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NK cells from malignant pleural effusions are potent antitumor effectors

A clue for adoptive immunotherapy?

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Natural killer (NK) cells exert potent antitumor activity. However, NK cells infiltrating solid tumors are severely impaired in their function. Remarkably, NK cells isolated from malignant pleural effusions kill very efficiently tumor cells upon exposure to interleukin-2, offering an important clue for the development of novel approaches for tumor immunotherapy.

A number of experimental evidences support the notion that the immune response can control oncogenesis and tumor progression, at least in some circumstances. This involves not only adaptive but also innate immunity, and a relevant role in this setting is played by natural killer (NK) cells. NK cells constitute innate effector cells that are capable of recognizing and killing virus-infected and malignant cells, while sparing their normal counterparts. Two major NK-cell subpopulations have been identified based on the level of expression of CD56 antigen: $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$ cells.[1] $CD56^{dim}$ NK cells predominate in the peripheral blood (PB) (> 90% of circulating NK cells), express the MHC Class I-specific inhibitory receptors CD94/NKG2A and/or killer-cell immunoglobulin-like receptors (KIRs), are highly cytolytic and rapidly release cytokines upon the engagement of activating NK-cells receptors. In contrast, $CD56^{bright}$ NK cells predominate in lymph nodes, produce cytokines upon interleukin (IL)-2/IL-15-induced activation and are poorly cytolytic. These cells express CD94/NKG2A but not KIRs and are considered as the precursors of $CD56^{dim}$ NK cells.[1]

The NK-mediated killing of tumor cells is regulated through a balance of opposite signals that are delivered by cell-surface activating or inhibitory receptors. The main activating receptors include NKp46, NKp30, NKp44, NKG2D and DNAM-1.[2] These receptors recognize ligands that are often expressed at low levels (or not at all) by normal cells but can be upregulated upon malignant transformation, viral infection or other forms of cellular stress.[2] The functions of NK-cell activating receptors can be counteracted by KIRs and CD94/NKG2A upon interaction with MHC Class I molecules, which are expressed by most normal cells but frequently absent or downregulated in neoplastic or virally infected cells.[3]

Tumors grow more aggressively and generate a higher metatastatic burden in mice depleted of NK cells or genetically deficient in NK-cell functions than in their wild-type coutnerparts. Furthermore, the infusion of cytokines such as IL-2, IL-12, IL-15, IL-21 and interferon α/β (IFNα/β), which strongly enhance NK-cell activity, can lead to the clearance of established tumors.[4] Notably, a reduced NK-cell lytic activity has been associated with an increased risk of cancer in humans.[5] These findings support the notion that NK cells play an important role in the control of tumor growth. In this context, tumor infiltration by NK cells has been reported to represent a positive prognostic marker. Another important evidence that NK cells can control human tumors stems from studies on patients receiving haploidentical hematopoietic stem cell (HSC) transplantation as a therapeutic option against high-risk leukemia. In these patients, NK cells generated from donor HSCs can efficiently prevent relapse by killing leukemic cells that survive the conditioning regimen.[6] However, only a few (anergic) NK cells can be detected within the majority of established solid tumors.[5] This may reflect an inefficient homing of NK cells and/or inhibitory signals mediated by cell-cell interactions or soluble factors that are released by neoplastic cells themselves or by tumor-associated regulatory T cells, tolerogenic myeloid cells or stromal cells, such as transforming growth factor β (TGFβ) prostaglandin E2 (PGE_2) and L-kynurenine.[7,8]

Limited information exists on NK cells residing in malignant pleural effusions (PEs). In a recent study, we have analyzed cells isolated from PEs from patients affected by primary or metastatic tumors of different origin, including mesothelioma as well as lung, breast, colon, gastric, bladder and uterine metastatic carcinomas (**Fig. 1A**).[9] PE-resident NK cells predominantly exhibit a $CD56^{bright}$ phenotype, express normal levels of the main activating receptors as well as of the MHC Class I-specific inhibitory

