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Tumor lysate-loaded Bacterial Ghosts as a tool for optimized production of therapeutic dendritic cell-based cancer vaccines

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ABSTRACT

Cancer immunotherapy with dendritic cell (DC)-based vaccines has been used to treat various malignancies for more than two decades, however generally showed a limited clinical success. Among various factors responsible for their modest clinical activity is the lack of universally applied, standardized protocols for the generation of clinical-grade DC vaccines, capable of inducing effective anti-tumor immune responses. We investigated Bacterial Ghosts (BGs) – empty envelopes of Gram-negative bacteria – as a tool for optimized production of DC vaccines. BGs possess various intact cell surface structures, exhibiting strong adjuvant properties required for the induction of DC maturation, whereas their empty internal space can be easily filled with a source tumor antigens, e.g. tumor lysate. Hence BGs emerge as an excellent platform for both the induction of immunogenic DC maturation and loading with tumor antigens in a single-step procedure. We compared the phenotype, cytokine secretion profile, functional activity and ability to induce immunogenic T-cell responses *in vitro* of human monocyte-derived DCs generated using BG platform and DCs matured with widely used lipopolysaccharide (LPS) plus interferon-γ cocktail and loaded with tumor lysate. Both approaches induced DC maturation, however BG-based protocol was superior to LPS-based protocol in terms of the ability to induce DCs with a lower tolerogenic potential, resulting in a more robust $CD8^+$ T cell activation and their functional activity as well as significantly lower induction of regulatory T cells. These superior parameters are attributed, at least in part, to the ability of BG-matured DCs to resist potential immunosuppressive and pro-tolerogenic activity of various tumor cell lysates, including melanoma, renal carcinoma and glioblastoma.

Abbreviations: BG-DCs, dendritic cells matured with oncolysate-pre-loaded bacterial ghosts plus interferon gamma; BG, bacterial ghost; CFSE, carboxyfluorescein succinimidyl ester; DAMP, damage-associated molecular pattern; DC, dendritic cell; ELISPOT, enzyme-linked immunospot assay; FCS, fetal calf serum; HLA, human leukocyte antigen; IFN (-α or-γ), interferon alpha or gamma; IL(-1β,-2,-4,-6,-10,-12,-17), interleukin (-1β,-2,-4,-6,-10,-12,-17); ILT3, immunoglobulin-like transcript 3; LPS, lipopolysaccharide; LPS-DCs, dendritic cells pre-loaded with oncolysate and matured LPS plus IFN-γ; MACS, magnetically-activated cell sorting; MFI, mean fluorescence intensity; MHC, major histocompatibility complex; MLR, mixed leukocyte reaction; OS, overall survival; PAMP, pathogen-associated molecular pattern; PBMC, peripheral blood mononuclear cells; PD-L1, programmed death (receptor) ligand 1; PHA, phytohemagglutinin; RCC, renal cell carcinoma; TAA, tumor-associated antigen; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; Treg, regulatory T cell.

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1. Introduction

Immunotherapy is one of the fastest developing cancer treatment strategies. Among various types of cancer immunotherapy [1] therapeutic cancer vaccination is one of the most extensively studied [2] with the first clinical trial in melanoma patients dating back to 1998 [3]. Therapeutic cancer vaccination exploits dendritic cells (DCs) and their unique intrinsic capacity to stimulate and orchestrate antigen-specific immune responses [4,5]. DCs can be therapeutically targeted *in vivo* or "educated" *ex vivo* by isolating their precursors from a cancer patient, manipulating them *ex vivo* and reinjecting back into the same patient [6]. Both strategies have their pros and cons and here we focus on *ex vivo*-generated autologous monocyte-derived DC vaccines.

Therapeutic DC vaccines have shown favorable safety profiles and ability to induce antitumor immune responses in cancer patients [7]. Although scattered results with long-term benefit were reported [8], their therapeutic activity has been disappointing in terms of objective clinical responses that generally do not exceed 15% [7]. As a result, DC vaccines mainly applied as a monotherapy in end-stage disease, have not fulfilled their promise and eventually attracted scepticism. However therapeutic DC vaccination can still hold a strong position among other cancer treatment approaches, especially in combinational settings, subject to addressing challenges associated with optimal production and application protocols of DC vaccines. First, the implementation of alternative immune-related response evaluation criteria has already started and was prompted by the observation that early objective responses might not represent the true clinical activity of immunotherapies [9,10]. Furthermore, clinical evidence indicates that DC-based vaccination may improve overall survival (OS) without inducing objective tumor responses or prolonging progression-free survival [11,12]. Second, to allow well-conducted large-scale multicenter phase III trials, the production of DC vaccines should be standardized between centers. Third, validation of one or several standardized, cost-effective, straightforward DC generation protocols is pending. Numerous phase I/II clinical trials with monocyte-derived DC vaccines have been performed or are ongoing, however comparison of their results is complex, since a variety DC production protocols (differentiation, maturation, tumor antigen loading) and administration regimens have been used [5].

