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Type II natural killer T cells foster the antitumor activity of CpG-oligodeoxynucleotides

Jie Zhao, Sreya Bagchi, and Chyung-Ru Wang*

Department of Microbiology and Immunology; Feinberg School of Medicine; Northwestern University; Chicago, IL USA

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Type II natural killer T (NKT) cells in cancer immunity are typically associated with suppression of tumor immunosurveillance through secretion of IL-13. We previously demonstrated that CpG oligonucleotide therapy activated Type II NKT cells to produce T helper type 1 (Th1) rather than T helper type 2 (Th2) cytokines. This cytokine skewing may manifest in Type II NKT cell antitumor properties in an immunotherapeutic setting.

Natural killer T (NKT) cells are a unique subset of T cells that recognize lipid antigens presented by the MHC class Ib molecule CD1d. There are two distinct types of NKT cells termed Type I and Type II NKT cells. Type I NKT cells bear "invariant" T cell receptors (TCRs) and can be detected by CD1d/α-galactosylceramide (α-GalCer) tetramers. In contrast, Type II NKT cells express diverse TCRs and are not CD1d/α-GalCer tetramer reactive. Due to the polyclonal nature of the TCR repertoire, specific markers to track Type II NKT cells remain largely unavailable, a caveat that has been a huge deterrent to their study. Since these cells are more prevalent than Type I NKT cells in humans,[1] it is immensely important to further our understanding of these lymphocytes, and even manipulate the functions of this unique T-cell subset, in order to harness their therapeutic potential.

Recently, we took advantage of a unique mouse model to track and characterize Type II NKT cells at a polyclonal level.[2] This model allowed us to evaluate the developmental requirements, antigen specificity, and functional potential of both Type I and Type II NKT cells. In comparison to Type I NKT cells, we found that Type II NKT cells produced more IL-13 relative to interferon γ (IFN-γ). This finding was particularly relevant in the context of tumor immunity, as previous studies have shown that IL-13 secreted by Type II NKT cells is crucial for mediating tumor immunosuppression.[3] Thus, our study suggested that the differential cytokine-secreting capacities of Type I and Type II NKT cells may contribute to the contrasting roles of these NKT cell subsets in tumor immunosurveillance.

To harness the beneficial effects of Type II NKT cells in tumor immunity, we sought to induce cytokine switching in these cells from a T helper type 2 (Th2) to a T helper type 1 (Th1) cytokine production pattern. It was known that CpG oligodeoxynucleotides (ODNs), the Toll-like receptor 9 (TLR9) agonists and potent adjuvants in cancer immunotherapy, promoted IFN-γ but not IL-4 production by Type I NKT cells.[4,5] However, it remained unclear whether CpG ODNs could skew Type II NKT cells to a Th1 phenotype, and if so, what the functional consequences of this phenotypic shift would be on tumor immunity. Importantly, Type II NKT cells produce significant amounts of IFN-γ, but not IL-4 and IL-13, following either in vitro or in vivo treatment with CpG ODNs. Furthermore, the production of IFN-γ was partially blocked by anti-CD1d antibody, indicating that full activation of Type II NKT cells by CpG ODNs was CD1d-dependent.

To assess the role of CpG-skewed and activated Type II NKT cells on tumor immunotherapy, B16-melanoma bearing mice lacking Type I NKT cells ($J\alpha18^{-/-}$) or lacking both Type I and II NKT cells ($CD1d^{-/-}$) were monitored for responses to CpG in comparison to WT controls. CpG treatment was observed to significantly reduce tumor size in all the mouse strains. Notably, CpG-treated WT mice exhibited the smallest tumor volume, whereas CpG-treated $CD1d^{-/-}$ mice had the largest, suggesting both Type I and II NKT cells contributed to the antitumor activity of CpG. Analyses of tumor-infiltrating cells revealed that CpG induced a significantly higher percentage of $CD45^{+}$ cells and more IFN-γ-producing NK cells in WT mice than in $J\alpha18^{-/-}$ and $CD1d^{-/-}$ mice. Although the composition of tumor-infiltrating leukocyte subsets was the same between $J\alpha18^{-/-}$ and $CD1d^{-/-}$ mice, a higher proportion of IFN-γ producing $CD8^{+}$ T cells was found in $J\alpha18^{-/-}$ compared with $CD1d^{-/-}$ mice. These data suggest that CpG activated Type II NKT cells contribute to antitumor immunity by augmenting $CD8^{+}$ T cell responses.