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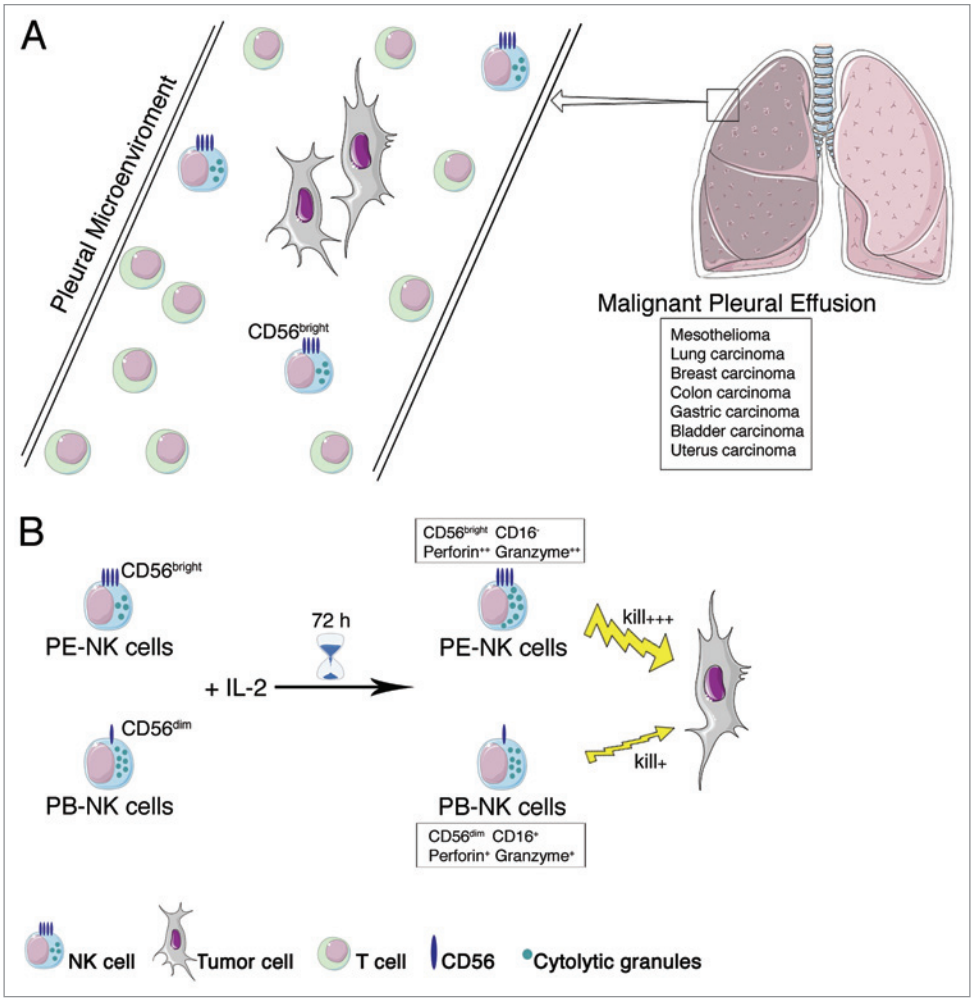

Figure 1. Natural killer cells derived from pleural effusions display strong antitumor activity upon activation with interleukin-2. (**A**) In malignant pleural fluids, natural killer (NK) cells are not functionally impaired due to the dilution of immunosuppressive factors and to the fact that they do not tightly interact with malignant or tumor-associated suppressor cells. (**B**) Upon short-term exposure to interleukin-2 (IL-2), pleural effusion (PE)-derived NK cells (predominantly manifesting a $CD56^{bright}$ phenotype), become able to kill tumor cells more efficiently than circulating NK cells (predominantly exhibiting a $CD56^{dim}$ phenotype) isolated from the same patient or even from healthy donors.

receptors, and they rapidly release cytokines including interferon γ (IFNγ) and tumor necrosis factor α (TNFα) while undergoing degranulation upon exposure to neoplastic cells. These data suggest that PE-resident NK cells are not substantially inhibited by pleural-infiltrating tumor cells, unlike NK cells obtained from other metastatic localizations. Furthermore, upon short-term culture in IL-2 (72 h), PE-derived NK cells maintained their $CD56^{bright}$ phenotype and displayed an increased expression of NKp30, NKp44, NKG2D and DNAM-1, as well as of perforin, granzyme A and B. Importantly, PE-derived NK cells acquired a potent cytolytic activity not only against allogeneic but also against autologous tumor cells (**Fig. 1B**). These data are remarkable because in healthy donors circulating $CD56^{bright}$ NK cells are much less cytolytic then their $CD56^{dim}$ counterparts. The cytolytic activity of PE-derived NK cells was higher than that of circulating NK cells isolated from the same patient or even from healthy donors (and cultured under the same conditions). Thus, our findings indicate that PE-derived NK cells constitute particularly efficient cytolytic effectors. The killing of tumor cells by PE-derived NK cells primarily involves NKG2D and NKp30 and, to a lesser extent, NKp46 and DNAM-1.

The finding that NK cells isolated from PEs are not functionally impaired may reflect the particular tumor microenvironment. It is conceivable that tumor-derived inhibitory factors/cytokines may be diluted in pleural fluids, not reaching effective concentrations. Moreover, the interactions of PE-derived NK cells with tumor cells or tumor-associated suppressor/regulatory cells may be limited, further contributing to the maintenance of efficient cytolytic functions.[7,8] The capability of PE-derived NK cells to rapidly respond to IL-2 in vitro, resulting in the efficient killing of autologous tumor cells, suggests the possibility of inducing/potentiating antitumor effects by the local (intra-pleural) infusion of IL-2 or even by the re-infusion of PE-derived NK cells activated with IL-2 ex vivo.[10]

In conclusion, our data suggest the relevance of NK cells as primary effectors

not only against high-risk leukemias, but also against solid tumors. Our study may have relevant implications for novel immunotherapeutic interventions against primary or metastatic tumors exhibiting a pleural localization.

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Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.