Changes in the Glycosylation Pattern of Total Serum IgG in Patients with Rheumatoid Arthritis analyzed by MALDI-TOF MS

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Introduction

Glycosylation of human serum immunoglobulin at position 297 in the antibody constant region is affected by different parameters, e.g. age, disease. The glycan is essential for maintaining a functional Fc region, which is a prerequisite for antibody mediated immune reactions. Variations in the composition of the glycan moiety can dramatically influence antibody activity. Rheumatoid arthritis (RA) is a widely prevalent chronic autoimmune disease and is known to be characterized by changes in the glycosylation pattern. Here, we present a simple method based on graphite purification and MALDI-TOF MS to investigate the relative distribution of N-glycans of total serum immunoglobulins. Patients with RA showed a relative increase of agalactosylated glycans compared to healthy individuals.

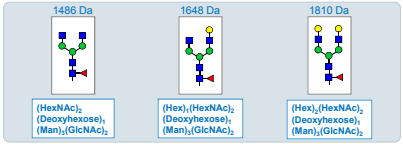

Fig. 1: Fucosylated bi-antennary complex glycan-structures represented by immunoglobulins

Methods

Total serum (5 µl) of patients and healthy volunteers were diluted with sodium phosphate buffer and subsequently digested at 37°C overnight in the presence of 0.4 U PNGase F (Roche). For purification of released N-glycans, 384-well filter plates were loaded with 100 µg of graphite by centrifugation. After washing with 50 % ACN/0.1 % TFA followed by equilibration with 0.1 % TFA, the digested serum was applied on the graphite loaded filter plates. Bound glycans were eluted off using 30% ACN/0.1 % TFA. 1 µl eluate was mixed with 1 µl DHB (10 mg/ml in 50 % ACN/5 mM NaCl) on AnchorChip2000™ targets. Reflector spectra were acquired and the peak areas of the three most prominent glycan peaks were determined. Fig.2 gives an overview of the workflow.

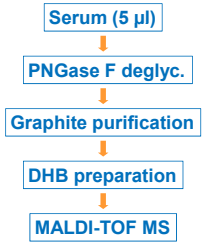

Fig. 2: Workflow describing the digestion, purification and MALDI-TOF MS measurement of serum immunoglonbulin glycans

Results

Serum of 48 healthy volunteers, 27 patients with RA and 17 patients with Osteoarthritis (OA) were processed according to the protocol described above. The resulting reflector spectra comprised about six to ten peaks in the mass range of 1000 to 3500 Da. Subjecting these peak masses to a database search employing Glycomod (http://www.expasy.org/tools/glycomo/) facilitated the correlation to defined N-glycan structures measured as $[M+Na]^+$. The three most prominent peaks at 1486, 1687 and 1810 Da formed a series of fucosylated bi-antennary complex glycans differing only by one hexose unit (162 Da) (Fig. 3).

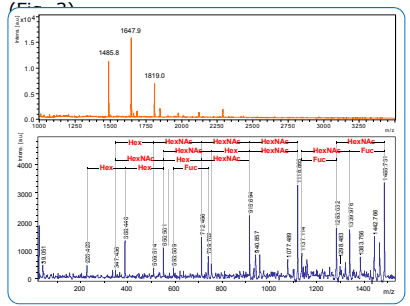

Fig. 3: MALDI-TOF MS spectrum of PNGase F digested and purified gkycans from human serum (a). MS/MS spectrum of the peak at 1485.7 Da confirming the presence of a glycan (b).

Since immunoglobulins are the most abundant glycoproteins in serum, these glycans are derived from immunoglobulin G and the hexose unit correspond to galactose. Calculation of the peak areas of these peaks and their normalization of the areas to the area of peak 1485 Da allowed for the relative quantitation of the extent of galactosylation. Over all samples the ratio of the mono-galactoslated form gave an average of 1.4 and the di-galactosylated form of 0.7. Subdividing the samples according to the different patient groups (healthy, RA OA) the averages for the mono-galactosylated form were 1.7, 0.9 and 1.1, respectively (Fig. 4).

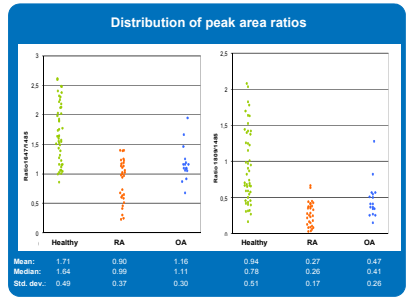

Fig. 4: Peak areas from MALDI-TOF MS spectra were normalized to the area of the peak at 1485 Da and plotted against the patient group

Correspondingly, the averages for the di-galactosylted form were 0.9, 0.3 and 0.5 for healthy donors, RA and OA patients. In healthy individuals the mono-galactosylated glycan is the main glycan form. In patients with RA the completely agalactosylated glycan form is the dominant form. A correlation between the degrees of de-galactosylation with clinical parameter, e.g. C-reactive protein, Cyclic citrullinated Peptide, Rheuma Factor, could not be observed. It will have to be investigated if the extend of galactosylation will be usefull as an additional clinical marker for RA.

Conclusions

- A simple, quick and reliable method based on MALDI-TOF MS for the analysis of N-glycans has been developed.
- This method allows for the relative quantitation of different glycan structures.
- The relative extent of galactosylated bi-antennary complex glycans of IgGs is decreased in patients with RA.

MALDI-TOF MS