Clin Exp Metastasis (2013) 30:945–947
DOI 10.1007/s10585-013-9582-9

TECHNICAL NOTE

Beware of NK cells in pre-clinical metastasis models

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Received: 11 February 2013 / Accepted: 4 April 2013 / Published online: 19 April 2013

Experimental metastasis models in rodents are commonly used to provide pre-clinical data on metastasis inhibitors upon which subsequent clinical trials in cancer patients are justified. The purpose of this communiqué is to bring to the attention of cancer researchers the profound anti-metastatic effect exerted by natural killer (NK) cells in these models and to recommend that studies assessing the effects of novel agents in tumour metastasis also take into account the effects on NK cells as even subtle alterations in NK function may impact significantly upon metastasis load.

The anti-tumour effects of NK cells were first demonstrated in vitro using lymphoma cells in 1975 [1] and in vivo in 1980 [2]. Since then, many papers have been published shedding light on the underlying mechanisms of NK cell mediated tumour clearance. Excellent reviews are available on the mechanisms of NK cell recognition of target cells, but to summarise briefly, NK cell killing relies on the balance between activating and inhibitory signals received by NK cell receptors from target cell ligands. Malignant transformation of a cell generally results in the expression of stress ligands that activate NK cell killing, and the down-regulation of self-recognition ligands that inhibit NK cell killing [3–7]. Furthermore, interactions between NK cells and dendritic cells have been described that result in the enhancement of NK cell activation as well as activation of adaptive immune responses against tumor cells [8].

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The magnitude of the anti-metastatic effect of NK cells in an acute metastasis model is shown in Fig. 1 where B16F1 melanoma and 4T1.2 breast cancer metastasis is dramatically increased (up to 68 fold, Table inset Fig. 1) in both NK cell-depleted (anti-asialo GM1 pAb) mice and mice genetically deficient of NK cells (NOD/SCIDγc-/-) [9]. Moreover, in the absence of NK cells the full extent of tumour cell invasion of organs is revealed. For example, liver metastases of B16F1 melanoma are rarely observed in the acute metastasis model, however, in the absence of NK cells liver invasion is clearly evident (Fig. 1). Similarly, in a lymphoma metastasis model, splenic metastases became apparent in NK-cell depleted mice [10]. The list of NK cell-sensitive murine tumor cell lines used as pre-clinical metastasis models is numerous and includes B16F1 and F10 melanoma, 4T1 and 4T1.2 breast cancer, the fibrosarcomas CFS1, S180 and UV-2237, the lymphomas YAC-1 and ASL1w, Lewis Lung carcinoma and colon-26 carcinoma.

Despite the wealth of evidence on the anti-tumor effects of NK cells, many experimental metastasis studies have been performed assessing prospective interventions without associated studies on the effect of the intervention on NK cells. The potential error is the assumption that the intervention is effective at the level of the tumor cell when it may, in fact, rely on NK cell activity for tumor elimination. Thus, trial anti-metastatic treatments may mediate their anti-tumour effects by enhancing NK cell anti-tumor activity directly or indirectly by (1) increasing NK cell production, (2) activating NK cells, or (3) altering the expression of NK cell inhibitory or activation ligands on tumour cells. Furthermore, whilst NK cell activity may be normal in the experimental mouse it is frequently impaired in cancer sufferers [11] which may explain the failure of some anti-cancer treatments to translate from the experimental mouse to the clinic.

