Anticancer immunotherapeutic regimens must activate multiple effectors of the innate and the adaptive immune system in order to efficiently counteract the escape mechanisms set in place by malignant cells. The cellular components of the immune system that are important for treatment outcome have been shown to vary, at least to some degree, with tumor type and treatment modality. Thus, while natural killer (NK) cells are needed for so-called “missing self”-based cellular cytotoxicity (resulting in the elimination of cells that do not express MHC class I molecules), γδ T lymphocytes eliminate cells that express altered self antigens, CD4+ T lymphocytes help B cells to produce antibodies that are necessary for antibody-dependent cellular cytotoxicity (ADCC), and CD8+ T lymphocytes exert direct, tumor-specific cytotoxic functions. Invariant natural killer T (iNKT) cells are important in that they integrate the activation of the innate and adaptive immune systems by secreting high amounts of immunostimulatory cytokines and cross-licensing antigen-presenting cells (APCs) such as dendritic cells (DCs). Exosomes are nanovesicles originating from the endosomal compartment that can carry immunostimulatory or immunosuppressive molecules, and are currently explored as therapeutic vehicles. We have recently shown that DC-derived exosomes loaded with the iNKT-cell ligand α-galactosylceramide (αGC) and the model antigen ovalbumin (OVA) induce strong innate and OVA-specific adaptive immune responses while preventing the anergy of iNKT cells. In particular, we demonstrated that the intravenous administration of αGC/OVA-loaded exosomes leads to a strong, sequential activation of iNKT cells, NK cells, γδ T cells, CD4+ T cells as well as OVA-specific CD8+ T and B lymphocytes. Moreover, we found that exosomes are more potent in inducing γδ T cell-dependent and OVA-specific T and B cell-mediated immunity than comparable amounts of soluble αGC and OVA. In contrast, soluble αGC induces more robust iNKT- and NK-cell responses than αGC-loaded exosomes, indicating that different mechanisms are responsible for the activation of cells of the innate and adaptive immune systems.

Despite the potent adjuvant activity of iNKT cells, their use in clinical settings has been limited since a single injection of soluble αGC is sufficient to render iNKT cells unresponsive (anergy). Conversely, αGC results in prolonged iNKT-cell responsiveness, even upon several injections. Similarly, an antigen-CD1d-αGC fusion protein has been shown to prevent the induction of iNKT-cell anergy. Moreover, the co-delivery of αGC with microbial or synthetic glycolipid antigens on latex beads reportedly increases its adjuvant activity through the iNKT cell-dependent cross-activation of APCs, resulting in the release of T cell-attracting chemokines. Our findings suggest that αGC- and OVA-loaded exosomes boost T- and B-cell immunity by a similar mechanism. We propose that an early activation of iNKT cells in the spleen leads to efficient antigen presentation by DCs and hence improved T- and B-cell responses (Fig. 1). We observed that the levels of circulating interferon γ (IFNγ) levels and OVA-specific antibodies are significantly increased upon the injection of αGC/
OVA-loaded exosomes as compared with that of soluble ligands. Based on our results and the aforementioned studies, we speculate that the delivery of both αGC and antigens to the same APC is crucial for the activation of antigen-specific T cells, thus having a critical impact on the outcome of immune responses. We also demonstrated that a single dose of αGC/OVA-loaded exosomes not only reduces the growth of established OVA-expressing B16 melanomas but also increases the survival of mice bearing these neoplasms. Importantly, a second dose of αGC/OVA-loaded exosomes significantly increased tumor infiltration by OVA-specific CD8+ T cells as well as the titers of OVA-specific antibodies, hence further prolonging the survival of tumor-bearing mice.

In summary, exosomes might provide a new delivery platform for antigens and αGC that optimally harnesses the adjuvant activity of iNKT cells. In addition to αGC and antigens, DC-derived exosomes contain various immunostimulatory molecules, including heat-shock proteins, the α chain of the interleukin-15 receptor (IL15Rα), killer cell lectin-like receptor subfamily K, member 1 (KLRK1) ligands and Toll-like receptor (TLR) agonists, and might be further modified to increase their immunostimulatory efficacy. The design of exosomes might therefore be adjusted depending on the desired immunological outcome. We propose that the next step in exosome-induced immune responses to be explored in clinical trials should include iNKT cell ligands for the induction of broad immune responses that engage both the innate and the adaptive arms of the immune system.

Disclosure of Potential Conflicts of Interest
The authors declare that there are no conflicts of interest.
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