Lymphomas are aggressive hematological malignancies and a high number of patients relapse and die following treatment. These cancers are typically poorly immunogenic, so therapeutic vaccination strategies that incorporate adjuvants are required to enhance recognition and targeting of tumor cells by the immune system. We are investigating an adjuvant strategy in a mouse model of Myc oncogene-driven B cell lymphoma involving the natural killer T (NKT) cell ligand \(\alpha\)-galactosylceramide (\(\alpha\)-GalCer), based on reports indicating that NKT cell stimulation generates potent immune responses against a range of malignancies including B-cell lymphomas.\(^1,2\)

We adopted the approach of loading \(\alpha\)-GalCer onto autologous tumors administered as an irradiated cellular vaccine product. The rationale for this approach lies in the fact that the tumor cell vaccine acts as a source of potentially undefined tumor antigens, in addition to delivering \(\alpha\)-GalCer, thus allowing for the generation of innate immunity and impending long-term tumor-specific T-cell adaptive immunity, owing to the presence of a NKT cell adjuvant effect. This approach is ideally suited for many hematological malignancies, in which a large number of tumor cells can be easily obtained from the blood or bone marrow, immediately incubated with \(\alpha\)-GalCer and irradiated prior to re-infusion, overall constituting a simple, inexpensive and patient-specific cancer vaccine.

We have recently demonstrated that a single therapeutic vaccination with irradiated, \(\alpha\)-GalCer-loaded autologous tumor cells substantially inhibits the development and outgrowth of aggressive, poorly immunogenic murine E\(\mu\)-myc B-cell lymphomas and significantly prolongs the survival of tumor-bearing mice.\(^3\) In vivo examination of the effector cells and cytokines that are required for the vaccine antineoplastic activity revealed that components of both the innate (NKT and NK cells) and adaptive (CD8\(^+\) T cells) immune system are critical (Fig. 1). CD8\(^+\) T cells enhanced the therapeutic efficacy of the vaccine, yet they were not critical for the response. The requirement for CD4\(^+\) T cell help or effector function is likely to be context-dependent as Chung et al. revealed a significant role for CD4\(^+\) T cells in vaccine-induced immunity against MHC Class II-expressing A20 lymphomas.\(^4\) Our vaccine was also tumor antigen-specific, since the injection of \(\alpha\)-GalCer-loaded AML-ETO tumor cells (a model of acute myeloid leukemia) provided no protection against E\(\mu\)-myc B-cell lymphomas.

In a separate but related study, we recently demonstrated that antitumor immunity as elicited against established B16F10 melanoma by \(\alpha\)-GalCer-loaded tumor cell vaccination is significantly enhanced by the transient depletion of immunosuppressive FOXP3\(^+\) regulatory T cells (Tregs).\(^5\) Treg depletion resulted in increased activation of NK cells and CD8\(^+\) T cells, and exacerbated the infiltration of tumors by CD8\(^+\) T cells upon vaccination. This study highlights two important points. First, combining NKT adjuvant-based anticancer vaccines with the short-term depletion of Tregs is likely to facilitate the generation of adaptive immune responses that are required for long-term tumor protection. Second, as CD1d expression on B16F10 tumor cells was not required for effective vaccine-induced immunity against tumor-associated \(\alpha\)-GalCer, this therapeutic approach appears to be extendable to solid tumors, many of which do not express CD1d. However, the application of this strategy to solid malignancies is restricted by the limited accessibility of viable solid tumor cells for ex vivo vaccine generation. It is currently unclear how \(\alpha\)-GalCer is incorporated into CD1d\(^+\) cells or how NKT cells become activated in response to vaccination with CD1d\(^+\) tumors. One possibility is that the lipophilic sphingosine tail of \(\alpha\)-GalCer becomes integrated into the plasma membrane\(^6\) and that

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Immune adjuvants aimed at initiating or re-activating host immunity against poorly immunogenic cancers represent a potential tool for immunotherapy. By employing the natural killer T (NKT) cell agonist \(\alpha\)-galactosylceramide in a whole tumor cell therapeutic vaccine approach, we have achieved potent suppression of established hematological cancers upon the elicitation of innate and adaptive antitumor immunity.
Additional or alternative glycolipid antigens might be superior to that of α-GalCer in this approach. Finally, we have demonstrated that α-GalCer-loaded tumor cells are effective as an anticancer vaccine in other oncogene-driven mouse models of hematological malignancies. These include rapidly growing AML-ETO9a leukemia and Vk\(^*\)myc multiple myeloma cells. The identification of neoplasms that are likely to benefit from α-GalCer-based vaccines may be obtained by investigating whether effective vaccine-elicited antitumor responses are linked to the sensitivity of the tumor.

