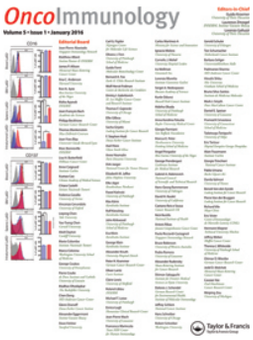

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Cytokine therapy restores antitumor responses of NK cells rendered anergic in MHC I-deficient tumors

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Recent extraordinary advances in cancer immunotherapy rely primarily on marshaling T cell responses. Here we discuss how NK cell responses can be amplified. We find that MHC I-deficient tumors induce anergy of NK cells but that cytokine therapy restores NK cell activity and increases the survival of mice bearing MHC I-deficient tumors.

Natural Killer (NK) cells are key effectors in the response to tumor. They mediate tumor rejection via cytotoxicity and production of cytokines such as IFNγ and TNF-α.[1] NK cells are known to attack MHC-deficient tumor cells, due to the failure of such tumor cells to convey inhibitory signals mediated by MHC-specific inhibitory receptors. Yet many primary tumors show loss of MHC I (MHC class I) molecules,[2] and it is not known how such tumors evade NK cell recognition. In our recent publication in *The Journal of Clinical Investigation*, we describe a new mechanism of tumor evasion acting on NK cells.[3] We found that tumors with low expression of MHC I molecules induce functional anergy of NK cells that depresses the antitumor response. Our results may account in part for findings that NK cells are often dysfunctional in human tumors.[1]

Our studies employed the NK cell-resistant cell line RMA and its MHC I-deficient counterpart cell line, RMA-S, which NK cells kill efficiently. Low or intermediate doses of RMA-S tumor cells were completely rejected in an NK cell-dependent fashion when injected in syngeneic C57BL/6 mice. However, when high doses of RMA-S were implanted, a large fraction of the mice grew solid tumors. Therefore, NK-mediated surveillance of MHC class I-deficient tumors is very effective at lower tumor cell doses but fails when the mice are challenged with a higher dose.

We found that NK cells in close proximity to MHC I-deficient tumors were impaired in their ability to degranulate or produce inflammatory cytokines after *ex vivo* stimulation (what we called a functionally anergic state). Induction of NK cell anergy also occurs in steady state conditions, as we showed by transferring responsive NK cells into MHC I-deficient mice.[4] Our new findings show that MHC I-deficient tumors impose similar anergy of NK cells, enabling the tumor cells to escape the NK cell response. NK cell anergy is induced in both the tumor bed and in the tumor draining lymph nodes, whereas in the distal lymph nodes or in the spleen the NK cells were fully responsive. When MHC I expression was restored on RMA-S cells, anergy of tumor infiltrating NK cells did not occur. Notably, NK cell anergy was associated with poor phosphorylation of intracellular kinases, such as ERK1/2 or AKT, after stimulation *ex vivo*.

Human tumors are usually heterogeneous, and this extends to expression of MHC I molecules.[2] To mimic the heterogeneity in MHC I expression, we challenged mice with MHC I-deficient tumor cells mixed with tumor cells expressing MHC I. We observed that: (i) the MHC I-deficient tumor cells in the mixture were never completely eliminated and (ii) the intratumoral NK cells became anergic, showing that MHC I-deficient tumor cells dominantly impose NK cell anergy. In light of the results as a whole, we hypothesize that NK cells are induced to become anergic by the persistent stimulation provided by the MHC I-deficient cells in the tumor bed.

Using pro-inflammatory cytokines to harness the immune system against tumor cells was one of the first examples of cancer immunotherapy. Promising results obtained from pre-clinical studies inspired many clinical trials that resulted in regulatory approval of IL-2 for treatment of kidney carcinoma and metastatic melanoma, and IFN-α for treatment of metastatic melanoma, Kaposi's sarcoma and certain leukemias.[5,6] However, both treatments are effective in only a small fraction of patients, and both show significant toxicity.

