Optimization of Natural Killer T Cell–Mediated Immunotherapy in Cancer Using Cell-Based and Nanovector Vaccines

C. Faveeuw\textsuperscript{1,2,3,4,5} and F. Trottein\textsuperscript{1,2,3,4,5}

Abstract

$\alpha$-Galactosyleramide ($\alpha$-GalCer) represents a new class of immune stimulators and vaccine adjuvants that activate type I natural killer T (NKT) cells to swiftly release cytokines and to exert helper functions for acquired immune responses. This unique property prompted clinicians to exploit the antitumor potential of NKT cells. Here, we review the effects of $\alpha$-GalCer in (pre)clinics and discuss current and future strategies that aim to optimize NKT cell–mediated antitumor therapy, with a particular focus on cell-based and nanovector vaccines.

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Introduction

Adjuvants trigger innate immunity, which represents a crucial step to initiate efficient and long-lasting acquired immune responses against pathogens and/or tumor cells. Modern vaccines incorporate molecularly defined adjuvants that directly or indirectly activate sentinel cells (e.g., dendritic cells, DC) through innate sensors including Toll-like receptors or the inflammasome. Activating pathways triggered by innate sensors induce a complex cascade of events that ultimately leads to the maturation of DCs and to the transactivation of other innate immune cells and conventional T and B lymphocytes.

Adjuvant properties of $\alpha$-galactosyleramide ($\alpha$-GalCer) have gained considerable interest over the past decade. $\alpha$-GalCer has been identified as a potent activator of a subset of nonconventional T lymphocytes termed as type I natural killer T (NKT) cells. Exposure to $\alpha$-GalCer induces an explosive cytokine response by NKT cells that triggers powerful innate and acquired immune responses. Of particular interest is the capacity of NKT cells to induce DC maturation and to display T helper–like functions that result in the development of cytotoxic T cell and/or antibody responses. This unique property is used in clinical situations, and there is a strong interest to exploit the adjuvant effects of NKT cells to develop more efficient vaccines (1–3). Here, we review ongoing strategies using $\alpha$-GalCer for cancer immunotherapy and discuss future approaches that might optimize $\alpha$-GalCer–based antitumor responses with a particular emphasis on vaccines against cancer.

Definition of NKT Cells

Type I or invariant NKT cells (here referred to as NKT cells) represent a highly conserved subset of innate-like nonconventional T lymphocytes. These cells recognize (glycolipid antigen (Ag) presented by the monomorphic MHC class I–like molecule CD1d expressed by Ag-presenting cells (APC), including DCs (for review, see ref. 4). NKT cells express a semi-invariant T-cell receptor (TCR) composed by a unique TCR-$\alpha$ chain paired with a restricted number of $\beta$ chains. Rapidly after activation, NKT cells produce copious amounts of Th1-, Th2-, and/or Th17-type cytokines. Through this unique property, NKT cells are viewed as crucial cells in the regulation of innate and adaptive immune responses. Their role in various pathologies, including cancer, infection, inflammation, and autoimmune diseases, has been underlined in experimental models and humans (4, 5).

NKT Cells in Innate Immune-Based Antitumor Responses

$\alpha$-GalCer in antitumor therapy

$\alpha$-GalCer is a marine sponge–derived glycosphingolipid originally discovered during a screen for anti-metastatic agents (6). Several studies have convincingly demonstrated the potent antitumor effect of $\alpha$-GalCer against solid tumors and hematologic malignancies in the mouse system, particularly in prophylactic settings (7, 8). Mechanisms include early production of IFN-$\gamma$ by NKT and, at later time points, by NK cells and secretion of IL-12 by DCs (9). On the basis of these encouraging preclinical studies, clinical trials in patients with advanced cancer have been conducted using free soluble $\alpha$-GalCer. Unfortunately, no or low clinical outcomes were reported among patients (10). These observations can be explained by the low (and variable) number of NKT cells in patients, their...
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NKT Cells in Acquired Immune Responses against Cancer

Apart from innate immunity, elimination of tumor cells is also strongly dependent on adaptive immune responses and particularly on CTL. Recent findings indicate that α-GalCer–activated NKT cells can substitute "classical" CD4+ T helper cells to license the DCs for cross-priming, a process leading to the development of potent CTL responses (29). In this process, α-GalCer–activated NKT cells induce the production of the chemokine CCL17 by cross-priming DCs, which in turn attracts naïve CCR4-expressing CTLs. Among DCs, the CD8α− subset (mouse system and BDCA3+ in humans) excels in Ag cross-presentation (30, 31). Different strategies using diverse delivery cell-based (DCs and tumor cells) systems and nanovectors have been developed to exploit the adjuvant activity of NKT cells and the cross-priming activities of endogenous DCs in cancer therapy (Table 1).