Monocyte-derived DC vaccines are most widely investigated due to the easy access and sufficient amounts of their blood precursors. It is accepted that mature rather than immature DCs should be used for therapeutic vaccination to achieve the desired immunological and clinical outcomes in cancer patients [13,14]. By using different DC maturation-inducing agents (maturation cocktails), it is possible to generate DCs with distinct or even functionally-opposite (immunogenic versus tolerogenic) functional states [15]. Strongly immunogenic DC vaccines capable of inducing long-term Th1-type adaptive antitumor immune responses are required for successful cancer immunotherapy [16]. Besides DC maturation, the source and loading approach of tumor-associated antigens (TAA) is another critical factor. Much effort has been made to optimize the maturation and TAA loading protocols for obtaining optimal clinical-grade DCs with various results [5].

In this study we analyzed Bacterial Ghosts (BGs) for the production of optimized clinical-grade DC vaccines. BGs are Gram-negative bacteria-derived cell envelopes devoid of all cytoplasmic content, but with a preserved intact external cellular morphology possessing all cell surface structures that exhibit intrinsic adjuvant properties and are potent activators of a broad range of cell types involved in innate and adaptive immunity [17,18]. Experiments in cell cultures and animal models showed promising efficacy and safety results [19,20]. In addition, empty internal space of BGs can be easily filled with a source tumor antigens [17]. These results suggest that BGs may serve as an excellent plaftform for the induction of DC maturation and their loading with TAAs in a single-step procedure. Indeed, among various DC maturation cocktails, a combination of gram-negative bacteria-derived lypopolysaccharide (LPS) and cytokine interferon (IFN)-γ has emerged as one of the most applicable for the generation of clinical-grade DCs [21,22]. In this regard, BGs may serve as a superior DC maturation-inducing tool, since they possess a natural combination of various innate immunostimulatory agonists.

In this comparative study we comprehensively evaluated the potential of tumor cell lysate(oncolysate)-loaded BGs and a combination of LPS and IFN-γ to induce maturation of DCs capable of triggering strong Th1-polarized antitumor immune responses. As a TAA source we used lysates of three different tumor cell lines, including melanoma, renal cell carcinoma (RCC) and glioblastoma. The use of oncolysate enables DC loading with various TAAs without the need of their characterization and allows the induction of polyclonal immune responses against various TAA epitopes thereby reducing the ability of tumor immune escape via loss of particular TAA(s) [23]. Since oncolysates may possess various immunosuppressive components interfering with immunogenic maturation of DCs [24], we also evaluated the impact of different oncolysates on the DC maturation state.

2. Materials and methods

2.1. Study population

DCs were prepared from monocytes of 15 volunteer donors. Blood was collected at the National Blood Centre (Vilnius, Lithuania). Donors were not affected with primary or secondary immunodeficiencies, autoimmune diseases, were not infected with hepatitis B or C viruses. All individuals signed a written consent approved by the Lithuanian Bioethics Committee.

2.2. Preparation of oncolysates

For oncolysate preparation, three different tumor cell lines were used, including SK-MEL-28 (melanoma), 786-O (renal cell carcinoma), and U-87 (glioblastoma), purchased from American Type Culture Collection (USA) and maintained in an appropriate culture medium (DMEM for SK-MEL-28 and U-87 cell lines and RPMI 1640 for 786-O cell line) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 °C in a humidified 5% CO_2 atmosphere. Tumor cells were harvested by trypsinization at a log growth phase, washed twice and resuspended in sterile distilled water. Oncolysate was prepared by five freezing-thawing cycles using −196 °C liquid nitrogen and +37 °C water bath. The obtained oncolysate was centrifuged at 10 000*g* for 12 min at 4 °C and sterile-filtered. Protein concentration in the oncolysate was measured with NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific, USA).

2.3. Generation of immature dendritic cells

Immature DCs were generated from peripheral blood mononuclear cells (PBMC) obtained from 50 ml of buffy coats of healthy donors. PBMC were isolated by Lymphoprep (Axis-Shield PoC AS, Norway) density gradient centrifugation at 1500*g* for 20 min at room temperature, washed twice, resuspended in X-VIVO medium (Lonza, USA) and cultured in 75 cm^2 plastic flasks (Thermo Fisher Scientific, USA) at a concentration of 5×10^6 cells/mL at 37 °C in a humified 5% CO_2 atmosphere. After 2 h of incubation,

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