In light of our results, MHC I-deficient tumors are a tempting target for cytokine-based immunotherapy. We hypothesized that a cocktail of pro-inflammatory cytokines could "re-awaken" the NK cells from the anergic state induced by the MHC I-deficient tumor cells. Therefore, we treated tumor bearing mice with a mixture of IL-12 and IL-18 or with the IL-2 mutant H9, whose binding to the IL-2R

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does not depend on the α-subunit of the receptor.[7] Interestingly, both treatments greatly increased the survival of mice challenged with MHC I-deficient tumors and the effects were entirely NK cell-mediated. Interestingly, the treatment was not effective in mice carrying the matched tumors with high MHC I expression, probably because such tumor cells are poor NK cell targets and therefore as resistant to highly active NK cells as they are to weakly active ones. Our results open a new possibility for the use of pro-inflammatory cytokines in the context of tumor immunotherapy. Indeed, one reason for the poor outcomes observed to date in clinical trials could be that the treatments provide benefit selectively to patients with anergic NK cells. Better results may be obtained when patients are stratified according to the MHC I expression of the tumor cells or status of NK cells within the patient's tumors. The toxicity of cytokine therapy may be ameliorated to some degree by employing engineered versions of cytokines that exhibit lower cytotoxicity. Notably, the H9 mutant of IL-2 we employed has been reported to exhibit decreased toxicity compared to wild type IL-2.[7]

The possible greater efficacy of combining cytokine therapies with checkpoint therapies remains to be explored in detail. In particular, our results suggest that efforts to amplify the antitumor effects of NK cells by blocking inhibitory MHC-specific receptors (KIR blockade),[8,9] or by inducing antibody-dependent cellular cytotoxicity mediated by NK cells, may benefit from combination with cytokine therapy. Such combinations may maximize the antitumor effects that NK cells can provide.

Disclosure of Potential Conflicts of Interest

Dr. Raulet is a member of the Scientific Advisory Board of Innate Pharma, SA, which develops cancer therapeutics that target NK cells.

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References

1. Marcus A, Gowen BG, Thompson TW, Iannello A, Ardolino M, Deng W, Wang L, Shifrin N, Raulet DH. Recognition of tumors by the innate immune system and natural killer cells. Adv Immunol 2014; 122:91-128; PMID:24507156; http://dx.doi.org/10.1016/B978-0-12-800267-4.00003-1
2. Garrido F, Algarra I. MHC antigens and tumor escape from immune surveillance. Adv Cancer Res 2001; 83:117-58; PMID:11665717; http://dx.doi.org/10.1016/S0065-230X(01)83005-0
3. Ardolino M, Azimi CS, Iannello A, Trevino TN, Horan L, Zhang L, Deng W, Ring AM, Fischer S, Garcia KC et al. Cytokine therapy reverses NK cell anergy in MHC-deficient tumors. J Clin Invest 2014; 124:4781-94; PMID:25329698; http://dx.doi.org/10.1172/JCI74337
4. Joncker NT, Shifrin N, Delebecque F, Raulet DH. Mature natural killer cells reset their responsiveness when exposed to an altered MHC environment. J Exp Med 2010; 207:2065-72; PMID:20819928; http://dx.doi.org/10.1084/jem.20100570
5. Coventry BJ, Ashdown ML. The 20th anniversary of interleukin-2 therapy: bimodal role explaining long-standing random induction of complete clinical responses. Cancer Manag Res 2012; 4:215-21; PMID:22904643; http://dx.doi.org/10.2147/CMAR.S33979
6. Cutler A, Brombacher F. Cytokine therapy. Ann N Y Acad Sci 2005; 1056:16-29; PMID:16387674; http://dx.doi.org/10.1196/annals.1352.002
7. Levin AM, Bates DL, Ring AM, Krieg C, Lin JT, Su L, Moraga I, Raeber ME, Bowman GR, Novick P et al. Exploiting a natural conformational switch to engineer an interleukin-2 'superkine'. Nature 2012; 484:529-33; PMID:22446627; http://dx.doi.org/10.1038/nature10975
8. Romagne F, Andre P, Spee P, Zahn S, Anfossi N, Gauthier L, Capanni M, Ruggeri L, Benson DM, Jr., Blaser BW et al. Preclinical characterization of 1-7F9, a novel human anti-KIR receptor therapeutic antibody that augments natural killer-mediated killing of tumor cells. Blood 2009; 114:2667-77; PMID:19553639; http://dx.doi.org/10.1182/blood-2009-02-206532
9. Vey N, Bourhis JH, Boissel N, Bordessoule D, Prebet T, Charbonnier A, Etienne A, Andre P, Romagne F, Benson D et al. A phase 1 trial of the anti-inhibitory KIR mAb IPH2101 for AML in complete remission. Blood 2012; 120:4317-23; PMID:23002117; http://dx.doi.org/10.1182/blood-2012-06-437558