Cell-based systems

DCs transduced with the mammary tumor–associated Ag Her-2 and loaded with α-GalCer induce potent antitumor responses, an effect amplified with combined treatment with the deoxyxycytidine analog gemcitabine, a drug known to relieve immunosuppression (32). The effectiveness of this strategy was further confirmed, both in prophylactic and therapeutic settings, in different models of tumors (33). Of interest, DCs genetically engineered to express ovalbumine (OVA) plus CCL21, a chemokine that attracts both T cells and NKT cells, are potent to eradicate OVA-expressing tumors (34). Finally, enforced expression of CD1d in human embryonic stem cell–derived DCs assists the priming of CD8+ T cells against tumor Ag (35). The potential benefit of this latter strategy in cancer immunotherapy is being studied.

Tumor cells have been used as the tumor Ag sources for developing cancer vaccines although their low immunogenicity requires combined adjuvants. The capacity of tumor cells loaded with α-GalCer to act as a cellular adjuvant was initially evaluated by Shimizu and colleagues. This strategy exploits the proinflammatory potential of dying tumor cells, together with the unique capacity of DCs to cross-present Ag from dying cells. The authors demonstrated the efficacy of this approach in prophylactic and therapeutic models of mouse melanoma, lymphoma, and leukemia. In this setting, dying tumor cells are selectively taken up by DCs, and the subsequent cross-presentation of α-GalCer to NKT cells and tumor Ags to CD8+ T cells leads to strong and long-lasting antitumor responses (36). The efficacy of tumor-cell vaccines incorporating α-GalCer has been confirmed and extended in other therapeutic models of solid tumor and hematologic malignancies (Table 1; refs. 37–39). Of interest, concurrent depletion of regulatory T cells amplifies the antitumor response in this system (40). More recently, Fujii and colleagues developed a novel strategy based on the use of allogeneic fibroblasts expressing tumor Ag and loaded with α-GalCer (41). The same group also designed "artificial adjuvant vector cells or aAVCs" consisting of human embryonic kidney cells stably transfected with CD1d, loaded with α-GalCer, and expressing the human melanoma MART-1 gene.

Reduced responsiveness upon repeated challenges, and by suboptimal activation due to uncontrollable delivery of α-GalCer (11, 12). In contrast to other APCs such as B cells, DCs are well equipped (e.g., appropriate costimulatory signals) to trigger efficient NKT-cell activation and to avoid NKT-cell unresponsiveness (13–15). α-GalCer presentation by DCs optimizes NKT cell–based antitumor responses in mice, even in therapeutic settings (16). In humans, adoptive transfer of α-GalCer–loaded autologous APCs, including DCs, induced clinical benefits in some patients, an effect that correlates with IFN-γ production (13, 15, 17, 18). Of interest, combined treatment with the immunomodulatory drug lenalidomide showed promising clinical responses (18). In the same vein, adoptive transfer of autologous NKT cells along with α-GalCer–loaded APCs also represents a promising approach, although technical and economic issues still persist (19, 20). Although not yet tested in the clinic, CD1d-based artificial APCs, an acellular system made by the covalent coupling of CD1d–α-GalCer and anti-CD28 monoclonal antibody to magnetic beads, might be of interest to activate and expand NKT cells in the future (21).

