

In vivo evidence for dendritic cell lysis by NK cells

Hints on improving cancer vaccines by targeting NK cell activation

Guido Ferlazzo

Laboratory of Immunology and Biotherapy; Department of Human Pathology; University of Messina; Messina, Italy

Keywords: Cancer vaccines, cytotoxic T lymphocytes, dendritic cells, natural killer cells, lymph nodes, perforin

By using an experimental model of anticancer vaccination, we have recently lent support to the assumption, so far only sustained by in vitro data, that natural killer cells can restrain less immunogenic, allegedly tolerogenic, dendritic cells (DCs). This in vivo selection of immunogenic DCs appears to depend on perforin and to be associated with a more protective tumor-specific T lymphocyte response.

In recent years, a number of studies have indicated that natural killer (NK) cells are not merely cytotoxic lymphocytes competent in containing viral and tumor spreading but also can be considered as crucial fine-tuning effector cells during protective immune responses. For instance, the exploration of the functional links between NK and dendritic cells (DCs) has shown that reciprocal activation ensue the NK/DC interaction.^{1,2} Therefore, NK cells can also shape adaptive immune responses by causing DC maturation and influencing the polarization of primary T-cell responses.³ These functions support protective immune responses against intracellular pathogens and tumors that are most efficiently promoted by Th1 polarized immunity.^{3,4} The NK cell-mediated differentiation and maturation of DCs primarily occurs via the release of pro-inflammatory cytokines by activated NK cells, including tumor necrosis factor α (TNF α) and interferon γ (IFN γ).⁴

Conversely, once activated by DCs, NK cells can acquire the capability of killing immature myeloid DCs.¹ This effect is due to the fact that immature DCs typically express low levels of MHC class I molecules, which would protect them from NK-mediated lysis. Conversely, DCs that, upon antigen uptake, undergo maturation, upregulate MHC Class I

expression and become resistant to NK cells.¹ During the process of maturation, DCs not only upregulate MHC molecules but also chemokine receptors, such as CCR7, and co-stimulatory molecules. The expression of these molecules is crucial for the subsequent DC migration to lymph nodes as well as for the priming of T lymphocytes. Thus, it has been suggested that the NK-mediated killing of DCs may serve to keep in check the quality and the quantity of DCs undergoing maturation (DC editing).⁵ According to this model, DCs that fail to express sufficient amounts of MHC molecules, which would induce inappropriate, low-affinity T-cell priming resulting either in Th2 responses or in the induction of tolerance, would be removed by NK cells.⁶ Indeed, in the absence of NK cells, the in vivo default development pathway of T cells appears strongly biased toward the acquisition of a Th2 phenotype.⁷

However, while in vivo evidence for DC activation by NK cells had previously been provided,^{8,9} the demonstration that autologous DC killing by NK cells is a functionally relevant event occurring in vivo has remained elusive for a long time. We addressed this issue in a recent study, showing that NK cells can efficiently select highly immunogenic DCs for the generation of optimal adaptive immune

responses in vivo.¹⁰ In an experimental model of anticancer vaccination, irradiated tumor cells were administered alone or together with cells devoid of MHC Class I molecules, i.e. cells capable of triggering NK cell activation. Adding MHC-devoid target cells in the vaccine boosted the expansion of tumor-specific CTLs, eventually resulting in enhanced survival of mice upon challenge with a lethal dose of tumor cells (Fig. 1). The depletion of NK cells as well as the use of *Pfn*^{-/-} mice (which lack the gene coding for perforin) impaired both this tumor-specific T-cell response and its protective role upon tumor challenge.

