OncoImmunology 2:4, e23432; April 2013; © 2013 Landes Bioscience

Immunotherapy with artificial adjuvant vector cells

Harnessing both arms of the immune response

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Keywords: α-galactosylceramide, adjuvant effects, cross-presentation, dendritic cells, iNKT cells

Both innate and adaptive immunity underpin cancer immunosurveillance. To stimulate both these arms of the immune system, we used allogeneic cells loaded with natural killer T (NKT) cell ligands and expressing tumor-associated antigens, resulting in NKT cell activation, dendritic-cell maturation and ultimately in the elicitation of adaptive T-cell responses. This approach holds great promise for the development of novel anticancer immunotherapies.

Dendritic cells (DCs) are powerful antigen-presenting cells (APCs) that activate innate lymphocytes and elicit antigen-specific T-cell responses. DC-based immunotherapy, for instance by means of autologous DCs loaded with tumor-specific antigens ex vivo, has been used in cancer patients with some success.[1] Unfortunately, ex vivo DC-based immunotherapy requires a large number of DCs to be generated from individual patients, and the quality of DCs largely depends on the patient's clinical conditions. More recently, strategies for the in vivo targeting of DCs have been developed based on chimeric proteins in which a selected tumor-associated antigen is fused to an antibody specific for C-type lectin receptors (CLRs).[2]

We have recently established a different in vivo DC-targeting strategy that exploits the pro-inflammatory potential of dying cells together with the adjuvant activity of invariant natural killer T (iNKT) cells. iNKT cells have several features that make them unique. iNKT cells express indeed a nearly invariant T-cell receptor (TCR) and rapidly produce both T_H1 and T_H2-type cytokines upon stimulation.[3] An exogenous glycolipid ligand, α-galactosylceramide (α-GalCer), is presented on a monomorphic, MHC Class I-like molecule, CD1d, and is widely used as a synthetic ligand for activating iNKT cells. Immunotherapeutic strategies based on DCs pulsed with α-GalCer have been shown activate iNKT cells and natural killer (NK) cells to mediate antitumor effects, both in mice and humans. In these settings, the number of interferon γ (IFNγ)-producing innate lymphocytes positively correlated with the extent of antitumor responses.[4,5] Of note, not only DCs, but other $CD1d^+$ cells including macrophages, B cells and even tumor cells, can act as APCs for iNKT cells in vivo.[6]

Activated iNKT cells have the ability to license DCs in vivo.[7] Mature DCs not only express increased co-stimulatory (i.e., CD40, CD80 and CD86) and MHC Class II molecules but also produce pro-inflammatory cytokines, such as tumor necrosis factor α (TNFα) and interleukin (IL)-12, and chemokines, including CCL17 and CCL21, hence recruiting both T and iNKT cells. Finally, mature DCs acquire the capacity to induce antigen-specific T-cell responses.

Many studies have demonstrated that the co-administration of soluble or cell-associated antigens plus α-GalCer leads to the generation of antigen-specific T_H1 $CD4^+$ T-cell responses and cytotoxic T lymphocytes (CTLs).[3,7] The timing of antigen delivery to DCs is crucial, as DCs exhibit reduced antigen uptake after maturation.[8] Based on these observations, we sought to design "artificial adjuvant vector cells" (aAVCs) that would be loaded with α-GalCer and transfected with an appropriate tumor antigen-coding mRNA,[9,10] combining the adjuvant effects of iNKT-cell activation with antigen delivery to DCs in vivo[6,9] (**Fig. 1**).

We first compared immune responses in mice administered with CD1d-expressing allogeneic vector cells vs. syngeneic DCs.[10] Mice receiving aAVCs exhibited stronger antigen-specific T-cell responses than mice treated with DCs transfected with antigen-coding mRNA or iNKT ligand-loaded DCs transfected with antigen-coding mRNA. Interestingly, the magnitude of CD1d expression on aAVCs correlated with the strength of T-cell response.[7] By means of this system, we demonstrated that α-GalCer-loaded tumor cells as well as α-GalCer-loaded allogeneic fibroblasts transfected with tumor antigen-coding mRNAs efficiently generate antigen-specific CTLs in murine models[6,9] (**Fig. 1**).

