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Toward the next generation of NK cell-based adoptive cancer immunotherapy

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Abbreviations: APC, antigen-presenting cell; NK, natural killer; RT, radiation therapy; Tregs, regulatory T cells

The adoptive transfer of interleukin (IL)-2-expanded natural killer (NK) cells has provided unsatisfactory clinical benefits to patients affected by solid tumors. Our study demonstrates that the activation of NK cells with IL-12/IL-15/IL-18 prior to transfer into tumor-bearing mice is critical for obtaining high recovery rates, effector functions in vivo and tumor regression.

Natural killer (NK) cells exert potent antitumor effector functions as they efficiently kill target cells expressing low levels of MHC Class I molecules. Many different tumor types express low levels of MHC Class I molecules and hence are attacked by NK cells. In addition, NK cells produce inflammatory cytokines and crosstalk with other cells of the innate and adaptive immune system.[1] So far, NK cell-based immunotherapeutic approaches have generated promising results only in hematological cancer patients. Conversely, targeting solid tumors with NK cells has been unsuccessful.[2,3]

In most current clinical protocols for adoptive cell transfer, NK cells are expanded ex vivo with interleukin (IL)-2 or IL-15 before reinfusion into patients. In this setting, to obtain high numbers of NK cells, cell expansion must be performed under good manufacturing practice (GMP) conditions and requires large amounts of cytokines. In addition, cytokines such as IL-2 are often administered to patients to allow for the expansion of transferred NK cells and for the acquisition of robust effector functions.[2] The systemic application of IL-2, however, has often been associated with severe side effects. In mouse tumor models, elevated numbers of IL-2-expanded NK cells and repeated cell infusions were required to achieve tumor regression.[4] In our study, we activated NK cells with three cytokines, namely IL-12, IL-15 and IL-18, overnight before adoptive transfer. This cytokine cocktail has previously been shown to increase NK-cell recovery and effector function upon adoptive transfer into naïve $Rag2^{-/-}$ mice.[5] We observed that one single dose of 10^6 IL-12/IL-15/IL-18-preactivated NK cells transferred into irradiated, tumor-bearing mice induced pronounced tumor regression. In contrast, IL-2- or IL-15-treated NK cells failed to achieve any therapeutic effect.[6] IL-12/IL-15/IL-18-preactivated NK cells proliferated rapidly and accumulated in the spleen, tumor and other organs, yielding much higher cell recovery rates than IL-15-treated NK cells. In addition, adoptively transferred IL-12/IL-15/IL-18-preactivated NK cells, but not IL-15-treated NK cells, exhibited high effector functions upon ex vivo re-stimulation with tumor cells. Importantly, in our study (1) NK cells were not expanded for long periods before adoptive transfer, and (2) no additional cytokines were administered to animals. Our protocol relied indeed on the in vivo expansion of adoptively transferred NK cells exhibiting robust effector functions. Taken together, our findings identify a simple and efficient protocol for NK cell-based immunotherapeutic approaches against solid tumors.

Our results reveal a critical role for host CD4+ T cells, which produced IL-2, in the therapeutic effects mediated by IL-12/IL-15/IL-18-preactivated NK cells (**Fig. 1**). IL-12/IL-15/IL-18-pulsed NK cells, compared with IL-15-pretreated NK cells, expressed increased levels of the IL-2 receptor α chain CD25 and host-derived IL-2 was important for their proliferation in vivo. In our study, both IL-12 and IL-18 were necessary for the induction of CD25. In peripheral blood mononuclear cell (PBMC) cultures, IL-12 has previously been reported to be the main inducer of CD25.[7] In our hands, IL-2 was required for the proliferation of IL-12/IL-15/IL-18-preactivated NK cells in vivo, but its role in the sustained effector functions of adoptively transferred NK cells remains a matter of investigation in our laboratory. We also found high numbers of CD4+ T cells in close proximity of NK cells within neoplastic leasions, suggesting that the crosstalk between these two cell populations might involve cell-to-cell contacts as well.

