Natural killer cell mediated immunosurveillance of pediatric neuroblastoma

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Until recently, the pathophysiological impact of natural killer (NK) lymphocytes has been largely elusive. Capitalizing on our previous discovery that NK cells mediate immunosurveillance against gastrointestinal stromal tumors (GISTs), we have now investigated the potential influence of immunostimulatory and immunosuppressive isoforms of the NK receptor NKp30 on the fate of infants with neuroblastoma. In three independent cohorts of high-risk neuroblastoma, we observed a similar prognostic impact of the ratio of immunostimulatory vs. immunosuppressive NKp30 isoforms. Patients with high-risk neuroblastoma that are in remission after induction chemotherapy have a higher risk of relapse if their circulating and bone marrow NK cells express the preponderantly immunosuppressive NKp30 C isoform, as determined by a robust RT-PCR-based assay. We also found that neuroblastoma cells express the NKp30 ligand B7-H6, which can be shed from the tumor cells. Elevated soluble B7-H6 levels contained in patient sera inhibited NK functions in vitro and correlated with downregulation of NK-p30 on NK cells, as well as with bone marrow metastasis and chemoresistance. Altogether, these results support the contention that NK cells play a decisive role in the immunosurveillance of neuroblastoma. In light of these results, efforts should be undertaken to investigate NK cell functions in all major cancer types, with the obvious expectation of identifying additional NK cell-related prognostic or predictive biomarkers and improving NK cell based immunotherapeutic strategies against cancer.

Anticancer immunosurveillance has been best characterized at the level of specific MHC class I and class II-restricted T lymphocyte mediated responses against tumor-specific antigens. Nonetheless, there has been growing awareness over the last decade that innate immune responses, including those mediated by NK cells, play a major role in immunosurveillance against some tumor types, especially at the level of metastatic dissemination. This chronological sequence (T before NK cells) most likely does not indicate a hierarchy of importance between T and NK lymphocytes, but rather reflects three interrelated facts, namely, (i) a relatively poor knowledge on the physiology of NK cells (as compared to T lymphocytes), (ii) the existence of major species differences between mouse and human NK cells, and (iii) the absence of standardized tools for the functional exploration of human NK cells.

On theoretical grounds, anticancer immunosurveillance may occur in two specific situations. First, natural immunosurveillance likely avoids most cancers to develop and may retard the progression of established neoplastic lesions. Second, immunosurveillance may be induced by therapeutic interventions against advanced cancers, for instance in the context of immunotherapies. Successful chemotherapies and immunotherapies may also re-establish a state of immunosurveillance through a variety of effects, ranging from the induction of immunogenic cancer stress or death to the direct stimulation of immune effectors or the reversal of immunosuppression. The therapeutic importance of NK cell-mediated immunosurveillance was first documented in one particular disease, namely GISTs that are treated with imatinib. This tyrosine kinase inhibitor has been used for the treatment of GIST based on the rational that it inhibits the oncogene product c-KIT, which is hyperactivated in GIST due to gain-of-function mutations. However beyond this direct effect on tumor cells, imatinib also inhibits the kinase activity of endogenous, non-mutated c-KIT in dendritic cells (DC), thereby activating a stimulatory crosstalk with NK cells. Indeed, in GIST patients treated with imatinib, circulating NK cells produce interferon-γ, and this effect correlates with the duration of the clinical response. Moreover, the phenotype of circulating NK cells related to NKp30 isoform expression predicted the long-term effects of imatinib therapy in GIST independently of the mutational status of c-KIT exon 11

The NCR3 gene (alternative protein name: CD337) encodes three isoforms of the NKp30 protein that are generated by alternative splicing and that have rather
Figure 1. Two possible scenarios describe the NKp30 involvement in controlling high-risk neuroblastoma (HR-NB): in the first one, a favorable ratio of the NKp30B over the immunosuppressive NKp30C isoform (NKp30ΔBC<sub>high</sub>) is associated with tumor growth control. In particular, the NKp30/B7-H6 recognition between NK lymphocytes and neuroblasts and/or monocytes elicits a Th1 response with consequent cytotoxic, cytostatic and anti-angiogenic effects. In the second possible scenario, the presence of a predominant immunosuppressive NKp30 isoform (NKp30ΔBC<sub>low</sub>) induces IL-10 production with a consequent immunosuppressive environment resulting in tumor growth. In addition, the soluble B7-H6 form (sB7-H6) is highly represented in both cases interfering with the NKp30/B7-H6 recognition. NB: neuroblastoma; φ: monocyte; NK: natural killer lymphocyte; TNFα: tumor necrosis factor α; IFNγ: interferon γ; IL-10: interleukin-10.

Altogether it appears that three NK cell-related biomarkers may prove useful for risk stratification in neuroblastoma: (i) the circulating levels of immunosuppressive soluble B7-H6 protein, the NKp30 ligand, (ii) the expression level of total NKp30 protein per cell, and (iii) the relative expression of distinct NKp30 isoforms. Among these parameters, the last appears to be the most robust one, based on the relative ease of its measurement (by RT-PCR).

Thus far, systematic analyses addressing the importance of NK-mediated immunosurveillance across distinct cancer cell types are elusive. At this stage, we may suspect that NK cell-dependent immune responses against malignant cells are far more important for the therapeutic success of targeted therapy than anticipated. However, formal proof in favor of this conjecture is still missing.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.
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