Collectively, our results demonstrated a novel immunotherapeutic approach aiming to manipulate the function of Type II NKT cells by CpG. This altered function appears to contribute to the protective role of Type II NKT cells in

*Correspondence to: Chyung-Ru Wang; Email: chyung-ru-wang@northwestern.edu
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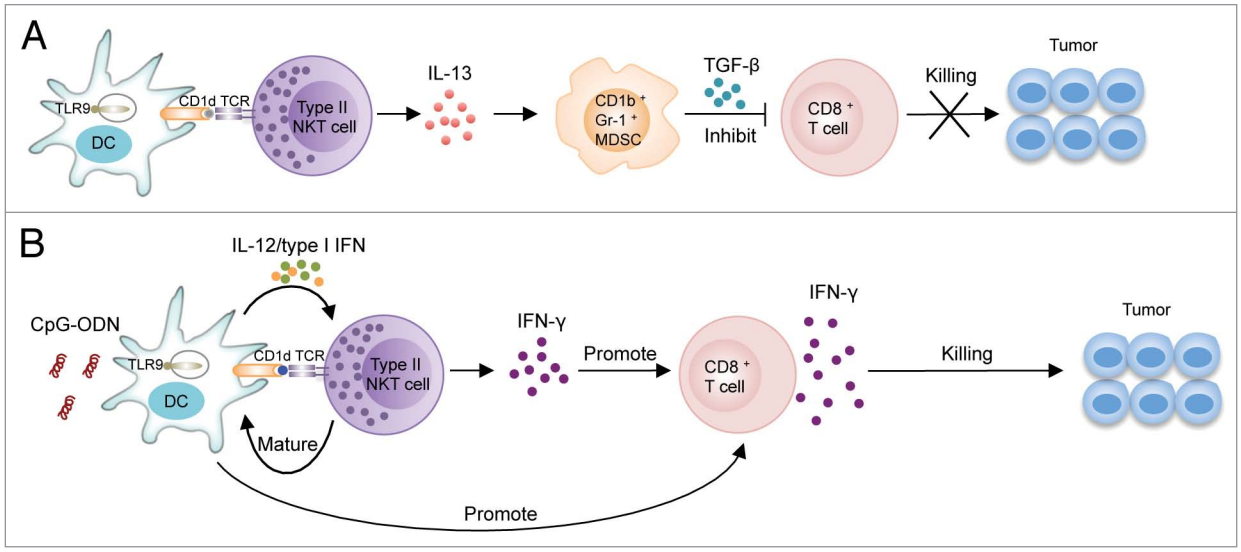

Figure 1. The role of Type II natural killer T cells in tumor immunity. **(A)** Type II natural killer T (NKT) cells suppress antitumor immune responses by producing IL-13, thereby activating myeloid-derived suppressor cells (MDSCs) that subsequently inhibit tumor-specific CD8+ T-cell responses via TGF-β. **(B)** CpG-stimulated dendritic cells (DCs) activate Type II NKT cells via cytokine production and CD1d-mediated lipid antigen presentation. CpG-activated Type II NKT cells produce interferon γ (IFN-γ), which in turn enhances DC maturation and promotes tumor-specific CD8+ T-cell responses.

tumor immunity (**Fig. 1**). It is worth mentioning that the mechanism of Type II NKT cells in suppressing tumor immunosurveillance was delineated in BALB/c mice. However, we used the B16 melanoma model in C57BL/6 mice to evaluate the contribution of Type II NKT cells in the CpG-mediated antitumor response. As C57BL/6 mice exhibit a Th1 bias and BALB/c mice tend to develop a Th2-predominant immune response, further studies are needed to rule out any strain specific effects on the role of Type II NKT cell in tumor immunity. Additionally, we anticipated that in our model, Type II NKT cells would contribute to tumor immunity primarily by altering dendritic cell and CD8+ T-cell function. However, our previous study showed that clonally-derived Type II NKT cells were capable of directly lysing CD1d-expressing lymphoma cells.[6] Thus, while CD1d-positive tumors could be either directly, or indirectly, killed by Type II NKT cells, CD1d-negative tumors are exclusively indirect targets of this NKT cell subset. Lastly, aside from CD1d, humans also possess the non-polymorphic group 1 CD1 molecules (CD1a, CD1b, CD1c). Interestingly, group 1 CD1-restricted autoreactive T cells also have an activated phenotype and diverse TCR repertoire similar to Type II NKT cells.[7-9] Therefore, exploring the role of group 1 CD1-restricted autoreactive T cells in tumor immunity is also warranted.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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