We next evaluated how efficiently human DCs cross-present tumor

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Submitted: 12/23/12; Accepted: 12/28/12
Citation: Fujii S, Shimizu K. Immunotherapy with artificial adjuvant vector cells: Harnessing both arms of the immune response. OncoImmunology 2013; 2:e23432; http://dx.doi.org/10.4161/onci.23432

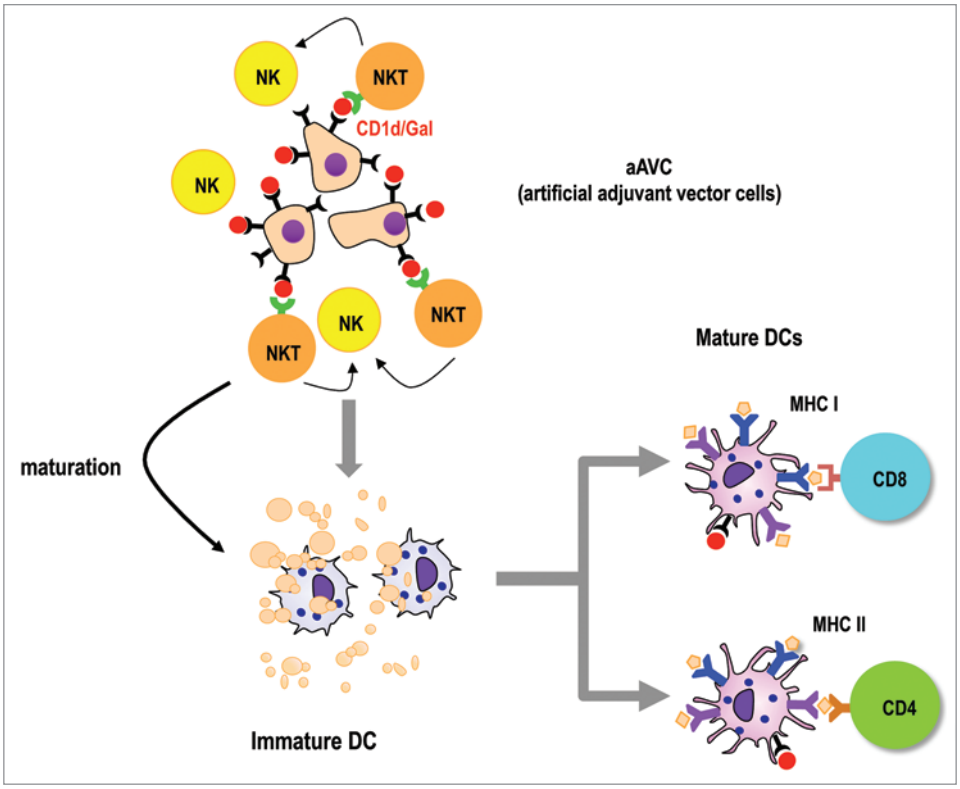

Figure 1. Efficacy of artificial adjuvant vector cells for the induction of innate and adaptive immunity. Artificial adjuvant vector cells (aAVCs) are loaded with α-galactosylceramide (α-GalCer) and engineered to express tumor-specific antigens. When mice are immunized with aAVCs, natural killer (NK) and invariant NK T (iNKT) cells kill aAVCs, leading to the uptake of aAVC debris (including tumor-associated antigens) by dendritic cells (DCs) in situ. Alongside, DCs mature in response to CD40-CD40L signaling and cytokines secreted by activated iNKT cells, hence becoming able to stimulate both $CD4^+$ T and $CD8^+$ T cells in an antigen-specific manner.

antigens delivered by aAVCs.[10] To this aim, we developed a novel system using *NOD/Shi*-scid/*IL-2Rγc*^{null} (NOG) mice as recipients for immature human DCs and T cells (hDC-NOG). T cells from healthy donors were engineered for the expression of the MART-1 tumor antigen and adoptively transferred into NOG mice. Autologous immature DCs were then injected i.v., followed by iNKT cells alone or combined with aAVCs. The efficiency of DC-mediated cross-presentation was evaluated by the expansion in vivo of T cells transduced with a MART-1-specific TCR. Interestingly, the efficient cross-presentation of tumor antigens to MART-1-specific T cells required the co-administration of aAVCs.

Translational studies using large animals, such as dogs or monkeys, serve as an important research intermediary, so that discoveries can ultimately be translated from the bench to the clinics. The promising results obtained in mice prompted us to investigate the safety of using human aAVCs in canines.[10] Physical examination, blood chemistry, autoantibody tests as well as tissue biopsies from the liver, lung and other organs from dogs receiving high-dose aAVCs confirmed the safety of this strategy and its ability to elicit immunological responses.

Our approach of combining antigen-expressing cells with α-GalCer closely reproduces the conditions that manifest during immune responses in vivo, leading to broad and effective adaptive immunity. We have shown that aAVCs safely initiate antigen-specific immune responses by activating both the innate and adaptive arms of the immune system. Our results support the development of aAVCs as immunotherapeutic tools against cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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