NK cells have been described as cells of the innate immune system that are capable of rapidly acquiring effector functions. However, relatively persistent memory NK-cell populations that mount a robust

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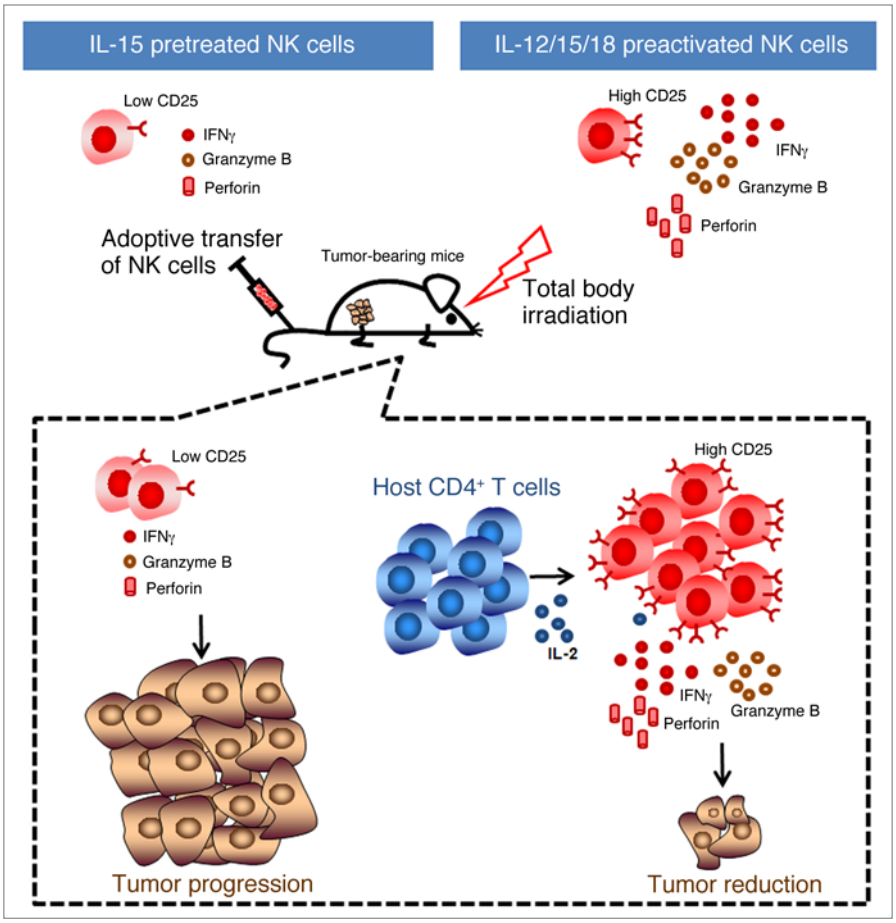

Figure 1. Adoptive transfer of interleukin (IL)-12/IL-15/IL18-preactivated natural killer cells mediates antineoplastic functions against established tumors. C57BL/6 mice were inoculated s.c. with 10^6 RMA-S tumor cells. After 7 days, tumor-bearing mice received total body radiation therapy (5 Gy) and 10^6 natural killer (NK) cells that were pre-activated in vitro with interleukin (IL)-15 (left panel) or IL-12/IL-15/IL-18 (right panel) for 16 h, i.v. Compared with IL-15-pretreated NK cells, adoptively transferred IL-12/IL-15/IL-18-preactivated NK cells proliferated more rapidly in a manner that was dependent on IL-2 produced by host $CD4^+$ T cells. In addition, IL-12/IL-15/IL-18-preactivated NK cells persisted in higher cell numbers and exhibited more robust effector functions than their IL-15-pretreated counterparts, two phenomena that also required the presence of host $CD4^+$ T cells. Most importantly, the adoptive transfer of IL-12/IL-15/IL-18-preactivated NK cells, but not IL-15-pretreated NK cells, into irradiated, tumor-bearing mice substantially reduced tumor growth.

recall response have recently been reported to arise during viral infection[8] and contact hypersensitivity reactions.[9] We found that IL-12/IL-15/IL-18-preactivated NK cells maintain a mature phenotype (i.e., $CD11b^{high}CD27^{low}KLRG1^{high}CD43^{high}$) that resembles that of memory NK cells arising during mouse cytomegalovirus infection.[8] NK cells originating from the adoptively transferred cells persisted for at least 150 days in recipients that had rejected tumors and produced high levels of interferonγ (IFNγ) upon restimulation (unpublished data). Intriguingly, the short (16 h) exposure of NK cells to IL-12/IL-15/IL-18 prior to adoptive transfer resulted in long-lasting changes that affected the behavior of the cell progeny 150 days later. The molecular mechanisms underlying this type of immunological memory are still elusive.

