Rapid Glycan Profiling with a Nine-Lectin Microarray for Therapeutic IgG1 Monoclonal Antibodies

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Abstract

Glycosylation is a critical quality attribute (CQA) of many therapeutic monoclonal antibodies (mAbs), and therefore must be adequately analyzed and controlled to ensure product quality and manufacturing consistency. Here, we describe a lectin microarray that comprises nine distinct lectins for detecting glycan epitopes of therapeutic IgG1 mAbs, a dominant class of biopharmaceuticals that are often produced as glycoproteins by living cell systems. We first analyzed the glycosylation patterns for 70 commercially marketed therapeutic IgG1 mAbs approved by the US Food and Drug Administration (FDA) based on the relevant information in the Biologic License Applications (BLAs). A set of nine abundant glycan epitopes was found to be consistently present across all therapeutic IgG1 mAbs produced by different expression systems and was used as a benchmark for analytical method development. After screening a large lectin library, we identified nine lectins that specifically recognize the benchmark glycan epitopes of IgG1 mAbs. We then developed a lectin microarray comprising the nine lectins and proved its suitability for high-throughput glycan profiling of IgG1 mAbs utilizing intact glycoprotein samples, which enables comparative glycosylation analyses for batch-to-batch or biosimilar-to-innovator products.

Introduction

IgG1 mAbs account for nearly 75% of all therapeutic mAbs approved by FDA. These products are normally produced as N-glycosylated proteins by CHO, NSo, or Sp2/0 cell lines. Such N-linked glycans are an integral part to glycoprotein folding, stability and biological activity, thus having direct impact on the product safety and efficacy. Moreover, recombinant mAbs may contain non-human glycoforms, such as N-glycolylneuraminic acid (NGNA) residues or Galα1-3Gal disaccharide (α-Gal) units that may trigger immunogenic responses in patients. Glycosylation naturally occurs with fair heterogeneity due to its non-template driven biosynthesis machinery, thus glycosylation patterns (e.g., glycan species and abundance) may vary from batch to batch, leading to variations in product quality. For these reasons, glycosylation is considered a CQA for certain therapeutic antibodies, requiring adequate characterization and control to ensure product quality and manufacturing consistency.

Several methods are available for glycosylation analysis, commonly relying on liquid chromatography to analyze N-glycans released from the protein backbone. Due to the time-consuming and low-throughput procedures, there is continued interest to improve method performance by using intact glycoproteins with high throughput capacity. Lectin microarray is a unique platform for intact glycoprotein analysis that exploits the selective interactions between lectin proteins and specific glycan epitopes. A commercial lectin microarray was previously evaluated, which can recognize a wide range of glycan epitopes but lack selectivity to N-glycans of mAbs, causing false-positive or uninterpretable binding signals. This study was aimed to develop a new generation of lectin microarray dedicated to IgG1 mAbs. An in-depth analysis of commercially marketed IgG1 mAbs identified nine consensus glycan epitopes, followed by a rationale-based design and qualification of the newly constructed lectin microarray suitable for characterizing IgG1 mAbs produced in different expression systems.

Materials and Methods

Lectin microarray analysis: Proteins were incubated with Cy3 fluorescent dye in the dark at 25°C for 1-2 hours, followed by removal of unbound Cy3 using spin desalting columns. The Cy3-labeled proteins were serially diluted to 125 ng/mL and 40 µL aliquot was applied to a well of the 14-well lectin chip. The chips were kept overnight in the dark at room temperature on an orbital shaker, followed by scanning (without washing steps) for fluorescence intensity at each lectin-coated spot using an evanescent-field fluorescence scanner GlycoStation.

In vitro glycoengineering of IgG1 mAbs: The terminal glycans of IgG1 mAbs were modified through in vitro enzymatic glycoengineering reactions, using a combination of glycosidases (which remove terminal glycan epitopes) and glycosyltransferases (which add terminal glycan epitopes).

LC-MS analysis: Liquid chromatography coupled with mass spectrometry (LC-MS) analyses of intact proteins were performed on an Agilent HPLC-Chip nano-electrospray-ionization Q-TOF MS system using a C8 chip. All IgG1 mAb samples were reduced in 50 mM DTT, centrifuged, then supernatant was transferred to an HPLC vial for LC-MS analysis.

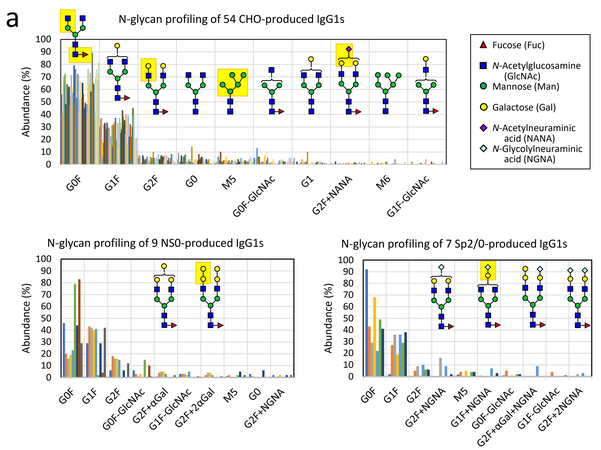

Figure 1. (a) Benchmarking N-glycan profiles of therapeutic IgG1 mAbs. (b) Workflow of devising lectin chips for therapeutic IgG1 mAbs.

