Xenogeneic DNA vaccination with Tyrosine hydroxylase against neuroblastoma

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Rationale:
To break self-tolerance of the cellular immune system against antigens overexpressed by tumor cells is an important strategy in cancer immunotherapy. A need for the development of such effective additional treatment strategies supporting “classical” therapy in pediatric oncology is emphasized in stage 4 neuroblastoma. The development of a vaccine to activate the cellular arm of the immune system, specifically CD8+ T-cells, which seem to be best equipped to eliminate tumor cells, may provide a therapeutic element completing the established neuroblastoma therapy (1, 2). Neuroblastoma cells are derived from sympathetic neuroblasts and therefore express high amounts of the enzyme tyrosine hydroxylase (TH), the first step enzyme in catecholamine biosynthesis. TH catalyzes the conversion of tyrosine to DOPA, which subsequently leads to the generation of dopamine, norepinephrine and epinephrine and its metabolites homovanillic acid and vanillylmandelic acid. In fact, catecholamine production and metabolism is a well-established clinical marker for diagnosis and follow-up of neuroblastoma patients (3, 4).

In earlier experiments we could demonstrate the efficacy of a syngeneic DNA vaccination approach using a minigene which encodes three murine TH (mTH) derived peptides with high binding affinity to MHC class I (Huebener et al., Mol Cancer Ther 2008, in print). It is known that xenogeneic immunization using highly homologous antigens which derive from a different species, can further enhance antitumor effects by inducing a cross reactivity. This is particularly important regarding the often weak immune responses induced in human patients by preclinically successful DNA vaccines (5). Another possibility to enhance a therapeutic DNA vaccine effect is the introduction of an adjuvant gene into the vector design, e.g. an inflammatory cytokine like IL12.
For this purpose, three different DNA vaccines, based on the mammalian ubiquitin expression plasmid pCMV-F3Ub (vaccines A and B) (6, 8) and pBUD-CE4.1 (vaccine C), were designed: A) encoding for the cDNA sequence of hTH, B) encoding for a DNA minigene comprising of the three major MHC class I antigens in our mouse model and C) encoding for hTH cDNA and single chain IL12 (scIL12) in two independent expression units. DNA vaccine application was achieved by oral gavage with plasmid bearing attenuated *Salmonella typhimurium* SL7207 three times at two-week-intervals. The syngeneic murine neuroblastoma A/J mouse model is best equipped in order to test both the vaccine’s influence on primary tumor growth and spontaneous liver metastasis after surgical tumor removal (7).

Results:

To compare the three vaccines’ efficacy they were applied in our first in vivo vaccination experiment. Interestingly, all three vaccines were equally efficient in suppressing subcutaneous primary tumor growth (Fig. 1). The same result was achieved regarding the suppression of liver metastasis which becomes visible by a significant reduction of liver weights and metastasis grades in vaccinated mice compared to empty vector controls (Fig. 2 and 3).
Fig. 1 and 2: Suppression of primary tumor growth (left panel) and of liver metastasis (right panel) by hTHcDNA, hTH3 and scIL12-expressing hTHcDNA vaccines. Differences were considered statistically significant when p<0.05 (*).
Fig. 3: Grade of liver metastasis after vaccination with hTHcDNA, hTH3 and scIL12-expressing hTHcDNA-vaccine. Counts of metastatic nodules on the liver surface and scoring the metastatic load according to 0% = 0, <20% = 1, 20-50% = 2, >50% = 3.

<table>
<thead>
<tr>
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<th>number of tumor nodes on liver surface</th>
<th>grade of liver metastasis</th>
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<tbody>
<tr>
<td>control</td>
<td>250, 250, 15, 12, 10</td>
<td>3, 3, 2, 1, 1</td>
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<tr>
<td>hTHcDNA</td>
<td>0, 0, 0, 0, 0, 5</td>
<td>0, 0, 0, 0, 1 (p&lt;0.05)</td>
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<tr>
<td>hTH3</td>
<td>0, 0, 0, 5, 6</td>
<td>0, 0, 0, 1, 1 (p&lt;0.05)</td>
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<tr>
<td>hTH-scIL12</td>
<td>0, 0, 3, 3, 4, 100</td>
<td>0, 0, 1, 1, 1, 3</td>
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In order to further characterize the anti-tumor immune response induced by the xenogeneic hTHcDNA vaccine, CD8⁺T cells were depleted in another in vivo vaccination experiment, before, during and after vaccination period. Importantly, the anti-tumor effect was totally abrogated in the CD8-depleted mice regarding subcutaneous tumor growth and liver metastasis (Fig. 4 and 5).

Fig. 4 and 5: Abrogation of the DNA vaccine’s effect (•) on 4) subcutaneous primary tumor growth and 5) liver metastasis in CD8-depleted mice (◊) compared to control mice. Counts of metastatic nodules on the liver surface and scoring the metastatic load according to 0% = 0, <20% = 1, 20-50% = 2, >50% = 3. Differences were considered statistically significant when p<0.05 (*).
This result indicates that cytotoxic T lymphocytes (CTLs) are the major cell type involved in the anti-neuroblastoma immune response.

Moreover, when T cells, isolated from vaccinated mice, were incubated with inactivated murine neuroblastoma cells over several days the produced significantly higher amounts of the Th1 cytokine IFNγ, which also clearly indicates CTL activation (Fig. 6).

![Graph showing IFN-γ production](image)

Fig. 6: IFN-γ production of T cells isolated from spleens of hTHcDNA and hTH3 vaccinated mice upon a 6-day-incubation with murine NXS2 neuroblastoma cells. Differences were considered statistically significant when p<0.05 (*).

In order to further validate the data achieved so far, cytotoxicity assays using T cells from splenocytes and NXS2 murine neuroblastoma cells are currently under investigation. Furthermore, frozen tumor material from control and vaccinated mice will be analyzed for infiltrating CD8⁺ T cells. Moreover, we will test therapeutic efficacy of the hTHcDNA and hTH3 vaccine in a long-term survival experiment.
Reference List