It is noteworthy that such an enhanced antitumor immune response was strongly associated with a decrease in the number of DCs found in vaccine-draining lymph nodes. We indeed observed that the addition of MHC-devoid cells to the vaccine was consistently accompanied by a decrease of DCs in draining but not in controlateral lymph nodes. The residual DCs in the draining lymph node displayed higher T-cell activating capability. This decrease in the number of nodal DCs was abrogated by depleting NK cells in mice, indicating a crucial role for NK cells in this phenomenon. In addition, no decrease of DCs occurred in draining lymph nodes of *Pfn*^{-/-} mice, strongly suggesting that

Correspondence to: Guido Ferlazzo; Email: guido.ferlazzo@unime.it
Submitted: 07/27/12; Accepted: 07/31/12
<http://dx.doi.org/10.4161/onci.21682>

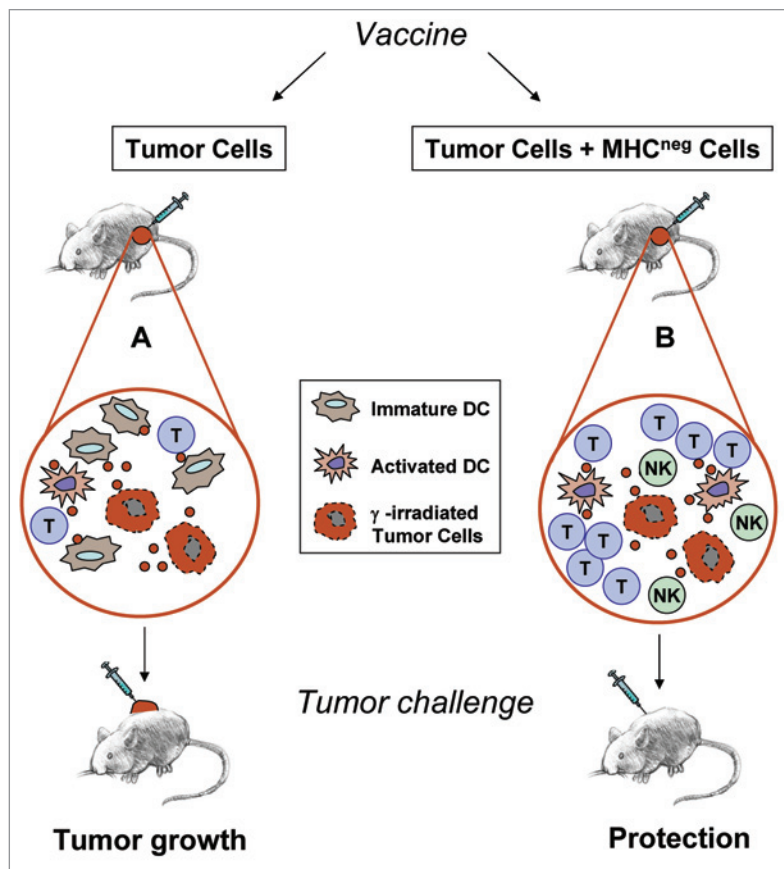


Figure 1. (A and B) In an experimental model of anticancer vaccination, mice were vaccinated with either γ -irradiated tumor cells alone or γ -irradiated tumor cells mixed with cells devoid of MHC molecules (MHC^{neg} cells). After 21 d, mice were challenged with a lethal dose of tumor cells and monitored for tumor growth twice a week for two months. **(A)** Improper tumor antigen presentation by immature dendritic cells (DCs) hinders the clonal expansion of tumor specific T cells. **(B)** Natural killer (NK) cells, as activated by MHC^{neg} cells, remove immature “tolerogenic” DCs. Residual activated DCs are highly immunogenic and can induce proper T-cell clonal expansion, which confers protection to mice against a subsequent challenge with tumor cells.

NK cells can restrain DCs number by a perforin-dependent mechanism.