Figure 1. Mechanisms of antitumor immunity as elicited by the administration of α-GalCer-loaded tumor cells. Loading ex vivo CD1d\(^+\) tumor cells (e.g., \(E\mu\)-myc lymphomas) with α-galactosylceramide (α-GalCer) allows for the direct, TCR-mediated recognition of reinfused tumor cells by natural killer T (NKT) cells, resulting in NKT-cell activation and cytotoxicity. The infusion of CD1d\(^+\) tumor cells (e.g., B16-F10 melanoma) labeled with α-GalCer also allows for the activation of NKT cells by the indirect cross-presentation of α-GalCer by host antigen-presenting cells (APCs) upon tumor cell death. Cell death also releases tumor-derived antigens which are co-presented by APCs on MHC molecules to CD8\(^+\) and CD4\(^+\) T cells. The combination of the NKT/APC crosstalk, dendritic cell (DC) maturation and CD4\(^+\) T cell help leads to the generation and activation of potent tumor-specific effector CD8\(^+\) T cells. The production of interleukin-12 (IL-12) from activated APCs also results in NK-cell mobilization as well as in the rapid, systemic production of interferon γ (IFN\(γ\)) by NKT and NK cells, which is critical for therapeutic efficacy of the vaccine. \(E\mu\)-myc tumor cells express high levels of MHC Class I molecules, which is further upregulated upon exposure to IFN\(γ\). Therefore, it is plausible that anti-lymphoma activity of CD8\(^+\) T cells is stimulated by vaccination due to increased expression of MHC Class I molecules by tumor cells as a result of IFN\(γ\) signaling.

Cross-presentation by host antigen-presenting cells (APCs) is required. Any cell death occurring in response to tumor irradiation or targeting by hitherto unidentified innate immune cells would lead to the release of α-GalCer and tumor antigens for cross-presentation to NKT cells and T cells, respectively, by APCs, ultimately resulting in the induction of antitumor immunity. Interestingly, Shimizu, et al. have shown that α-GalCer cross-presented by host dendritic cells leads to long-lived T cell-mediated antitumor immunity.

Our work in the \(E\mu\)-myc tumor model also extended the relevance of a single report on the antitumor activity of β-ManCer,\(^6\) by showing that the therapeutic vaccination with β-ManCer-loaded \(E\mu\)-myc tumor cells conveys prolonged antitumor effects against established \(E\mu\)-myc lymphoma when compared with α-GalCer-loaded malignant cells. In line with previous results,\(^9\) the underlying mechanism appeared to be independent of interferon γ (IFN\(γ\)), at odds with the one that accounts for the antitumor effects of α-GalCer. We are further investigating mechanisms by which β-ManCer induces long-term immune responses against \(E\mu\)-myc tumors, and whether the use of additional or alternative glycolipid antigens might be superior to that of α-GalCer in this approach.

Finally, we have demonstrated that α-GalCer-loaded tumor cells are effective as an anticancer vaccine in other oncogene-driven mouse models of hematological malignancies. These include rapidly growing AML-ETO9a leukemia and Vk\(^*\)myc multiple myeloma cells. The identification of neoplasms that are likely to benefit from α-GalCer-based vaccines may be obtained by investigating whether effective vaccine-elicited antitumor responses are linked to the sensitivity of the tumor.
which activates NK-cell and T-cell immunity, may be an effective means of enhancing anti-CD20 monoclonal antibody (rituximab)-induced antibody-dependent cellular cytotoxicity (ADCC), and hence of improving disease outcome in patients affected by B-cell malignancies.

Although NKT cells constitute a small cell population in humans, it is well established that they are very potent regulators of cellular immunity. Moreover, NKT cells to IFN-γ, given the prominent role for this pro-inflammatory cytokine in antitumor immunity. In addition, studies that are currently investigating the pathways that lead to tumor escape/relapse in murine models will establish the feasibility of using whole tumor cell-based vaccines for the induction of anti-lymphoma immunity and identify potential strategies for combination immunotherapies. For instance, α-GaLCer adjuvant-based immunotherapy, from cancer patients can be expanded and activated both in vivo and in vitro to stimulate antitumor immunity. Given the current availability of clinical grade α-GaLCer that is deemed safe for use in humans, the rapid translation of our findings to a clinical setting seems highly feasible.9

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

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