**ABSTRACT**

α-Gal is a glycosphingolipid. Function-Space-Lipid (FSL) molecule being developed as an intratumoral immunotherapy for solid tumors. This synthetic compound is comprised of the α-Gal sugar antigen (Gabi-3Galβ1-4GlcNAc-R), a β-hexosaminide, a linker and a lipid A.

α-Gal is present on the glycolipids and glycoproteins of mammalian species, and is synthesised by the α-L-fucosidase (α-L-Fuc) enzyme. Human and Old World monkey organs, on an ancient version of the α-Gal gene and therefore lack the α-Gal antigen. However, constant exposure to α-Gal-bearing commensal gut bacteria results in the development of high titer anti-α-Gal antibodies in Gal, called α-Gal IgG.

α-Gal is responsible for the hyperacute rejection of xenogenic tissue implants in humans, through rapid recruitment of anti-Gal to the transplanted tissue and activation of the complement cascade. Hyperacute rejection of a cute estimate of α-Gal glycolipids from rabbit erythrocytes, in vivo B10.F1 melanoma xenograft on 13G1 mice, has been shown to induce a tumor-specific T cell response that protected the mice from development of distant lesions. We have previously presented data showing the same protection from lesion formation by AGI-134 in the B10.F1 melanoma model, as well as survivors of AGI-134 anti-α-Gal-IgG monoclonal antibodies.

We now present in vitro data demonstrating the multiple anti-Gal mediates effector functions, induced by treating α-Gal-negative melanoma cells with AGI-134, that drove the observed in vivo tumor immunity.

**METHODS**

Anti-αGα were quantified by capture onto α-Gal coated ELISA plates, followed by detection with isotype- and subclass-specific enzyme-conjugated antibodies. Human Ig subclass and subclasses standards (BioLegend, Nordic-Mibio) were used to generate standard curves.

For assessment of anti-Gal recruitment and complement opsonization, target cells were treated with AGI-134 by incubation at 37°C for 1 h before further incubation with normal human serum (NHS; heat-inactivated or protein G purified), as specified in figure legends. For assessment of downstream effector functions, target cells treated as described above were co-cultured with effector cells as described in figure legends. Antibody was performed by flow cytometry.

**RESULTS**

Anti-αGα IgG and IgM are present in human serum, with the predominant IgG subclasses being IgG1 and IgG2 in both serum and purified polyclonal α-Gal IgG.

**CONCLUSIONS**

- Anti-αGal in human serum is predominantly IgG1, IgG2 and IgG3.
- AGI-134-treatment recruits the predominant anti-αGal isotypes and subclasses to the cell surface.
- Anti-αGal IgG effectively drives the deposition of, and activation of, complement, resulting in CDC of AGI-134-treated target cells whilst anti-αGal IgG drives ADCC.
- Ossification of AGI-134-treated target cells with anti-Gal and complement enhances their uptake by both human MDMs and CD8+ T-cells.

**REFERENCES & ACKNOWLEDGEMENTS**


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