A major challenge in cancer therapy is the destruction of undetectable micrometastases that persist after tumor resection/ablation. In many patients, indeed, these micrometastases eventually develop into lethal lesions. A lasting protection against micrometastases may be achieved by immunotherapy, promoting the activation of the immune system against the one or many autologous tumor-associated antigens (TAA). The immune system is capable of protecting against tumor cells presenting TAA, as it can be inferred from the correlation between the extent of T-cell infiltration observed in resected tumors and patient survival. Many TAA are unique to each cancer patient and are generated by coding mutations, and are thus capable of protecting against micrometastases. A lasting protection against micrometastases may be achieved by immunotherapy, promoting the activation of the immune system against the one or many autologous tumor-associated antigens (TAA). The immune system is capable of protecting against tumor cells presenting TAA, as it can be inferred from the correlation between the extent of T-cell infiltration observed in resected tumors and patient survival. Many TAA are unique to each cancer patient and are generated by coding mutations, and are thus capable of protecting against micrometastases.

Effective tumor vaccines require both the recruitment of APC into the tumor and the active targeting of tumor cells for uptake by APCs. We have developed an immunotherapeutic regimen that promotes the recruitment of APCs into the tumor and in situ targets tumor cells for uptake by APCs, based on the intratumoral injection of α-gal glycolipids that interact with the natural anti-Gal antibody. Anti-Gal is the most abundant antibody in humans, constituting ~1% of immunoglobulins. Its ligand, the α-gal epitopes (Galα1–3Galβ1–4GlcNAc-R), is absent in humans and is produced in nonprimate mammals by the glycosylation enzyme α1,3-galactosyltransferase (α1,3GT). The anti-Gal antibody interacts very effectively in vivo with α-gal epitopes and activates the complement system, as indicated by the rapid rejection of pig xenografts following anti-Gal binding to α-gal epitopes on pig cells. Tumor cells can be manipulated to express α-gal epitopes by the intratumoral injection of α-gal glycolipids, hence becoming a target for anti-Gal antibodies. α-Gal glycolipids present linear or branched carbohydrate chains capped by α-gal epitopes. These glycolipids are extracted in large amounts from rabbit red cell membranes and dissolve in water as micelles. When injected into tumors, α-gal glycolipids insert into tumor cell membranes because their hydrophobic lipid “tail” is energetically much more stable when surrounded by cell membrane phospholipids than in micelles within aqueous environments (Fig. 1A). This spontaneous process results in the presentation of multiple α-gal epitopes on tumor cells.

In vitro studies indicate that the incubation of tumor cells lacking α-gal epitopes with 0.1 or 1 mg/mL α-gal glycolipids results in their extensive insertion into tumor cell membranes and cytolysis of these cells in the presence of anti-Gal antibodies and complement. The in vivo effects of α-gal glycolipids injected intratumorally were studied in a preclinical model. The immunogenicity of autologous tumor-associated antigens (TAA) is markedly increased upon the intratumoral injection of α-gal glycolipids, which insert into tumor cell membranes. The binding of natural anti-Gal antibodies to these glycolipids activates the complement system and recruits antigen-presenting cells (APC), which internalize anti-Gal-coated tumor cells upon Fc/FcγR interactions. Eventually, TAA-derived peptides presented by APC activate T cell-mediated tumor-specific immune response.

In situ conversion of tumors into autologous tumor-associated antigen vaccines by intratumoral injection of α-gal glycolipids

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The antitumor immune response elicited by the intratumoral injection of α-gal glycolipids appears to be primarily mediated by CD8+ T cells and to be potent enough to overcome the immunosuppressive effect of regulatory T cells.

The safety of α-gal glycolipids as an immunotherapeutic intervention was evaluated in a Phase I clinical trial in patients bearing various malignant solid tumors at an advanced stage of the disease. Patients received intratumorally 0.1 mg, 1 mg, or 10 mg α-gal glycolipids and kept under observation for 24 h. Subsequently, patients were monitored at regular intervals. None of the patients developed clinical or laboratory signs of toxicity, symptoms of allergic responses, autoimmune conditions, anti-nuclear antibodies or autoantibodies to normal tissue antigens. Thus, the treatment does not seem to cause a breakdown in immune tolerance to normal antigens. Injected tumors did not regress and patients developed evidence of disease progression at various time points after the four-week endpoint. However, several patients are alive with disease for 13+ to 48+ months, even though disease progression is evident by imaging. In addition, two patients with pancreatic adenocarcinoma had an unexpectedly long survival of 18 and 23 mo.

The therapeutic effect of intratumoral α-gal glycolipids injection may not be limited to advanced diseases, but may improve outcome also when used as neoadjuvant immunotherapy. Injection of primary solid tumors such as colon or mammary carcinoma 2–4 weeks prior to resection may convert treated lesion into a temporary vaccine that “educates” the immune system to recognize and destroy metastatic cells presenting autologous TAAs. Such an induced immunosurveillance may protect against micrometastases long after the primary tumor is resected. This immunotherapeutic approach may synergize with treatments that nonspecifically expand the tumor-specific T-cell clones that are activated following the injection of α-gal glycolipids.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.
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