Does it take sugar? A clinical role for measuring the glycosylation of IgG?

'Does He Take Sugar?' is a long running BBC radio programme describing the plight of the handicapped who are ignored frequently and whose worth is often doubted. By analogy in the study of immunoglobulins, and many other proteins, scant attention has been given to the oligosaccharides that are actually an integral part of them.

Glycosylation of proteins is widespread in eukaryotes, yet practically absent in prokaryotes. This is perhaps unsurprising in view of the fact that the major function of glycoprotein molecules lies in recognition of intercellular signals. Such recognition can involve membrane bound proteins on cell surfaces—for example the selectins, which mediate the attachment of leucocytes to endothelial cells, permitting their extravasation. The oligosaccharide binding domains of these molecules are homologous and essential for their function. Alternatively, glycoprotein signals may operate over long distances. The gonadotrophins and thyroid stimulating hormone are all glycoproteins. If these hormones are deglycosylated, biological activity is reduced and clearance rate increased. In the immune system, major histocompatibility complex antigens, T cell receptors, and immunoglobulins have all been shown to be glycosylated. Some divergent antibodies become monovalent after glycosylation, as a result of blockage of one of the antigen binding sites by oligosaccharide. Effector functions are influenced by Fc linked oligosaccharide. Thus binding to macrophage Fc receptor and complement C1q in vitro are reduced when sugars are enzymatically removed from IgG molecules. The increasing interest in the subject of glycoimmunology is reflected in the three meetings on the subject that have taken place in the past six years. The introduction of the journals Glycobiology and Glycosylation and Disease also reflects the greater awareness of glycosylation research.

It is 10 years since the first detailed analysis of the variation in oligosaccharide structures attached to serum IgG was published. Human IgG is mainly glycosylated with N-linked carbohydrates, and glycosylation of the Fc region occurs at asparagine 297, on which two opposing biantennary arms of oligosaccharide protrude and interact. Figure 1 depicts a space filling model of this arrangement. The study, published in 1985, followed an earlier, much smaller study and showed that serum IgG from patients with rheumatoid arthritis (RA) and, to a lesser extent, osteoarthritis had an increased number of oligosaccharide structures in which the outer arms lacked galactose and terminated in N-acetyl glucosamine (GlcNAc). Theseagalactosyl oligosaccharides was generally referred to as Gal(0).

With hindsight it was easy to see that an important difference between those patients with RA and osteoarthritis reported to have increased Gal(0) was the older age of those with osteoarthritis. In a subsequent study of 151 normal healthy individuals (both men and women) varying in age from one to 70 years, it was demonstrated that the relative incidence of agalactosyl IgG was indeed age dependent. It has now been established that at birth most IgG is fully glycosylated, though during the first year of life about 50% of the IgG produced is agalactosylated. From one to approximately 15 years of age, the proportion decreases to around 15-20%; it remains virtually constant up to the age of 40, but then increases steadily. These observations have been confirmed using both a complex biochemical method and a lectin binding assay. This latter assay takes advantage of the fact that different lectins bind selectively to different sugars. This method also offers...
with 25 different monosaccharide sequences Gal(0) to age for IgG from 179 healthy subjects aged 1 to 90 years. The solid curve depicts the regression function for healthy subjects generated by the least squares method and is given by the equation $y = 27.8 - 0.49x + 0.009x^2$, $n = 179$. The dashed curves represent the 95% confidence limits of the fit.

Figure 2  Relation of the percentage incidence of agalactosyl monosaccharide sequences Gal(0) to age for IgG from 179 healthy subjects aged 1 to 90 years. The solid curve depicts the regression function for healthy subjects generated by the least squares method and is given by the equation $y = 27.8 - 0.49x + 0.009x^2$, $n = 179$. The dashed curves represent the 95% confidence limits of the fit.

a much faster way of obtaining results. It is thus evident that measurement of Gal(0) must make an age allowance (Fig 2).

In a much larger study of approximately 250 patients with 25 different diseases, the specificity of an increased agalactosyl IgG was explored. It was observed that increased Gal(0) is confined mainly to patients with active RA, pulmonary tuberculosis, Crohn’s disease, and some patients with lupus complicated by Sjögren’s syndrome. In contrast, patients with various viral infections, including AIDS, bacterial infections, a variety of other autoimmune diseases, diabetes, and rheumatic fever have normal levels of Gal(0). It was thus concluded that increased Gal(0) is not merely an acute phase response akin to the erythrocyte sedimentation rate or C reactive protein.

In later studies, however, it was shown that Gal(0) levels were occasionally increased in patients whose diseases had not previously been associated with high levels. For example, patients with leprosy undergoing the erythema nodosum leprosum reaction had increased Gal(0) levels that corresponded to high levels of interleukin-6. Some patients with active sarcoidosis have also been found to have high levels of Gal(0) (Rook G, personal communication).

Serial studies in patients with adult onset RA who were pregnant, and in children with juvenile chronic arthritis, of all three types (polyarticular, pauciarticular, and systemic onset) showed that the level of Gal(0) reflected the degree of disease activity. However, the criteria on which this judgment was based were relatively crude.