Results and Discussion

Through analyzing glycosylation information in BLAs for 70 IgG1 mAbs, we identified 10 mostly abundant N-glycans produced by each of the 3 cell lines (Fig.1a), and found a set of 9 glycan epitopes that are commonly seen in therapeutic IgG1 mAbs (Fig.1b), including 2 epitopes (bisecting-GlcNAc and triantennary) in less abundant glycans. Using the 9 epitopes as a benchmark, after screening 74 commercial lectins, we developed a IgG1-mAb-LecChip consisting of 9 distinct lectins to recognize the 9 glycan epitopes (Fig.1b & Table 1). The suitability of the new LecChip for high-throughput glycan profiling of IgG1 mAbs was proved by using intact protein samples (Fig.2), including model protein samples with well-defined glycan profiles and IgG1 mAbs with modified glycan epitopes, generated by glycoengineering and confirmed by LC-MS (data not shown). The utility of the new LecChip was demonstrated by comparative glycan profiling of an innovator IgG1 mAb and its biosimilar (Fig.3), which showed similar levels of the major glycan epitopes but different levels of minor epitopes such as high mannose and NGNA. Therefore, the 9-lectin microarray enables comparative glycosylation analyses for batch-to-batch or biosimilar-to-innovator products.

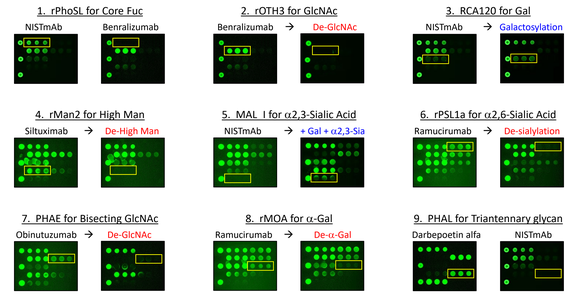

Figure 2. Qualification of IgG1-mAb-LecChip using either model proteins with well-defined glycan profiles (panels 1 & 9) or IgG1 mAbs with modified terminal glycan epitopes obtained through targeted glycoengineering (panels 2 to 8).

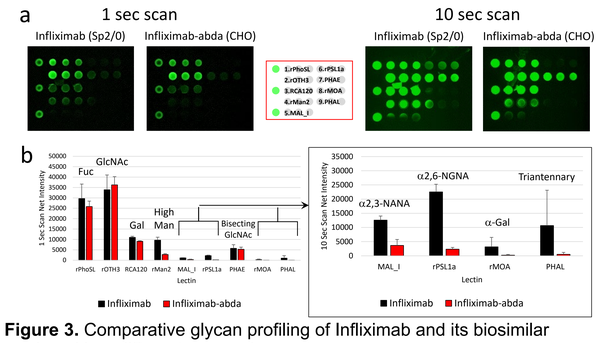

Figure 3. Comparative glycan profiling of Infliximab and its biosimilar produced in a different expression system.

Table 1. Selected lectins (n=9) for capturing the common glycan epitopes of therapeutic IgG1 mAbs

#	Lectin (origin)	Reported N-glycan epitope selectivity*	Targeting N-glycan epitope on IgG1 mAb
1	Recombinant PhoSL or **rPhoSL** (*Pholiota squarrosa*)	α(1,6)fucosylated N-glycans	Core fucose (Fuc)
2	**rOTH3** (*Ulva limnetica*)	Unknown	Terminal N-acetylglucosamine (GlcNAc)
3	RCA120 (*Ricinus communis*)	Galβ(1,4)GlcNAc (up with increasing the number of terminal Gal), Galβ(1,3)Gal (weak), no affinity for agalactosylated N-type	Terminal galactose (Gal)
4	**rMan2** (*Kappaphycus alvarezii*)	High Mannose (High Man)	High mannose (High Man)
5	**MAL_I** (*Maackia amurensis*)	Siaα(2,3)Galβ(1,4)GlcNAc	Terminal α2,3-linked N-acetylneuraminic acid (NANA)
6	**rPSL1a**	Siaα(2,6)Galβ(1,4)GlcNAc	Terminal α2,6-linked N-glycolylneuraminic acid (NGNA)
7	**PHA-E** (*Phaseolus vulgaris*)	Bi-antennary complex-type N-glycan with outer Gal and bisecting GlcNAc, no affinity for fully sialylated N-type	Bisecting GlcNAc
8	**rMOA** (*Marasmius Oreades*)	α-Gal (Galα(1,3)Galβ(1,4)GlcNAc)	α-Galactose (α-Gal)
9	**PHA-L** (*Phaseolus vulgaris*)	Tri/tetra-antennary complex-type N-glycan	Triantennary N-glycan

**For naturally occurring lectins, refer to Lectin Frontier DataBase (LfDB)(https://acgg.asia/lfdb2/).*

Conclusion

Glycosylation is a CQA of many therapeutic mAbs and must be adequately analyzed and controlled during product development and throughout a product lifecycle. Through benchmarking of the 9-glycan epitopes that are commonly present in commercially marketed IgG1 mAbs (see Table 1), we have developed a new lectin microarray chip consisting of 9 distinct lectins to recognize the 9 glycan epitopes that are commonly seen in therapeutic IgG1 mAbs. This 9-lectin microarray provides a high-throughput platform for comparative testing of glycan profiles attached to intact IgG1 mAbs without the need for releasing the glycans. Upon testing a panel of therapeutic IgG1 mAbs, as well as their glycoengineered forms and biosimilars, we showed the utility of the nine-lectin microarray for glycan profiling of a broad array of IgG1 mAbs. The assay uses a simple procedure, has a high throughput capacity, and enables comparative testing of a large number of samples in a short time. By including an appropriate internal reference control, which is tested at a series of dilutions, the lectin microarray could provide a semi-quantitative measurement of terminal glycan epitopes within an IgG1 mAb sample. Therefore, the new IgG1-mAb-LecChip may be adopted by pharmaceutical companies when developing IgG1 mAbs to assess batch-to-batch or biosimilar-to-innovator glycan epitopes.