Cover Page

Universiteit Leiden

The handle http://hdl.handle.net/1887/22975 holds various files of this Leiden University dissertation.

Author: Pahl, Jens Heinz-Werner
Title: Innate immune responses of natural killer cells and macrophages against bone sarcomas : towards cellular immunotherapy
Issue Date: 2014-01-09

SUMMARY OF THIS THESIS

Introduction

Osteosarcoma and Ewing sarcoma are the most common bone cancers in children and young adults. Despite advanced surgical techniques and multi-drug chemotherapy, one-third of the patients still succumb to recurrent disease with poor prognosis. Likewise, patients with metastatic and chemotherapy-resistant disease have a poor outcome. Thus, novel targeted therapies are needed that combine potent and specific anti-cancer activity with limited toxicity toward normal tissues.

Previous research lines have provided evidence that natural killer (NK) cells and macrophages, both cell types of the innate immune system, are able to contribute to anti-cancer responses against osteosarcoma and Ewing sarcoma cells. NK cells are able to eliminate virus-infected and tumor-transformed cells and can support adaptive immune responses. The cytotoxic function of NK cells is not MHC class I-restricted; thus, while being able to evade cytotoxic T lymphocyte responses, tumor cells with reduced MHC class I expression can be recognized by NK cells. NK cells are additionally attractive for the design of anti-tumor immunotherapies, since NK cells have not been associated with severe adverse effects such as graft-versus-host disease if therapeutically introduced into patients. During cancer development, cancer cells are presumed to evolve immune escape variants, a process which is supported by the immunosuppressive milieu of the cancer microenvironment, inhibiting anti-cancer immune responses. Thus, to harness NK cells and macrophages for immunotherapeutic approaches, their anti-cancer potential may need to be induced in such a way that it can resist inhibitory influences.

In the **introductory chapter 1**, clinical and biological properties of osteosarcoma and Ewing sarcoma are discussed followed by an overview of cancer immunology and immunotherapy. The results of the preclinical studies described in the **chapters 2–6** are further discussed in the **concluding chapter 7**, addressing implications for anti-cancer immunotherapeutic strategies involving NK cells and macrophages.

Results

In **chapter 2,** an experimental model for measuring NK cell activation is explored in response to a prototypical viral infection by adenovirus type 5 (HAdV5) and HAdV35. It is described that increased expression of the NK cell activation marker CD69 and enhanced NK cell cytotoxic activity in response to HAdV5 relied on the contribution of T cells and IL-2. In contrast, NK cell activation in response to HAdV35 occurred in the absence of T cells and was mediated by the significantly higher production of interferon-α by pDC. Interferon-α production by pDC was dependent on toll-like receptor-9 signaling and was enhanced by the reciprocal interaction of NK cells and pDC.

In the following research chapters it is explored how the anti-cancer potential of NK cells and macrophages can be enhanced and directed to osteosarcoma and Ewing sarcoma cells. In **chapter 3** it is shown that the cytotoxic activity of NK cells can be more specifically directed to sarcoma cell lines if the cancer cells are coated with a therapeutic antibody like cetuximab, targeting the epidermal growth factor receptor (EGFR) on the cancer cell surface. In the presence

of cetuximab, FcγRIII/CD16-mediated, antibody-dependent NK cell cytotoxicity (ADCC) against autologous osteosarcoma cells was substantially enhanced and as effective as by cytokine (IL-15)–activated NK cells. ADCC by NK cells required only minimal EGFR expression on the cancer cell surface. Thus, NK cells are able to exert substantial cytolytic activity against osteosarcoma and Ewing sarcoma cell lines as demonstrated in short-time 4-hour cytotoxicity assays.

However, as illustrated in **chapter 4**, prolonged two-day sarcoma–NK cell interactions, potentially occurring in the cancer microenvironment *in vivo*, can alter NK cell functionality. It is shown that certain sarcoma cell lines can trigger down-regulation of NK cell-activating receptors, such as NKG2D, DNAM-1 and NKp30, even in the presence of the NK cell activating cytokine IL-15. This inhibiting effect was dependent on physical contact and resulted in impaired NK cell cytotoxicity mediated through these receptors, inhibiting lysis of osteosarcoma and Ewing sarcoma cells. Notably, when activated with IL-15 for five days prior to sarcoma cell interaction, these pre-activated NK cells were resistant to the immunosuppressive effects of sarcoma cells. Moreover, the FcγRIII/CD16-mediated antibody (cetuximab)-dependent cytotoxic potential of NK cells remained functional after prolonged sarcoma cell interactions. Hence, a combination of cytokine activation with therapeutic antibodies may improve and sustain the capacity of NK cells to contribute to cancer cell elimination in a potentially immunosuppressive cancer microenvironment.

Macrophages frequently infiltrate solid cancers. In osteosarcoma, macrophage infiltration is correlated with favorable patient outcome, suggesting anti-cancer activity of macrophages in osteosarcoma. In **chapter 5** it is demonstrated that human M1-type macrophages are able to inhibit osteosarcoma cell growth if activated with a bacterial stimulus like liposomal muramyl tripeptide (L-MTP-PE). Importantly, induction of anti-cancer activity by L-MTP-PE required the presence of IFN-γ, a notion which is relevant for improving the outcome of currently applied L-MTP-PE therapy in osteosarcoma patients. IL-10–polarized M2 macrophages, which are considered to support cancer development, were able to inhibit growth of some osteosarcoma cells if directed to antibody (cetuximab)-coated cancer cells. Hence, tumor-infiltrating macrophages may be manipulated to exert anti-cancer responses if activated through factors reminiscent of an infection in the cancer microenvironment and/or by the application of cancer cell-reactive therapeutic antibodies.

In **chapter 6**, it is documented that the immunoregulatory protein CD70 is expressed on the majority of osteosarcoma cell lines and patient-derived osteosarcoma cultures, whereas only few Ewing sarcoma cell lines expressed CD70. CD70 expression in primary and recurrent osteosarcoma lesions was heterogeneous and restricted to tumor cells and not attributed to tumor-infiltrating T cells. CD27, the receptor for CD70, was expressed neither on tumor cells nor on tumor-infiltrating T cells in CD70-positive or CD70-negative osteosarcoma lesions. CD70 gene expression did not correlate with the occurrence of metastasis and survival of osteosarcoma patients. Since CD70 expression in normal tissue is restricted and transient, CD70 may represent a novel, more cancer-specific target for anti-cancer immunotherapy in patients with CD70 positive tumors.

Conclusion

The research performed in this thesis demonstrates that human NK cells and macrophages have the potential to exert anti-cancer responses if sufficiently activated by immunostimulators, and directed to osteosarcoma and Ewing sarcoma by therapeutic antibodies. These findings may help to improve currently applied therapies and design novel immunotherapeutic strategies which counteract potential cancer escape and immunosuppression and contribute to cancer cell elimination. For an effective contribution of NK cell-based immunotherapy to cancer cell elimination, it will be important to further investigate how tumor cell escape from immune control can be overcome and how the migration and penetration of NK cells into the cancer microenvironment can be enhanced.