Most importantly, we demonstrated that human IL-12/IL-15/IL-18-preactivated NK cells also proliferate more rapidly than their IL-15-pretreated counterparts, yielding higher cell recovery rates in low-dose IL-2 culture conditions. Notably, IL-12/IL-15/IL-18-pulsed NK cells responded with higher levels of IFNγ to tumor cells or cytokines in vitro. Our data are in line with a recent report from Romee et al., demonstrating that IL-12/IL-15/IL-18-preactivated NK cells maintain high IFNγ production in the presence of IL-15.[10]

A major challenge remains the translation of our findings to a clinical setting of cancer immunotherapy. In our study, radiation therapy (RT) was indispensable to obtain pronounced therapeutic effect from adoptively transferred murine IL-12/IL-15/IL-18-preactivated NK cells. Thus, before the initiation of clinical trials, optimal patient pre-conditioning conditions (involving radiation therapy and/or chemotherapeutic regimens) that allow for the efficient engraftment of adoptively transferred IL-12/IL-15/IL-18-preactivated NK cells and their homing to tumor tissues will have to be established. Therapeutic strategies based on the adoptive transfer of IL-12/IL-15/IL-18-preactivated NK cells might set the stage for a paradigm shift in cancer immunotherapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Cerwenka A, Lanier LL. Natural killer cells, viruses and cancer. Nat Rev Immunol 2001; 1:41-9; PMID:11905813; http://dx.doi.org/10.1038/35095564
2. Terme M, Ullrich E, Delahaye NF, Chaput N, Zitvogel L. Natural killer cell-directed therapies: moving from unexpected results to successful strategies. Nat Immunol 2008; 9:486-94; PMID:18425105; http://dx.doi.org/10.1038/ni1580
3. Parkhurst MR, Riley JP, Dudley ME, Rosenberg SA. Adoptive transfer of autologous natural killer cells leads to high levels of circulating natural killer cells but does not mediate tumor regression. Clin Cancer Res 2011; 17:6287-97; PMID:21844012; http://dx.doi.org/10.1158/1078-0432.CCR-11-1347
4. Pegram HJ, Haynes NM, Smyth MJ, Kershaw MH, Darcy PK. Characterizing the anti-tumor function of adoptively transferred NK cells in vivo. Cancer Immunol Immunother 2010; 59:1235-46; PMID:20376439; http://dx.doi.org/10.1007/s00262-010-0848-7
5. Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama WM. Cytokine-induced memory-like natural killer cells. Proc Natl Acad Sci U S A 2009; 106:1915-9; PMID:19181844; http://dx.doi.org/10.1073/pnas.0813192106
6. Ni J, Miller M, Stojanovic A, Garbi N, Cerwenka A. Sustained effector function of IL-12/15/18-preactivated NK cells against established tumors. J Exp Med 2012; 209:2351-65; PMID:23209317; http://dx.doi.org/10.1084/jem.20120944
7. Lee SH, Fragoso MF, Biron CA. Cutting edge: a novel mechanism bridging innate and adaptive immunity: IL-12 induction of CD25 to form high-affinity IL-2 receptors on NK cells. J Immunol 2012; 189:2712-6; PMID:22888135; http://dx.doi.org/10.4049/jimmunol.1201528
8. Sun JC, Beilke JN, Lanier LL. Adaptive immune features of natural killer cells. Nature 2009; 457:557-61; PMID:19136945; http://dx.doi.org/10.1038/nature07665
9. O'Leary JG, Goodarzi M, Drayton DL, von Andrian UH. T cell- and B cell-independent adaptive immunity mediated by natural killer cells. Nat Immunol 2006; 7:507-16; PMID:16617337; http://dx.doi.org/10.1038/ni1332
10. Romee R, Schneider SE, Leong JW, Chase JM, Keppel CR, Sullivan RP, et al. Cytokine activation induces human memory-like NK cells. Blood 2012; 120:4751-60; PMID:22983442; http://dx.doi.org/10.1182/blood-2012-04-419283