OncoImmunology 3, e28582; April 2014; © 2014 Landes Bioscience

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

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Keywords: natural killer cell, cancer, melanoma, dendritic cell, immunotherapy

Multipronged immunotherapies that activate both T cells and natural killer (NK) cells may result in more robust and durable anticancer responses. The successful outcome of dendritic cell (DC)-based vaccination therapy involves a hitherto unrecognized role for NK cells. Combinatorial regimens that enhance the contribution of NK cells to the anticancer immune response may therefore improve clinical outcomes.

Concerns that cancer is a heterogeneous disease and thus unlikely to respond to a single agent[1] 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.[2] Immunotherapeutic DC-based strategies have limited efficacy, yet tantalisingly, durable responses are noted in a limited set of patients, even those with advanced disease.[2] Self-renewing, clonal cells surviving therapeutic interventions present additional recalcitrance to treatment partially through genomic instability and alterations in their anatomic location.[1] Further complicating treatment outcome is the predisposition of the immune system to systemic tolerance in the latter stages of cancer therapy.[3] 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.[4] 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.[5,6]

In our recent report on the requirement of NK cells for DC vaccination, we focused our attention on melanoma.[7] 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.[6]

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

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Submitted: 03/14/2014; Accepted: 03/18/2014; Published Online: 04/29/2014
Citation: McLellan AD. A critical role for natural killer cells in dendritic cell-based anticancer immunotherapy. OncoImmunology 2014; 3:e28582; http://dx.doi.org/10.4161/onci.28582

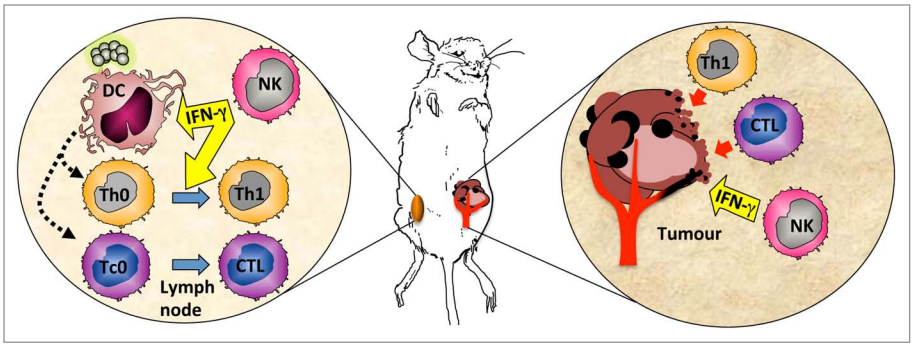

Figure 1. Possible mechanisms of NK cell contribution to DC-immunotherapy. Natural killer (NK) cells can foster dendritic cell therapy anticancer responses mediated either in the lymph node draining the vaccination site (left) or at the tumor environment (right). In the lymph node, bidirectional activation between DCs responding to microbial stimulus and NK cells influence the differentiation of type 1 T helper (Th1) $CD4^+$ T cells and cytotoxic T lymphocytes (CTLs). At the time of tumor challenge, NK cells and $CD4^+$ and $CD8^+$ T cells home to the tumor with the release of interferon-γ (IFNγ) being critical for the combined antitumor action. IFNγ may act to inhibit angiogenesis or to enhance the antitumor activities of $CD4^+$ and $CD8^+$ T cells.

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, anticancer 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 bacterial[8] or viral[9] 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 radiotherapy,[10] 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.

Acknowledgments

The author would like to thank Sarah Saunderson and Remy Muhsin for critical reading of the manuscript and Amy Dunn for assistance with the graphics.

References

1. Fidler IJ. Biological heterogeneity of cancer: implication to therapy. Hum Vaccin Immunother 2012; 8:1141-2; PMID:22854675; http://dx.doi.org/10.4161/hv.19643
2. Radford KJ, Tullett KM, Lahoud MH. Dendritic cells and cancer immunotherapy. Curr Opin Immunol 2014; 27C:26-32; PMID:24513968; http://dx.doi.org/10.1016/j.coi.2014.01.005
3. Andersen MH, Keikavoussi P, Bröcker EB, Schuler-Thurner B, Jonassen M, Søndergaard I, Straten PT, Becker JC, Kämpgen E. Induction of systemic CTL responses in melanoma patients by dendritic cell vaccination: cessation of CTL responses is associated with disease progression. Int J Cancer 2001; 94:820-4; PMID:11745484; http://dx.doi.org/10.1002/ijc.1536
4. Callahan MK, Wolchok JD. At the bedside: CTLA-4- and PD-1-blocking antibodies in cancer immunotherapy. J Leukoc Biol 2013; 94:41-53; PMID:23667165; http://dx.doi.org/10.1189/jlb.1212631
5. Mentlik James A, Cohen AD, Campbell KS. Combination Immune Therapies to Enhance Anti-Tumor Responses by NK Cells. Front Immunol 2013; 4:481; PMID:24391651; http://dx.doi.org/10.3389/fimmu.2013.00481
6. Bouwer AL, Saunderson SC, Dunn AC, Lester KL, Crowley LR, Jack RW, McLellan AD. Rapid interferon-gamma release from natural killer cells induced by a streptococcal commensal. J Interferon Cytokine Res 2013; 33:459-66; PMID:23659669; http://dx.doi.org/10.1089/jir.2012.0116
7. Bouwer AL, Saunderson SC, Caldwell FJ, Damani TT, Pelham SJ, Dunn AC, Jack RW, Stoitzner P, McLellan AD. NK cells are required for dendritic cell-based immunotherapy at the time of tumor challenge. J Immunol 2014; 192:2514-21; PMID:24477907; http://dx.doi.org/10.4049/jimmunol.1202797
8. Kuhn S, Hyde EJ, Yang J, Rich FJ, Harper JL, Kirman JR, Ronchese F. Increased numbers of monocyte-derived dendritic cells during successful tumor immunotherapy with immune-activating agents. J Immunol 2013; 191:1984-92; PMID:23858033; http://dx.doi.org/10.4049/jimmunol.1301135
9. Zamarin D, Holmgaard RB, Subudhi SK, Park JS, Mansour M, Palese P, Merghoub T, Wolchok JD, Allison JP. Localized oncolytic virotherapy overcomes systemic tumor resistance to immune checkpoint blockade immunotherapy. Sci Transl Med 2014; 6:226ra32; PMID:24598590; http://dx.doi.org/10.1126/scitranslmed.3008095
10. Sheng Sow H, Mattarollo SR. Combining low-dose or metronomic chemotherapy with anticancer vaccines: A therapeutic opportunity for lymphomas. Oncoimmunology 2013; 2:e27058; PMID:24498564; http://dx.doi.org/10.4161/onci.27058