The Role of NK Cells in the Treatment of Hepatic Micrometastases of Colorectal Cancer Using Adenoviral-Mediated Interferon-Beta Gene Therapy.

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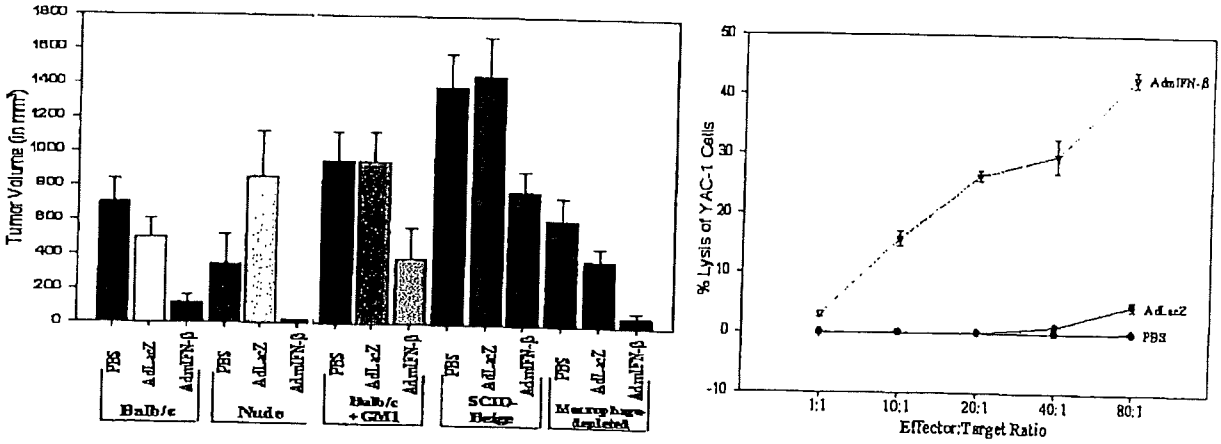
Introduction: We have been working to develop a treatment of hepatic colorectal metastases using systemically administered adenoviral vectors. We have previously demonstrated the effectiveness of adenoviral-mediated human interferon-β gene therapy in a colorectal liver metastases xenograft model. We sought to evaluate this in a syngeneic model to assess the role of host immune defenses. Experiments involving the murine colorectal cancer cell line CT-26 in an immunocompetent mouse and an adenovirus expressing murine IFN-β failed to show evidence of apoptosis as in the xenograft model, but showed similar evidence of tumoricidal activity. We hypothesized that a potential mechanism included the activation of the immune system by murine IFN-β.

Methods: Immunocompetent Balb/c mice, Nude mice (*nu/nu*), SCID-Beige mice, and Balb/c mice treated with anti-asialo GM1 serum (effective at depleting natural killer cells in mice) or liposome-encapsulated dichloromethylenediphosphonic acid (effective at depleting macrophages in mice) underwent a laparotomy during which 10^4 CT-26 cells were injected into the hepatic parenchyma. Five days later, the mice in each group were randomized to receive 5 x 10^{10} particles of AdCMVmIFN-β, 5 x 10^{10} particles of AdCMVLacZ (control vector), or 100 μl of PBS via tail vein injection. Fourteen days later mice were sacrificed and the livers were harvested for tumor measurement. In a separate experiment, Balb/c mice were sacrificed 3 days following administration of vector. Natural killer cells isolated from the liver and spleen using magnetic cell sorting were cultured for 48 hours in media supplemented with serum from the sacrificed animals. NK activity was then assessed via cytolysis of ^{51}Chromium-labeled YAC-1 murine hybridoma cells (a specific target of NK cells).

Results: Treatment with AdmIFN-β resulted in an 88% reduction in tumor volume ($p < 0.05$) in Balb/c mice, a 93% reduction in tumor volume ($p < 0.1$) in Nude mice, and a 91% reduction in tumor volume ($p < 0.05$) in macrophage-depleted Balb/c mice as compared with PBS and vector controls. AdmIFN-β treatment resulted in only a 60% reduction in tumor volume ($p < 0.05$) in Balb/c mice which were administered the anti-asialo GM1 serum and a 44% reduction in tumor volume ($p < 0.05$) in NK-deficient SCID-Beige mice as compared with PBS and vector controls. In addition, NK cells harvested from mice treated with AdmIFN-β showed a marked induction of cytolytic activity as compared with control animals.



Conclusion: Similar reductions in tumor volumes in the immunocompetent mice and Nude mice suggest that T-cell mediated immunity is not a dominant effector of inhibition of tumor growth in this model. Depletion of macrophages in Balb/c mice also did not affect the efficacy of AdmIFN-β treatment. Decreased effect of treatment in the NK-depleted (GM1) and NK-deficient (SCID-Beige) mice as well as induction of NK cell cytolytic activity following AdmIFN-β, however, suggests that natural killer cells may have a significant role in tumor killing following murine interferon-β gene therapy.