A critical role for natural killer cells in dendritic cell-based anticancer immunotherapy

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Concerns that cancer is a heterogeneous disease and thus unlikely to respond to a single agent also extend to efforts to develop immunotherapeutic regimens. Although T cell-based responses are detectable in the majority of patients vaccinated with tumor antigens, complete responses are rare and generally observed in <10% of patients. Immunotherapeutic DC-based strategies have limited efficacy, yet tantalizingly, durable responses are noted in a limited set of patients, even those with advanced disease. Self-renewing, clonal cells surviving therapeutic interventions present additional recalcitrance to treatment partially through genomic instability and alterations in their anatomic location. Further complicating treatment outcome is the predisposition of the immune system to systemic tolerance in the latter stages of cancer therapy. Therefore, there is a pressing need to develop combinatorial immunotherapeutic strategies with complementary activities to combat cancer as a multifaceted disease.

Promising clinical results with immune modulators, such as antibodies targeting programmed cell death 1 (PD-1) and cytotoxic T lymphocyte associated protein 4 (CTLA-4), have renewed interest in immunotherapy and have offset growing pessimism in response to the incomplete efficacy of conventional and vaccine-based cancer treatment regimens. The majority of immunotherapeutic endeavors have focused on the in vivo stimulation or adoptive transfer of tumor antigen-specific T cells. However it is now feasible to enhance anticancer T-cell responses by recruiting the effector activity of non-conventional “helper cells” such as mucosal-associated invariant chain T cells (MAIT), γδ-T cells, natural killer T (NK-T) cells, natural killer (NK) cells, and macrophages. In particular, NK cells offer cytolytic and cytokine-based helper and effector functions as ideal “adjuvants” for T cell based therapy. NK cell recruitment and activation may be triggered by a variety of mechanisms including agents targeting NK cell surface molecules, such as activating receptors and Fc receptors as well as cytokines, such as IL-12 / IL-18. Furthermore, NK cells may also be recruited and activated by exposure to DC and microbial stimuli.

In our recent report on the requirement of NK cells for DC vaccination, we focused our attention on melanoma. To improve DC based strategies, we first examined a panel of pattern recognition receptor ligands for their ability to induce a therapeutic response to melanoma. In these experiments, DC were pulsed with nominal tumor antigen (ovalbumin) and then matured with microbial products or synthetic mimics. The commonly utilised immunostimulants lipopolysaccharide, Pam3Cys synthetic lipopeptide, CpG oligonucleotides, and polyI:C RNA mimic were surprisingly poor at inducing a therapeutic response against melanoma (ref. 7 and data not shown) In contrast, we found that the Gram-positive bacterium S. salivarius and the Gram-positive cell wall component lipoteichoic acid were significantly more effective. We have previously noted that such Gram-positive preparations induce a rapid release of interferon-γ (IFNγ) from human or murine leukocytes. In mice, the activity was mediated exclusively by CD49b−TCR− cells and this property was maintained in RAG-1−/− and CD1d−/− mice, consistent with a role for NK cells.

Our tumor study utilized both therapeutic and prophylactic settings in order to determine whether NK cells were required for effective DC-immunotherapy during priming or tumor challenge. Strikingly, the absence of NK cells at the time of tumor challenge resulted in a significant loss of tumor protection, while depletion of NK cells at the time of vaccination had little impact on vaccine efficacy. These results suggested that NK cell activity was either enhancing the activation of tumor specific T cells, or increasing the susceptibility of the tumor to T-cell mediated destruction. When we analyzed the phenotype...
of NK cells in the draining lymph node, NK cells from DC-immunised animals showed an increased potential to release IFNγ. However, immigrant NK cells did not display enhanced cytotoxic activity against tumor cells, neither did they demonstrate enhanced cytotoxic activity as evaluated by granzyme B expression or LAMP-1 (CD107a) exposure. However, IFNγ release was shown to be essential for the therapeutic benefit of NK-mediated enhancement of DC-vaccination, as shown by in vivo antibody neutralisation studies, and by the attenuation of the response in mice genetically deficient in IFNγ. The therapeutic effect was maintained in perforin-deficient mice, ruling out perforin-mediated killing for both NK cells and T cells as a key mediator of the observed antitumor response. Despite the critical role of NK cells and IFNγ in this setting, CD4+ and CD8+ T-cell depletion did not consistently reduce vaccine efficacy.

The effects of NK cells were antigen-dependent, suggesting that either NK cells were essential for antigen-specific, T-cell responses, or alternatively, that NK cells were directly involved in some form of memory response. The latter seems unlikely, as ovalbumin has not previously been identified as an antigen capable of inducing NK cell memory. Our findings that treating B16 melanoma with IFNγ markedly upregulated both MHC-I and MHC-II on tumor cells supports a key role for IFNγ-producing NK cells in promoting antigen-dependent, anticaner T-cell responses.

IFNγ had no effect on tumor cell division or viability,7 suggesting that IFNγ effects were mediated by enhanced antigen presentation of tumor antigen to tumor specific T cells or by inhibition of angiogenic pathways (discussed in ref. 7; Fig. 1). Similar to our results, recent, independent studies have also noted the NK-cell dependence of tumor therapy when a potent bacterial9 or viral9 stimulus is utilized as an adjuvant.

In conclusion, activation of NK cells offers an additional strategy to enhance T-cell activation, either in conjunction with conventional chemotherapy or radiotherapy10 or together with more recent innovative immunotherapeutic strategies such as adoptive transfer of T cells or DCs, or tumor-antigen vaccination treatment regimens.

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

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