

# Cytotoxic and antigen presenting functions of T helper-1-activated dendritic cells

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**Abbreviations:** DT, diphtheria toxin; DTR, diphtheria toxin receptor; Fas-L, Fas-ligand; GFP, green fluorescent protein; IFN- $\gamma$ , interferon gamma; iNOS, inducible nitric oxide synthase; KDCs, killer dendritic cells; LPS, lipopolysaccharide; MHC, major histocompatibility complex; NMMA, N<sup>G</sup>-methyl-L-arginine; NO, nitric oxide; ROS, reactive oxygen species; Th-1, T helper-1 lymphocytes; TLR, Toll-like receptor; TNF, tumor necrosis factor; TRAIL, TNF-related apoptosis-inducing ligand

Although primarily defined by their cardinal antigen-presenting function, dendritic cells (DCs) are also equipped with cytotoxic properties. We have recently reported that DCs activated by IFN $\gamma$ -secreting Th-1 lymphocytes can kill cancer cells and subsequently present the acquired tumor-derived antigens to T lymphocytes both in vitro and in vivo.

The prospect of exploiting the antigen presenting function of DCs to generate adaptive anti-tumor immunity has prompted extensive research and clinical trials to evaluate the therapeutic efficacy of DC-based cancer vaccines. Besides their primary role as orchestrators of adaptive and innate immune responses, many studies conducted in rodents and humans have highlighted the possibility that DCs can also function as direct cytotoxic effectors against tumors.<sup>1-5</sup> This less conventional aspect of DC biology has however received limited attention and controversy has arisen as it relates to the true origin of these cells, the mode of induction of and the mechanism(s) underlying their killing activity.<sup>2,3,6,7</sup> Whether and how the cytotoxic function of DCs may influence their antigen-presenting function and ability to activate effector lymphocytes is still debatable.<sup>2-5,8,9</sup>

The killing activity of different DC subpopulations (native DC differentiating in vivo or DC generated in vitro from dedicated precursors) may be innate or triggered by distinct signals including Toll-like receptor (TLR) agonists such as LPS

or CpG, different cytokines, or CD40L. In our recent work,<sup>10</sup> we investigated the possible modulation of mouse bone marrow-derived DC cytotoxic function by T lymphocytes. Using B16 melanoma or 4T1 breast cancer cells as targets, we demonstrated that pro-inflammatory CD4<sup>+</sup>Tbet<sup>+</sup> T helper-1 (Th-1) lymphocytes promoted the killing potential of purified CD11c<sup>+</sup> DC generated from C57BL/6 or BALB/c mouse bone marrow. Further investigation of the mechanisms underlying Th-1-mediated induction of DC cytotoxic activity indicated that IFN $\gamma$  played a prominent role in this process. Indeed, anti-IFN $\gamma$  blocking antibodies prevented the induction of DC cytotoxic function and Th-1 lymphocytes failed at triggering the tumoricidal function of DCs generated from IFN $\gamma$  receptor knockout mice.

A variety of cytotoxic mechanisms responsible for DC-mediated tumor cell killing have been described which include the perforin/granzyme system, death receptor ligands (Fas-L, TRAIL and other TNF-family members), ROS and/or NO. We did not detect significant expression of

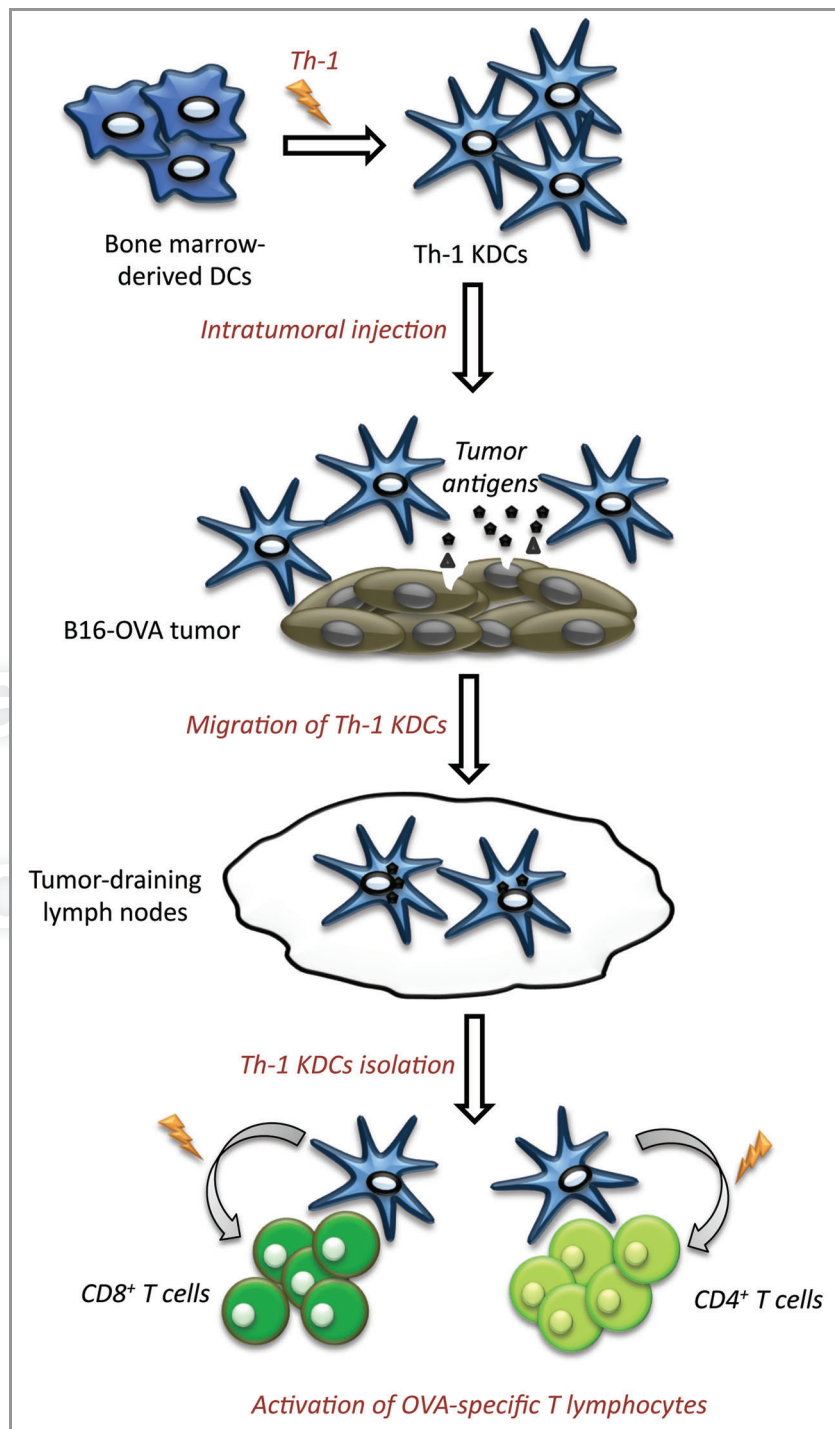
TRAIL, Fas-L, perforin and granzyme by Th-1-activated killer DCs (Th-1 KDCs). Moreover, the tumoricidal function of Th-1 KDCs generated from Fas-L<sup>-/-</sup>, TRAIL<sup>-/-</sup> or perforin<sup>-/-</sup> mice was not impaired compared with that of DC generated from wild-type mice, excluding a possible role for these molecules. Importantly, iNOS expression was significantly upregulated in Th-1 KDCs, which correlated with increased nitrite concentration in the culture supernatants. Confirming these observations, the inhibitor of iNOS, NMMA, abrogated Th-1 KDC-mediated tumor cell killing and the cytotoxic activity of DC generated from iNOS<sup>-/-</sup> mice was significantly impaired, highlighting the central role of NO in the tumoricidal function of these cells.