Our results indicate that, *in vivo*, NK cells can edit DCs for the generation of optimal adaptive immune responses not only by inducing DC maturation and improving DC survival, as previously demonstrated,^{8,9} but also by killing poorly immunogenic DCs.¹⁰ Cell vaccines are under intense investigation and represent a feasible and inexpensive tool for cancer immunotherapy. Our data suggest that cancer cell vaccines could be improved by strategies aimed at

NK cell activation, such as the use of NK-sensitive cancer cells. In the attempt to develop novel antitumor vaccines, appropriate NK cell activation could be accomplished by the employment of histotype-matched allogeneic tumor cells expressing MCH class I molecules that are adequately mismatched with the NK cell inhibitory receptor repertoire of the patient. In this scenario, allogeneic tumor cells would be recognized and killed by NK cells providing a sufficient source of tumor-associated antigens to be loaded on highly immunogenic DCs, as selected by

activated NK cells. Allogeneic tumor cells in these cancer vaccines could be coupled with autologous tumor cells if available. Nevertheless, since patient cancer cells are often not accessible, immunotherapy protocols foreseeing the use of suitable allogeneic cells might allow to both enroll large cohorts of cancer patients and virtually improve the efficacy of anticancer vaccines.

References

1. Ferlazzo G, Tsang ML, Moretta L, Melioli G, Steinman RM, Münz C. Human dendritic cells activate resting natural killer (NK) cells and are recognized via the NKp30 receptor by activated NK cells. *J Exp Med* 2002; 195:343-51; PMID:11828009; <http://dx.doi.org/10.1084/jem.20011149>.
2. Ferlazzo G, Münz C. Dendritic cell interactions with NK cells from different tissues. *J Clin Immunol* 2009; 29:265-73; PMID:19280325; <http://dx.doi.org/10.1007/s10875-009-9283-y>.
3. Martín-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, et al. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. *Nat Immunol* 2004; 5:1260-5; PMID:15531883; <http://dx.doi.org/10.1038/ni1138>.
4. Ferlazzo G. Natural killer and dendritic cell liaison: recent insights and open questions. *Immunol Lett* 2005; 101:12-7; PMID:15941591; <http://dx.doi.org/10.1016/j.imlet.2005.04.015>.
5. Moretta A. Natural killer cells and dendritic cells: rendezvous in abused tissues. *Nat Rev Immunol* 2002; 2:957-64; PMID:12461568; <http://dx.doi.org/10.1038/nri956>.
6. Langenkamp A, Messi M, Lanzavecchia A, Sallusto F. Kinetics of dendritic cell activation: impact on priming of TH1, TH2 and nonpolarized T cells. *Nat Immunol* 2000; 1:311-6; PMID:11017102; <http://dx.doi.org/10.1038/79758>.
7. Byrne P, McGuiirk P, Todryk S, Mills KH. Depletion of NK cells results in disseminating lethal infection with *Bordetella pertussis* associated with a reduction of antigen-specific Th1 and enhancement of Th2, but not Tr1 cells. *Eur J Immunol* 2004; 34:2579-88; PMID:15307190; <http://dx.doi.org/10.1002/eji.200425092>.
8. Andrews DM, Scalzo AA, Yokoyama WM, Smyth MJ, Degli-Esposti MA. Functional interactions between dendritic cells and NK cells during viral infection. *Nat Immunol* 2003; 4:175-81; PMID:12496964; <http://dx.doi.org/10.1038/ni880>.
9. Mocikat R, Braumüller H, Gummy A, Egeter O, Ziegler H, Reusch U, et al. Natural killer cells activated by MHC class I(low) targets prime dendritic cells to induce protective CD8 T cell responses. *Immunity* 2003; 19:561-9; PMID:14563320; [http://dx.doi.org/10.1016/S1074-7613\(03\)00264-4](http://dx.doi.org/10.1016/S1074-7613(03)00264-4).
10. Morandi B, Mortara L, Chiosso L, Accolla RS, Mingari MC, Moretta L, et al. Dendritic cell editing by activated natural killer cells results in a more protective cancer-specific immune response. *PLoS One* 2012; 7:e39170; PMID:22723958; <http://dx.doi.org/10.1371/journal.pone.0039170>.