

SEA technology: a novel strategy for enhancing antibody effector function

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1. Background

The activity of monoclonal antibodies (mAbs) can be enhanced by a number of chemical and genetic strategies. We describe a novel strategy, Sugar Engineered Antibody (SEA) technology, for enhancing antibody-dependent cellular cytotoxicity (ADCC) through modification of the mAb carbohydrate. A series of small molecule fucose analogs were added to mAb-expressing Chinese hamster ovary (CHO) cells, with the resulting mAbs showing a significant reduction in their carbohydrate fucosylation. We demonstrate that these mAbs show markedly increased ADCC activity and improved CD16 binding. The fucose analogs inhibit GDP-mannose dehydratase (GMD), the first enzyme in *de novo* synthesis of GDP-fucose, and lead to global depletion of intracellular GDP-fucose. We also demonstrate that this strategy yields mAbs with significantly reduced fucosylation in large scale CHO cell culture and is broadly active across a variety of mAbs and expression systems. Since genetic modification of the mAb-producing cell line is not required, SEA technology can be readily applied from the mAb screening to manufacturing stage to generate effector function enhanced therapeutic antibodies.

2. Antibody-Dependent Cellular Cytotoxicity

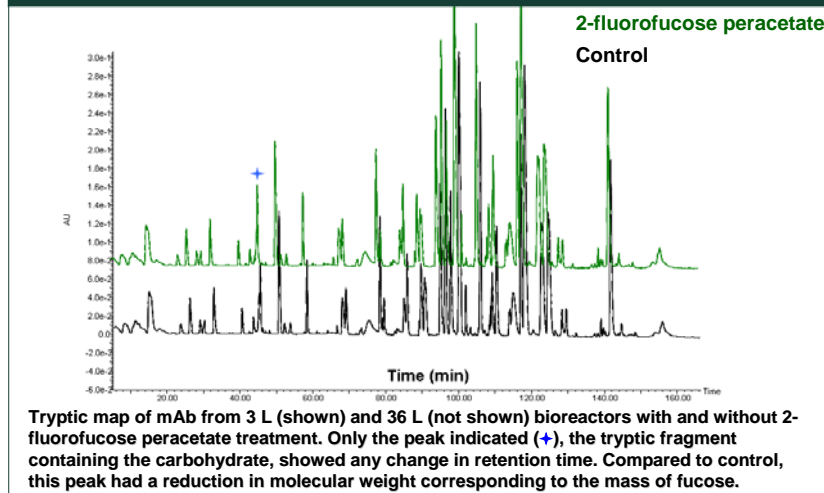
Antibody-dependent cellular cytotoxicity (ADCC) is an important component of antibody efficacy. Antigen-bound mAbs engage CD16 on the surface of effector cells (NK and macrophages), which release pore-forming proteins and proteases to lyse the target cell. ADCC activity can be improved through a variety of techniques, including removing the fucose residue from the mature, biantennary mAb carbohydrate.

Legend:
 ▽ = Fucose
 □ = N-acetylglucosamine
 ● = Mannose
 ○ = Galactose

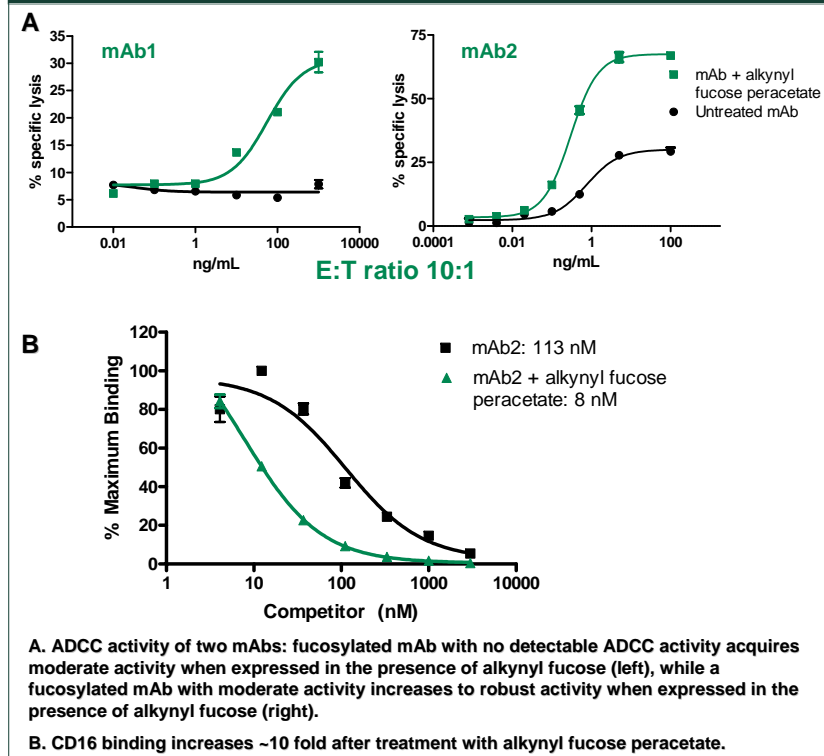
3. Unexpected Activity of Fucose Analogs

Fucose analogs 2-fluorofucose peracetate and alkynyl fucose peracetate (50 μM) were added to the growth media of mAb-producing CHO cells. The resulting mAb was trypsinized and analyzed by mass spectrometry. About 1-2% of mAb was fucosylated, with some incorporation (3%) of alkynyl fucose and minimal incorporation of 2-fluorofucose observed.

