (inset). (D) Ziehl–Neelsen staining revealed absence of BCG bacilli in the vaccine site. (E) A positive control for bacilli staining is shown (bowel tuberculosis). Bars = 2 mm (A); 50 µm (B–E).

FIGURE 2: Immune profiling and MART-1 distribution in CSF-470 vaccination site biopsy from patient #1. Formalin-fixed, paraffin-embedded tissue sections were stained with appropriate antibodies, amplified with avidin-biotin-peroxidase (ABC) system (Vectastain, Vector Labs), and revealed with 3,3′-diaminobenzidine. Brisk CD8+ and CD20+ lymphocyte infiltration was observed in zones (II, III), with scarce CD4+ or Foxp3+ infiltration (A–D). Expression of MART-1 Ag was observed throughout the inoculation site (E). CD11c+ cells were observed in dense nested structures (F). Quantification of the immune infiltrate (n° cells/mm² tissue surface, mean ± SD, two determinations) (G). Bars = 50 µm. Antibodies: CD8 (clone C8/144, Dako, CA, USA), CD20 (clone L26, Dako), CD4 (clone 1F6, Novocastra, Wetzlar, Germany), Foxp3 (clone 236A/E7, Abcam, MA, USA), MART-1 (clone A103, Dako), CD11c (clone EP1347Y, Abcam).

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investigation (2). Therapeutic cancer vaccines are aimed at promoting tumor-specific and long-term immunity. Cancer vaccines have been assayed using different strategies such as the use of inactivated whole tumor cells, Ag-specific peptides or purified proteins, among others, in combination with adjuvants to create an immunogenic microenvironment for Ag presentation and expansion of cytotoxic T lymphocytes (3). The classic paradigm proposes that following inoculation in the dermis, Ags are incorporated and processed by APCs, such as macrophages and dendritic cells (DCs), which then migrate to lymph nodes (LN), where processed Ags are presented to naïve T lymphocytes (1). However, the vaccination system of choice, combining suitable Ags, adequate adjuvants, and an appropriate immunization schedule is a delicate equation that may give rise to tolerance or immunogenicity. Therefore, dissection of the events that take place at the vaccination site is important to unravel the induction of an effective immune response.

We have developed multi-Ag allogeneic vaccines for CM treatment. The CSF-470 vaccine, consisting of four lethally irradiated allogeneic CM cell lines plus BCG and rhGM-CSF as adjuvants, is currently being assayed versus medium-dose IFN-α2b (2:1 ratio) in a randomized open trial (CASVAC-0401) in stages IIB, IIC, and III post-surgery CM patients in adjuvancy (Clinical trials.gov NCT01729663). The study has been approved by the Comité de Ética en Investigación del Instituto Médico Especializado Alexander Fleming, and it has so far recruited 32 patients (21 patients in the vaccine arm and 11 patients in the IFN-α2b arm). The rationale for the use of this formulation is to immunize patients with an inert scaffold that spans a broad repertoire of tumor Ag, thus counteracting heterogeneity in Ag expression (4). Adjuvant BCG induces a potent local inflammatory reaction with a T H 1-polarizing immune response and epitope spreading (5); rhGM-CSF stimulates local attraction of APCs and favors a T H 1 response (6). In a previous Phase I study with escalating GM-CSF dosage, 400 μg rhGM-CSF per vaccine was found to be the optimal dose. The combination was safe and induced a predominantly cellular immune response (7).

DISCUSSION

The results presented here constitute the first in situ evidence of the afferent arm of the immune response to our cell-based
Concluding Remarks

The vaccine inoculation site is the gateway for the induction of an immune response. In this study, we gained access to a vaccine site biopsy from a CM patient following a single immunization. We propose that the generation of an immune response toward tumor Ags by using allogeneic vaccines involves a potent local inflammation, driven by allograft rejection, and fueled by BCG and rhGM-CSF. This would favor a proper immunogenic environment for APC affluence, which subsequently may interact locally with Ags or migrate toward peripheral LN for Ag presentation. Since the CASVAC-0401 study comprises a total of 13 vaccinations over a period of 2 years, it will be important to analyze the evolution of vaccination sites at several time points in an ad hoc Phase I trial.

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