The fact that a same population of cells can unify cytotoxic and antigen presenting functions has been subjected to intensive debate and reservations were raised on the true antigen presenting capability of KDCs. A critical question was therefore to determine whether Th-1 KDCs were capable of presenting antigens from the tumor cells they had killed. To address this

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point, Th-1 KDCs were first co-cultured with B16 melanoma cells expressing the model antigen ovalbumin (B16-OVA), re-isolated and either stained with anti-CD11c and an antibody recognizing MHC class I-SIINFEKL complexes (H-2Kb/OVA<sub>257-264</sub>) or cultured for 24 h with B3Z (a CD8<sup>+</sup> T cell line expressing a TCR which specifically recognizes the SIINFEKL peptide of OVA in the context of MHC Class I). H-2Kb-SIINFEKL complexes were detected at the surface of isolated CD11c<sup>+</sup> DCs and purified Th-1 KDCs were capable of activating B3Z cells. Similar results were obtained using lymphocytes from OT-I transgenic mice that specifically recognize H-2Kb-OVA<sub>257-264</sub> complexes or from OT-II mice (recognizing I-A(d)-OVA<sub>323-339</sub>) (see ref. 10 and unpublished results). These results therefore indicate that in vitro Th-1 KDCs were able to kill OVA-expressing B16 and acquire, process and present ova peptides. In a next step we explored the significance of these observations in vivo. CD11c-GFP-DTR mice bearing established B16-OVA tumors were treated with DT to deplete endogenous CD11c<sup>+</sup> DCs and Th-1 KDCs or control non-killer DCs were injected into the tumor beds. After 36 h the ability of CD11c<sup>+</sup> DCs isolated from the tumor draining lymph nodes to activate specific T lymphocytes was examined. Interestingly, Th-1 KDCs were significantly more efficient than non-killer DCs at inducing B3Z activation and OT-I and OT-II T lymphocyte proliferation. These results therefore demonstrate that Th-1-activated killer DCs were capable of migrating from the tumor site to the lymph nodes, and that functionally these killer DCs had the ability to process and present in a MHC Class I and Class II-restricted manner the tumor-derived antigens acquired in vivo (Fig. 1). This capacity of Th-1 KDCs to efficiently present antigens was contingent upon induction of their killing function.

Harnessing the potential of KDC to act as killers and messengers may provide multiple advantages which may revitalize their attractiveness as anti-cancer vaccines. By participating in the direct elimination of tumor cells, KDC may contribute to



**Figure 1.** Antigen presenting function of Th-1 KDCs administered in vivo. Th-1-activated KDCs injected intratumorally to DT-treated B16-OVA-bearing CD11c-GFP-DTR mice migrated to the tumor draining lymph nodes and were capable of presenting acquired OVA-derived antigens to specific CD4<sup>+</sup> or CD8<sup>+</sup> T lymphocytes.

diversify the immune cytotoxic effector mechanisms. More importantly, from an immunological perspective, by allowing for the rapid uptake of released tumor antigens, before their clearance by

scavenger neutrophils or macrophages, KDC-mediated tumor cell killing is of considerable relevance for the acquisition of tumor-derived material in a more efficient manner. In line with this idea,

further studies are required to determine whether KDC may promote a more immunogenic type of cancer cell death which may foster tumor antigen uptake,

processing and presentation. It will also be essential to clearly delineate the potential advantages of using KDC (either KDC purified after killing of

tumor cells in vitro, or KDC directly injected into the tumor beds) over conventional tumor-antigen loaded DC as cancer vaccines.

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