Adaptive Immune Response to Nano-Pulse Stimulation (NPS)

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Abstract

Nano-Pulse Stimulation (NPS) is a technology that applies ultrashort electric pulses to initiate a cascade of cellular responses that stimulate tumor cells to undergo immunogenic apoptosis (see poster 345). This physical treatment is drug-free, non-thermal and highly localized to the treatment area (see Figure 1,2). We have previously shown treatment of a primary tumor with NPS can inhibit the growth of a secondary tumor and that depletion of CD8+ T-cells can reverse this effect (1). Using the MCA205 mouse fibrosarcoma model, we confirm that NPS treatment can inhibit the growth of secondary tumors. We also further characterize the adaptive immune response to NPS treatment by measuring levels of T-lymphocytes infiltrating the tumor, and in draining lymph nodes and spleen using flow cytometry.

Methods

Murine MCA205 fibrosarcoma cells were injected into the left flank of syngeneic B6 albino mice. After 7 days of growth, we either treated tumors in vivo with NPS (1300 pulses, 300 ns, 30 kV/cm) or surgically removed them prior to treatment. NPS-treated tumors from two groups of mice were excised at 2 and 5 days post-treatment.

The mice in the remaining groups were permitted to heal after NPS treatment and then received a secondary challenge of MCA205 cells in the right flank 3 weeks later. Naive mice were injected in parallel and used as primary tumor controls. 5- and 9 days after injection, tumors were removed. Tumors, draining lymph nodes and spleens were all analyzed on a flow cytometer to measure the % of CD4+, CD8+ and T-reg lymphocytes.

Experimental Timeline

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Primary
Day 1
10^6 MCA205 cells
CD4+ T-cells
CD8+ T-cells
Tumor removed

Secondary
Day 5
10^6 MCA205 cells
CD4+ T-cells
CD8+ T-cells
Tumor removed


Results

Primary tumors regressed after NPS treatment, but the percent of infiltrating T-lymphocytes measured was not significantly different than in untreated tumors. We did see a significant increase in the percent of T-regulatory cells in the draining lymph nodes 2 days after treatment. We suspect these cells may have served to protect the solid tumor from anti-tumor assaults, but left the site once the tumor microenvironment was disrupted by NPS treatment.

100% of secondary tumor growth was inhibited after re-injection with MCA205 cells. In contrast, primary tumor growth was uninhibited despite a similar percent of lymphocytes infiltrating both tumor sites. The percent of CD4+ T-cells was increased to levels just below significance in secondary tumors and increased significantly in the draining lymph nodes. T-regulatory cells were upregulated at Day 9 in the spleens of mice that received secondary injections. The percent of CD8+ cytotoxic T-cells present did not differ significantly in tumors, lymph nodes or spleens.

Infiltration of lymphocytes in primary tumors increased over time as the tumor grew. The percent of CD4+ T-cells increased significantly between Days 5 and 9 after injection.

Conclusions

Secondary tumor growth is significantly inhibited after NPS treatment, while primary tumor growth continues despite similar levels of TILs. We suspect that infiltrating lymphocytes in primary tumors may be exhausted and have therefore lost their tumoricidal ability. Previous data demonstrated that the adaptive immune response mediated by NPS is at least partially CD8+ T-cell dependent, as their depletion reversed the inhibition of tumor growth induced by NPS treatment.

Currently we are conducting experiments to determine whether NPS is invoking an immune response that increases the functionality of CD8+ T-cells and other lymphocytes at the site of secondary tumors, thus serving to inhibit their growth.

References