CYCLOPHOSPHAMIDE AUGMENTS THE EFFICACY OF AN IN SITU VACCINE IN A MOUSE MELANOMA MODEL

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Background We have previously shown that a direct intratumoral (IT) injection of the hu14.18-IL2 immunocytokine (IC), an anti-GD2 antibody linked to interleukin 2, can serve as an in situ vaccine and synergize with local radiotherapy (RT) to induce T cell-mediated antitumor effects. We hypothesized that cyclophosphamide (CY), a chemotherapeutic agent capable of depleting T regulatory cells (Tregs), would augment in situ vaccination.

Methods GD2+ B78 mouse melanoma cells were injected intradermally in syngeneic C57BL/6 mice. Treatments with RT (12Gy) and/or CY (100 mg/kg i.p.) started when tumors reached the size of 100–200 mm³ (day 0) followed by five daily injections of IT-IC (25 mcg) on days 5–9. In some experiments, CY was given prior or after RT. Tumor growth and survival were followed. In addition, tumors were analyzed by flow cytometry.

Results A single injection of CY led to an enhanced antitumor effect of IC comparable to that of RT. The strongest antitumor effect was achieved when CY, RT and IC were combined, as compared to combinations of CY+RT, CY+IC or RT+IC. This augmented effect of the triple combination was seen when CY was given on the same day as RT. Flow cytometric analyses showed that CY treatment decreased Tregs and increased the ratio of CD8+ cytotoxic cells to Tregs within the tumors. Moreover, the combination of RT, CY and IT-IC led to a systemic antitumor effect against the untreated tumor in a two-tumor model. Cured mice developed immunological memory as they were able to reject B78 tumor rechallenge.

Conclusions CY can augment the antitumor efficacy of IT-IC given alone or in combination with local RT in tumor-bearing mice. These preclinical results suggest the value of initiating clinical testing of the combination of CY, RT and IT-IC as an in situ vaccine.

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