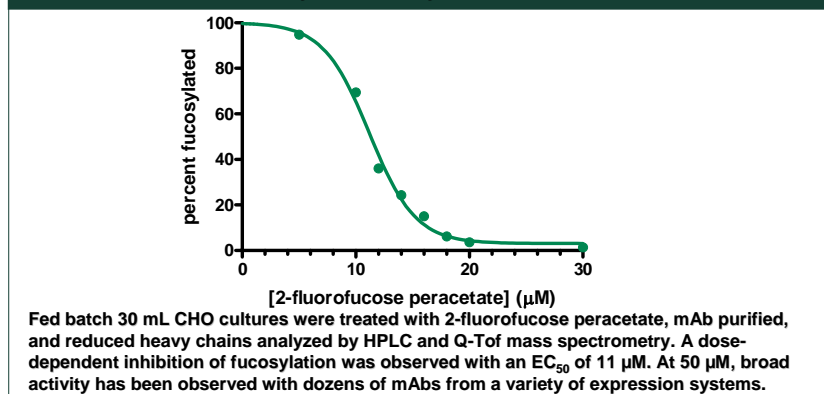
4. Product Characterization



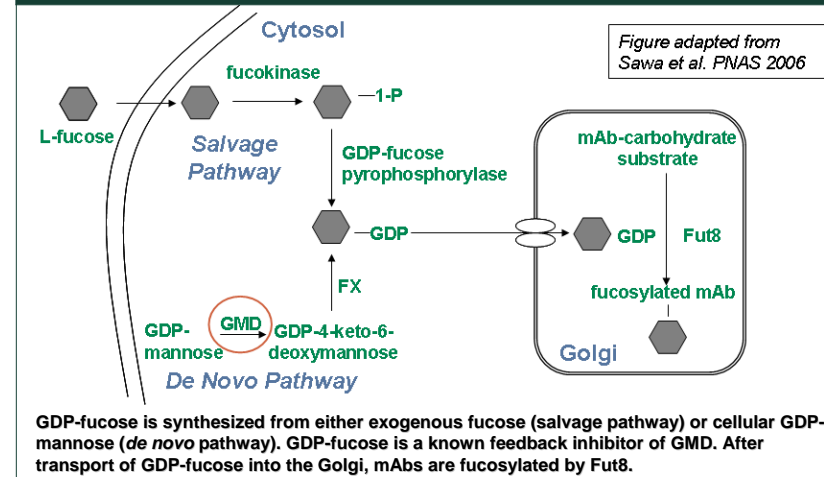
5. ADCC Activity and CD16 Binding



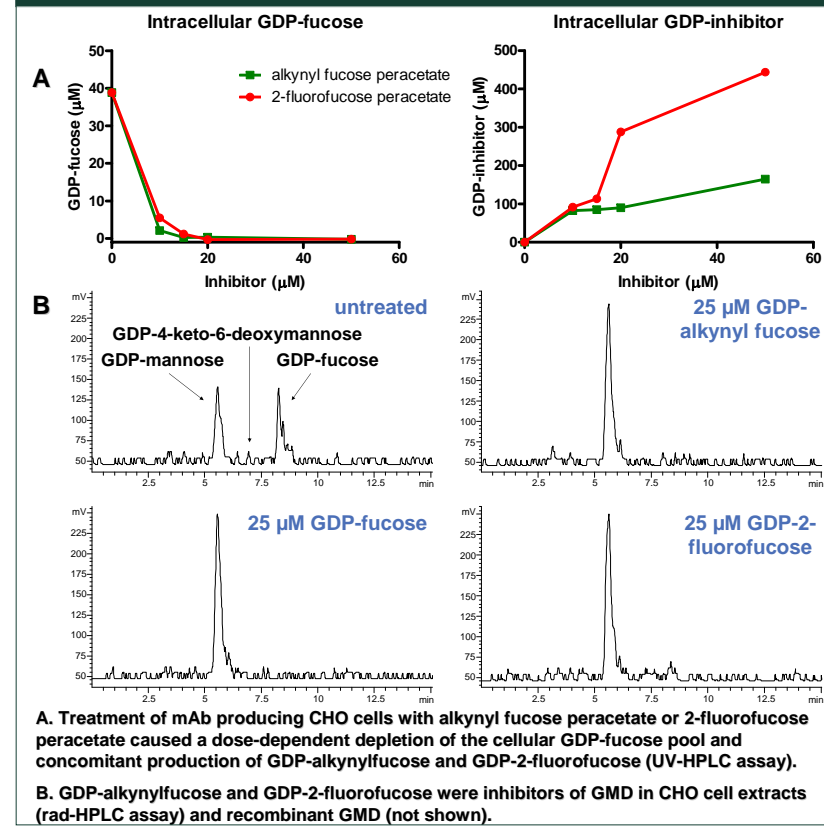
6. Potency of Fucosylation Inhibition



7. Fucose Biosynthesis



8. Mechanism of Inhibition



9. Conclusions

- ▶ We have identified modified sugars that inhibit the incorporation of fucose into the mature, biantennary carbohydrate chains of mAbs
- ▶ The resulting mAbs show enhanced ADCC activity due to increased CD16 binding
- ▶ The fucose analogs are converted to their corresponding GDP analogs by the salvage pathway and inhibit *de novo* synthesis of GDP-fucose by GMD, resulting in depletion of the cellular pool of available GDP-fucose
- ▶ Sugar Engineered Antibody (SEA) technology has several key advantages over current approaches for improving the ADCC activity of mAbs:
 - ▶ Can be applied to existing mAb producing cell lines without genetic modification of the cell line or changing the manufacturing process or product quality
 - ▶ Enables screening of mAb libraries for those with optimal ADCC activity
 - ▶ Does not alter the mature, biantennary oligosaccharide structure other than minimizing core fucosylation