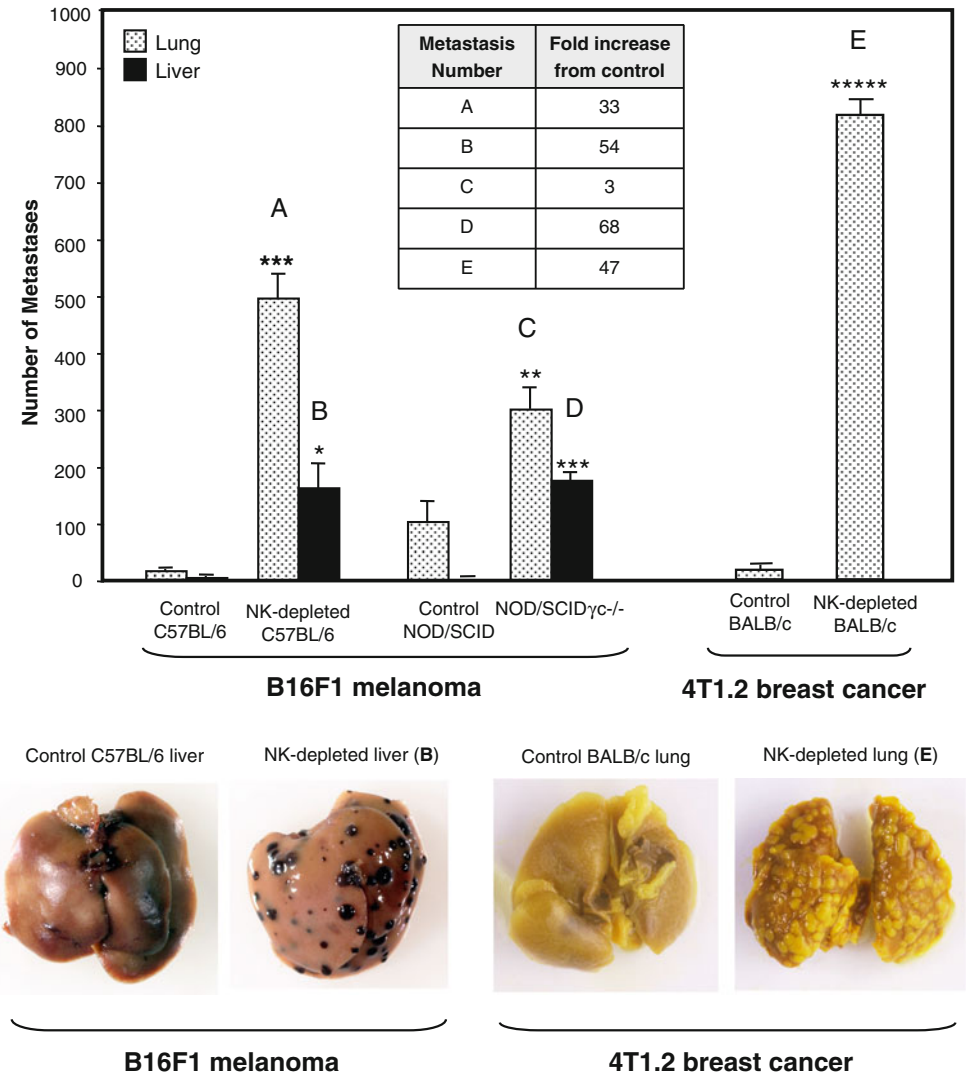

Fig. 1 Natural killer (NK) cells are profound inhibitors of B16F1 melanoma lung and liver metastasis, and 4T1.2 breast cancer lung metastasis. C57BL/6 and BALB/c mice depleted of NK cells (anti-asialo GM1 pAb) 48 h earlier, or NOD/SCID γc-/- (NK, T and B cell deficient) were injected i.v. with 2×10^5 B16F1 melanoma or 4T1.2 breast cancer cells as indicated. Fourteen days later organs of interest were removed and tumors counted under a dissecting microscope. Columns, mean; bars, SEM; significant changes in metastasis numbers relative to controls. $^*P \leq 0.05$; $^{**}P \leq 0.005$; $^{***}P \leq 0.0005$; and $^{*****}P \leq 0.000005$. Table inset details fold increase in metastasis numbers relative to controls. Photos represent livers from control C57BL/6 mice and NK-depleted mice (B) injected with B16F1 melanoma cells, and lungs from control BALB/c mice and NK-depleted mice (E) injected with 4T1.2 breast cancer cells. Figure based on data published in Coupland et. al. [9]

In conclusion, the anti-metastatic effect of NK cells in experimental metastasis models is profound. Studies investigating novel agents that prevent metastasis need to take this into account by confirming that anti-metastatic effects observed in wild type mice are also seen in NK cell depleted or deficient mice.

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