A potential survival strategy for human follicular lymphoma cells involving oligomannose glycans in the antigen binding site of IgG

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INTRODUCTION: FL cells express a strikingly high incidence of glycosylation sites in the variable regions of their surface immunoglobulins (Igs), a feature that is uncommon in normal B cells or in tumours outside the germinal centre (GC). Sites are introduced by somatic mutation and are positively selected, pointing to their involvement in tumour growth/survival in the GC site which is mainly a result of cell survival rather than a high cell proliferation rate, and this results in a slow-evolving disease that is difficult to treat.

RESULTS By analyzing FL-associated IgG from five patient-derived mouse/B cell lymphoma heterohybridoma cell lines, we have identified the sugars in the Fab regions as oligomannose-type glycans. Importantly, the sugar pattern differs from that in the Fc region, which contains the expected range of complex type glycans, indicating that the normal glycan processing pathway is not significantly altered in the tumour cells. The presence of α-galactose and the absence of bisected sugars is characteristic of mouse glycosylation.

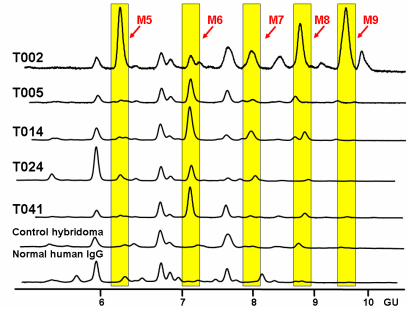


Fig 1 shows the NP HPLC chromatograms of the glycans released from 5 FL IgGs, control hybridoma and normal IgGs. The presence of oligomannose is clearly demonstrated in all the FL samples.

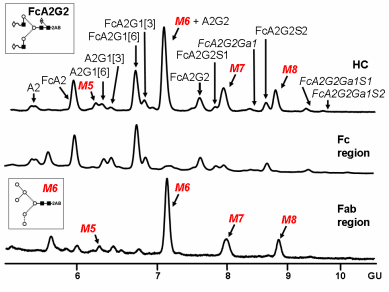


Fig 2 shows the NP HPLC chromatograms of the glycans released from FL T014 IgG, the Fc and Fab. The Fc has the usual profile for IgG whereas the Fab has oligomannose sugars.

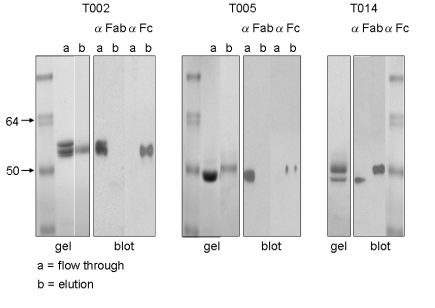
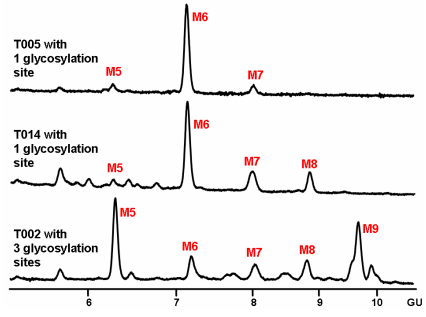


Fig 3 shows the Coomassie stained gels and western blots of the Fab and Fc fractions of 3 patient samples. This shows the cleavage of the Fab from the Fc and also three bands in the T002 Fab.

The analysis of Fab and Fc regions cleaved by papain digestion revealed that the oligomannose sugars are on the Fab alone. All the samples except T002 have one glycosylation site in the Fab whereas T002 has 3. Western blotting, glycan analysis and PNGase F in solution have revealed that there is variable site occupancy in T002.

Fig 4 shows the NP HPLC chromatograms of the oligomannose glycans of 3 Fab fractions.



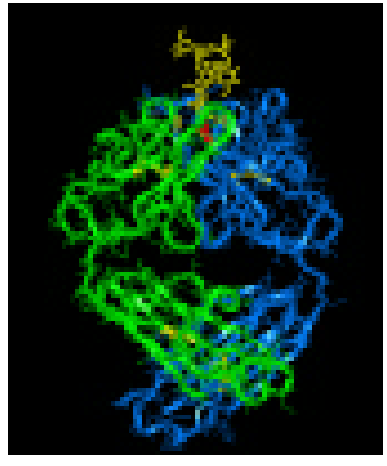


Fig 6 shows the model of Fab of T014. The oligomannose glycans, in yellow, can be seen protruding from the antigen binding site. HC is blue, LC is green

Molecular modelling showed that the glycosylation sites are in the antigen binding region. T002 has 3 sites; 2 sites in the antigen binding region and 1 that is more accessible to the glycosylation pathway enzymes, with the addition of some complex and hybrid sugars as a result.(see fig 4) It also showed that the oligomannose sugars may be accessible for ligand binding. This was demonstrated (fig 5) with HCs binding to mannose-binding lectin with normalisation to account for any binding to ungalacosylated glycans present on the Fc. The binding to T002 is significantly greater than that to T014.

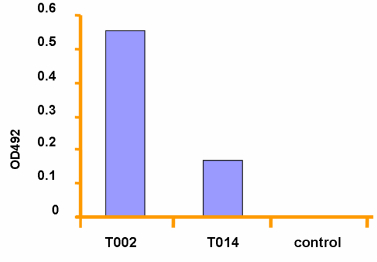


Fig 5 shows the combined results of ELISAs of calcium dependent MBL binding to HCs of T002, T014 and the control hybridoma after normalisation to account for any binding to ungalactosylated glycans in the Fc

CONCLUSION: The presence of oligomannose glycans on the heavy chain suggests that the combination of heavy and light chains that occurs in the ER prevents further processing of the sugar chains on the Fab. The 3D structure prevents access of the mannosidases that initiate the processing of complex type sugars. Molecular modelling indicates, however, that the diequatorial hydroxyl groups at C3 and C4 on the terminal mannose residues on FL IgG are exposed and this was confirmed by specific binding to mannose-binding lectin. This raises the possibility that there may be an alternative strategy for B-cell growth/survival that primarily involves molecules of the innate immune response and is independent of classical antigen recognition. The presence of the oligomannose glycans on FL IgGs may provide the basis of a strategy for interrupting the binding of FL cells to dendritic cells and lead to a novel and specific targeted therapy for FL.

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