Oligosaccharides of IgG in rheumatoid arthritis

ALEXANDER J. MACGILLIVRAY, MABEL IWIO, LUKE FERNANDEZ* and GEORGE PAPASAWAS*

School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 9QG.
*Department of Rheumatology, Royal Sussex County Hospital, Eastern Road, Brighton BN2 5BE.

IgG contains two major glycosylation sites in the CH2 domains at which complex biantennary oligosaccharides are attached at asparagine residues. A current interest is based on the finding that, compared to normal individuals, RA patients have increased levels of IgG oligosaccharide side chains lacking galactose and terminating in N-acetylgalactosamine [1]. Not only is the agalactosylation of IgG detectable prior to the onset of RA, [2] but the effect is also associated with a highly progressive disease course [3]. Consequently, IgG agalactosylation shows potential for use in the RA field.

Currently these changes in IgG glycosylation are detected by chemical means or by using lectin-binding assays [4] whilst identification of oligosaccharides attached to a glycoprotein involves their release from the protein by chemical or enzymatic means followed by radioactive or fluorescent labelling and separation by gel filtration or HPLC [1, 5]. The availability of the enzyme PNGase and the introduction of FACE [6] provide alternative means of releasing oligosaccharides and the separation of their fluorescent labelled derivatives by polyacrylamide gel electrophoresis. We have investigated the use of PNGase and FACE in a preliminary study of IgG oligosaccharides in rheumatoid arthritis.

IgG was prepared from the sera of seven definite RA patients and five healthy normal individuals using the combined G25/DEAE-C column procedure of Sumar et al. [3]. Pooled human and bovine IgG preparations and other glycoproteins (ovalbumin, fetuin) were also used. 1-5 mg of glycoprotein were digested at pH 2 for 48 h, followed by treatment with PNGase at pH 5 for 16 h according to Takahashi et al. [4]. The digests were then desalted on G25 columns and after concentration by freeze-drying the labelled products were separated by non-SDS Laemmli electrophoresis in the presence of Tris/borate buffer and after treatment with PNGase at pH 5 for 16 h according to Jackson [16]. ANTS and AMAC-labelling showed a range of fluorescent components in the range 7-10 CUE. The major components were of 7, 9 and 10 mg of wheat starch marker; lane 2: pooled human IgG; lane 3: RA1; lane 4: RA2; lane 5: RA3; lane 6: RA; lane 7: RA5; lane 8: RA6; lane 9: normal 1; lane 10: normal 2; lane 11: sample buffer. The gel was photographed under UV light.

Application of the ANTS-labelling procedure to IgG oligosaccharides released from pooled normal subjects gave electrophoretic patterns which were very similar to that obtained from oligosaccharides from pooled IgG, although in some the component of GUE 7 showed the weakest intensity (see Fig. 1, lanes 9, 10). In contrast, IgG oligosaccharides from six of the seven RA patients showed patterns in which the fluorescence intensity of the components of GUE 7 and 10 were increased and diminished respectively, relative to the pattern given by the normal samples (see Fig. 1, lanes 3, 4, 6, 7, 8). This pattern was reproducible using different aliquots from the same RA serum or from the same patient at different times. The remaining RA sample gave a pattern of oligosaccharides similar to that of normal IgG (Fig. 1, lane 5). No significant differences were found in the patterns given by AMAC-labelled oligosaccharides from RA and normal IgGs using both borate and non-borate electrophoresis conditions.

Of the two FACE techniques used in this study that using ANTS-labelling of oligosaccharides released by PNGase appears to have potential for comparing oligosaccharide patterns from different sources. It is of interest, therefore, that differences were obtained in the patterns of IgG oligosaccharides from IgGs of the RA and normal patients studied, suggesting that in RA smaller oligosaccharides may predominate. Similar results have been reported by Parekh et al. [11] for oligosaccharides released chemically from normal and RA IgGs, radioactively labelled and separated by gel filtration. Both procedures separate oligosaccharides on the basis of differences in size. These results may reflect the shift in RA from IgG oligosaccharide chains containing galactose and sialic acid to shorter complexes terminating in N-acetylgalactosamine. i.e. agalactosylation. We conclude that the FACE technique has potential for investigations into the nature of RA since it can be readily used as a quantitative analysis through densitometric scanning of the gels or photographs.

Abbreviations used: AMAC, 2-aminoacridione; ANTS, 8-aminonaphthalene-1, 3, 6-trisulphonic acid; DEAE-C, diethylaminoethyl-cellulose; FACE, fluorophore assisted carbohydrate electrophoresis; GLC, gas liquid chromatography; GUE, glucose unit equivalent; HPLC, high performance liquid chromatography; IgG, immunoglobulin G; PNGase, peptide-N-glycosidase; RA, rheumatoid arthritis.