Nanovectors for passive and active delivery

Along with cell (DC)-based approaches, passive and active in vivo delivery of α-GalCer in DCs by means of biodegradable vectors might enhance NKT cell–based responses and antitumor immunity. In this context, nanovectors (<1 μm) represent an interesting class of targeted delivery vehicles. Encapsulating α-GalCer into nanoparticulate carriers might offer several advantages compared with soluble α-GalCer, including preferential uptake by APCs, slower release of α-GalCer in CD1d–containing endosomes, and minimal side effects thanks to the lower amount needed for a similar biological effect. Recent reports suggest that passive delivery of α-GalCer to APCs is of potential interest. For instance, silica microspheres coated with lipid bilayers containing α-GalCer target DCs and CD169+ macrophages in mice (22, 23), both cell types being important in primary NKT-cell activation. PLGA, that is, poly(D/L lactic-coglycolic acid) is a biocompatible polymer that is widely used in the clinic. We and others have shown that PLGA-based nanoparticles can be taken up by DCs to activate NKT cells (24, 25). On the other hand, α-GalCer incorporated in octaarginine-modified liposomes, a process that enhances cellular internalization by APCs, strongly stimulates NKT cells, and protects against B16F10 lung metastasis in a therapeutic setting (26). To improve the specific targeting of α-GalCer to APCs, nanovectors can be decorated with Abs or ligands that bind to specific markers. Liposomes, bearing on their surface oligomannose that binds to mannose receptor and dendritic cell–specific intercellular adhesion molecule-3-grabbing non-integrin (DC-SIGN), target DCs in vivo and activate NKT cells toward a Th1 bias (27). Furthermore, encapsulating α-GalCer in liposomes decorated with glycans specific for the sialoadhesin CD169 potently activates NKT cells in vivo (28). Finally, our recent data indicate that α-GalCer inserted into PLGA-based Ab–armed nanoparticles that target CD8α− DCs enhance NKT cell–based innate immune responses, compared with nonvectorized α-GalCer (Macho Fernandez and colleagues; submitted for publication).
Table 1. NKT cell-based vaccines in cancer therapy

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Ag (42, 43). Of importance, this construct has strong antitumor activities in humanized mice. B lymphocytes were also shown to have potent antitumor properties when loaded with α-GalCer and the tumor-associated Ag Her-2 (44). In conclusion, cell-based vaccines to optimize α-GalCer activity in vivo are promising, although technical, logistical, and financial difficulties might limit the development of such vaccines. The in vivo targeted delivery of α-GalCer to tumor cells by means of fusion proteins might represent an alternative strategy and has already proved beneficial. Indeed, α-GalCer/CD1d tetramer fused to a single-chain antibody fragments specific for Her-2 induced efficient antitumor activities (45).

**Nanovectors carrying α-GalCer and tumor Ags**

Developing new “low-cost” tools that deliver α-GalCer and tumor Ag directly to DCs represents another attractive approach to optimize NKT-cell responses and cancer immunotherapy. In this context, the use of nanovectors that carry α-GalCer and tumor Ag and actively target DCs might offer an interesting immunotherapeutic strategy for the future (Table 1). McKee and colleagues were the first to investigate the consequences of α-GalCer and Ag co-encapsulation in antitumor responses (46). The authors inserted α-GalCer and the lymphocytic choriomeningitis virus–derived peptide gp33, used as a model Ag, into virus-like particles. Relative to free α-GalCer and gp33, encapsulated α-GalCer plus gp33 was 10-fold more active in triggering gp33-specific CTL responses. Moreover, the composite particle efficiently eliminated gp33-expressing melanoma cells in a prophylactic setting. Although the nature of APC(s) involved in this effect was not addressed, it is likely that α-GalCer and gp33 delivery in the endosomal compartment of APCs facilitates CD1d and MHC class I presentation (47). Targeting cross-priming DCs (mouse CD8α+ or human BDCA3+ DCs) offers an opportunity to trigger potent CTL responses. We have recently shown that targeting DEC205 by means of PLGA-based nanoparticles successfully delivers α-GalCer and OVA to mouse CD8α+ DCs, leading to Ag cross-presentation and potent antitumor responses (Macho Fernandez and colleagues; submitted for publication).

**Concluding Remarks and Future Perspectives**

The breadth of studies that highlight adjuvant properties of NKT cells and demonstrate that α-GalCer, or related lipid analogues, might be successfully used in cancer therapy cannot be contested. However, clinical studies have proved the necessity to design innovative strategies to better exploit the antitumor potential and the adjuvant effect of NKT cells to develop more efficient antitumor vaccines. In this context, cell-based vaccines that prevent NKT-cell unresponsiveness upon multiple challenges and that promote strong and long-lasting CTL responses should be encouraged. Nanovectors that passively or actively target cross-priming DCs are also of potential clinical benefit. Complementary/additive strategies, including approaches that boost the number of NKT cells in patients (e.g., infusion of autologous NKT cells), that aim to relieve immunosuppression (e.g., immunomodulatory drugs), and/or that combine NKT cell and Toll-like receptor agonists to amplify the strength and the quality of the immune response, might also represent strong added values in some circumstances. It is likely that, in a near future, the development of more sophisticated delivery systems (nanovectors) and the design of more potent NKT-cell activators will optimize antitumor responses and benefit cancer patients. Finally, the development of better animal models (humanized mice) to accurately replicate the NKT-cell response in humans is also an important area for future researches.

**Disclosure of Potential Conflicts of Interest**

No potential conflicts of interest were disclosed.

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