The studies of pregnant patients with RA, however, are particularly pertinent, as the majority of these individuals go into remission during pregnancy but relapse shortly after parturition. In the study of pregnant patients, by the third trimester all had normal age corrected Gal(0) levels, and at the last blood test before parturition seven of eight patients were thought to be in clinical remission. Post partum, six of these patients showed an increase in their Gal(0) levels, in each case associated with a clinical relapse. One patient, studied in detail, was of particular interest in that she went into remission six weeks before the birth of her child, as her Gal(0) decreased for the first time to less than 33%. Following the birth, the patient’s arthritis relapsed at day 7, as her Gal(0) increased above this level. Although the correlation with disease activity was not as good, the same fluctuation in Gal(0) levels has been found in patients with Crohn’s disease and ulcerative colitis (Pilkington et al, in preparation).

Various prospective and retrospective studies have been performed to determine whether the measurement of Gal(0) can provide additional and clinically useful information. A study of 60 patients presenting to early onset synovitis clinics demonstrated the potential usefulness of measuring Gal(0). Blood samples were taken at the first clinical visit (within one year of disease onset) and the rheumatoid factor and Gal(0) levels were recorded. The patients were followed for two years and the usefulness of measuring these parameters was assessed. The positive predictive power of these measurements individually was shown to be around 80%. However, those patients presenting with early onset synovitis who had both a positive rheumatoid factor (titre greater than 1:80) and an increased Gal(0) (more than 1 SD greater than the age corrected mean) produced a positive predictive value of 94% for those going on to develop RA.

These patients, and some others, have now been followed up for a minimum of four years to determine which factors at presentation might be able to predict clinical outcome and the degree of disease related damage. The patients were grouped as those who went into clinical remission (often after a more ‘dramatic’ onset) and those who had persistent or relapsing or remitting disease. This division was based on an overall clinical and serological assessment. Gal(0) alone was not significantly different between the two disease outcome groups. However, by combining several factors in a discriminant functional analysis, it was shown that Gal(0) was ranked as the most powerful parameter for predicting disease outcome at four years. The features which best predicted a poor prognosis in RA in these two groups of patients were Gal(0) between 1 and 1.8 SD above the age corrected mean at first bleed, grip strength <170 mm Hg, age of onset <51 years, and female gender.

In a similar study of a different cohort, 127 well characterised female RA patients were followed up for a mean duration of six years. Gal(0) levels in the first available serum sample more than 2 SD above the mean level of controls positively predicted more erosions, increased disease activity, and requirement for more second-line drugs compared with patients without an increased Gal(0) level. However, the first available sample was obtained on average at 3-4 years after onset of symptoms—rather later than in the study referred to above.

There are very few instances in medicine of it being possible to study a disorder before its clinical development. Longitudinal studies of the Pima Indians, however, afforded a rare opportunity to study the development of RA. The mean galactose content of the serum IgG from 11 patients with RA collected before disease onset was significantly lower than IgG from seronegative individuals without the disease from within the same population, but was not significantly different from healthy controls who did have a positive rheumatoid factor.

In order to explore the possibility that increase in Gal(0) is caused by an environmental agent, a family study of 31 members and spouses, including eight patients with RA (supplemented by a further 13 rheumatoid patients and spouses) was undertaken. It was shown that the glycosylation defect was present both in the relatives of the RA probands who also had the disease and, intriguingly, in 13 of 21 spouses of the patients, but rarely (4/26) in their
healthy first degree relatives. These data support the view that an environmental factor has a key role in the development of the impaired glycosylation.

Taken together, these observations strongly support the view that, though it remains to be proven that abnormal glycosylation is a truly aetiologic factor in RA, measuring agalactosyl IgG clearly does have clinical relevance.

Recent investigations have focused on the observation that binding of human monoclonal IgG rheumatoid factor to Fc is influenced by carbohydrate, and a suggestion that the level of agalactosyl IgG in immune complexes in RA might be increased. Furthermore, it has been shown that passive transfer of an acute synovitis in T cell primed DBA/I mice (known to have a predisposition to develop collagen induced arthritis) can be enhanced by using IgG containing autoantibodies to type II collagen, provided the antibodies are in the agalactosyl form. However, as Axford has commented, caution is required before it is assumed that the pattern of disease associated glycosylation changes are analogous in different diseases and in all ethnic groups. It would nevertheless be of interest to undertake a much larger, perhaps multicentre, study of early onset synovitis to confirm the principal finding referred to above, namely that increased agalactosyl IgG helps to predict the development of rheumatoid arthritis. Further longitudinal studies are also required to confirm the predictive power of Gal(0) early in disease development for identifying those likely to succumb to more serious disease. Given the recent descriptions of more sophisticated disease activity scores, the relationship between disease activity and Gal(0) also requires re-examination. A point to be borne in mind here is that the half life of IgG is approximately three weeks and such studies must reflect this turnover time. Finally, and perhaps most importantly, the development of a commercially available kit to measure agalactosylated IgG would enormously facilitate studies of this nature and promote the wider availability of the test. Perhaps only then will we be able to realise the full potential for measuring the levels of abnormally glycosylated IgG and be able to answer the question ‘Does it take sugar?’

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