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The Role of IgG Glycoforms in the Pathogenesis of Rheumatoid Arthritis

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Introduction

Most serum-derived and cell-surface proteins are *N*-glycosylated, i. e., have oligosaccharides covalently attached to Asn through an *N*-glycosidic linkage. The structure of these highly branched oligosaccharides is often very complex, since their constituent monosaccharides can be linked in many different ways [28]. Consequently, the potential information encoded into an oligosaccharide via its monosaccharide sequence and three-dimensional structure is considerable. Further, an individual *N*-glycosylation sequon usually does not contain a single oligosaccharide, but rather a qualitatively and quantitatively conserved *population* of structures, and this is usually referred to as microheterogeneity. This implies that polypeptides are diversified by glycosylation into populations of glycoproteins differing with respect to the structure and disposition of oligosaccharides on a common polypeptide (i. e., glycoforms). Each glycoform may be involved in different and unique cellular functions (e. g., 'homing', clearance, etc.). It follows that disease-associated changes in the incidence of individual naturally occurring glycoforms, or the generation of new glycoforms, will affect the carbohydrate-dependent functions of a polypeptide. Changes in oligosaccharides may in some cases, therefore, contribute directly to disease pathogenesis. Moreover, the final population of *N*-linked oligosaccharides carried by a polypeptide is the result of a large number of intra-cellular events, some of which are tissue specific [42]. Therefore, irrespective of the normal functions of the oligosaccharide, a comparative analysis of the *N*-glycosylation of particular glycoproteins provides an excellent probe for acquired or inherited cell type-specific or tissue-specific dysfunction.

All normal IgG isotype antibodies are glycoproteins carrying predominantly *N*-linked oligosaccharides [41, 49]. Briefly, human serum IgG carries, on average, 2.8 *N*-linked oligosaccharides, of which 2.0 are invariably located in the Fc at the conserved *N*-glycosylation site of Asn 297 (Fig. 1). The additional oligosaccha-

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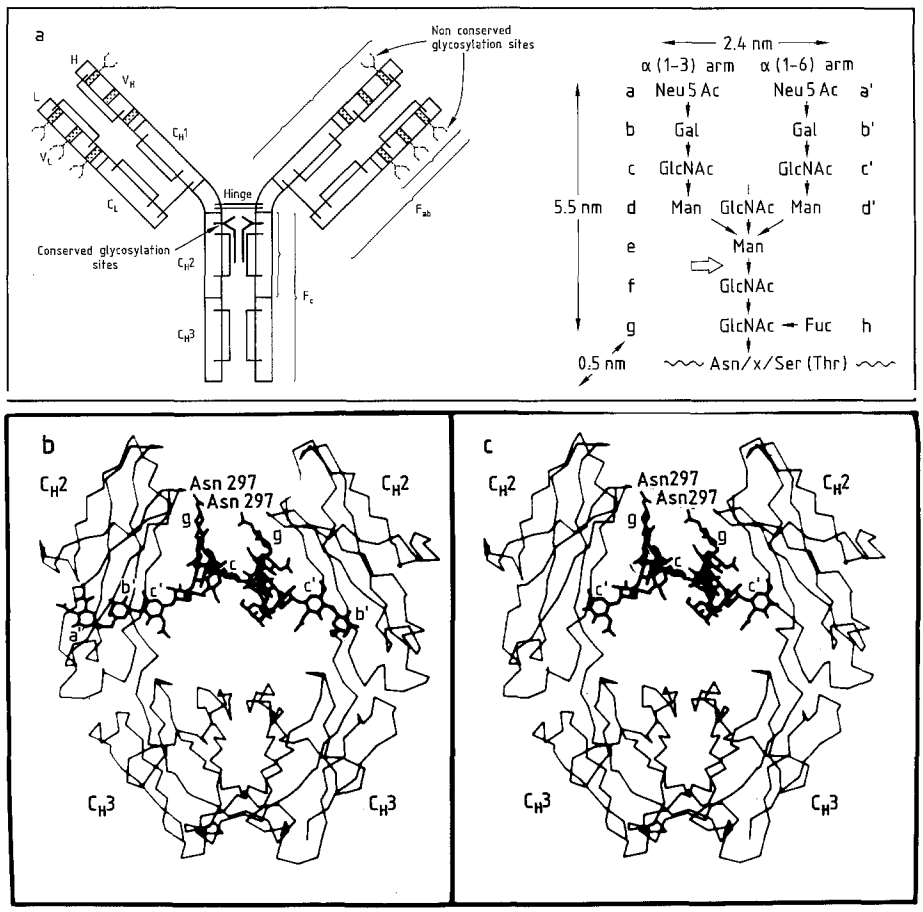

Fig. 1. a A schematic representation of an IgG molecule indicating the positions of conserved *N*-glycosylation sites (at Asn-297 in the CH2 domains) and non-conserved *N*-glycosylation sites [in the hypervariable regions (*dotted*) of the Fab region]. The relative size of an immunoglobulin domain and a fully extended *N*-linked biantennary complex oligosaccharide are similar [4]. Complex-type oligosaccharides present on IgG can be subdivided into an outer arm region (a, a′, b, b′, c, c′), and the core, which is composed of a trimannosyl unit (d, d′, e) and a *N*,*N*′-diacetylchitobiosyl unit (f, g). The 'bisect' GlcNAc (residue i) is linked $\beta(1\rightarrow4)$ and the fucose (residue h) is linked $\alpha(1\rightarrow6)$. The *arrow* between residues e and f indicates the site of interaction between the two oligosaccharides in b and c. **b** Refined structure at 2.8 A of rabbit Fc fragment [54]. The two carbohydrate chains, each attached at Asn 297, differ in conformation and may also differ in sequence and bridge the two CH2 domains. The $\alpha(1\rightarrow3)$ arm of the chain (left side) is always devoid of galactose and interacts through its $\beta(1\rightarrow2)$-linked GlcNAc residue (c) with the Man$\beta(1\rightarrow4)$ GlcNAc segment of the opposing (*right side*) oligosaccharide chain (see **a**). The $\alpha(1\rightarrow3)$ arm of the right chain extends outwards between the domains with no apparent steric constraints on its length. A Neu5Ac unit (a′) is shown on one $\alpha(1\rightarrow6)$ arm only (*left*). The extend of oligosaccharide heterogeneity in a single crystal is identical to that found in pooled Fc fragments [49], consequently the X-ray data represent the composite structure. **c** Fc fragment containing oligosaccharides devoid of galactose and sialic acid on each of the $(\alpha1\rightarrow6)$ arms. Since these residues in normal IgG are in contact with the surface of the protein (see **b**), their absence vacates oligosaccharide-binding sites in IgG from arthritic patients and could make the IgG 'sticky' by creating a lectin-like activity. It is not known to what extent the remaining sugar residues remain in contact with the peptide

rides are located in the variable region of the light and heavy chains, with a frequency and position dependent on the occurrence of the *N*-glycosylation sequon [Asn/Xaa/Ser(Thr)]. Approximately 30 different biantennary oligosaccharides are found to be associated with total human serum IgG (Fig. 2). These are distributed non-randomly between the Fab and Fc. Characteristics of Fc *N*-glycosylation include the absence of disialylated structures, a low incidence of monosialylated ones (~10%), a low incidence of cores carrying a 'bisecting' GlcNAc, and the absence of galactose on the $\alpha 1 \rightarrow 3$ arm of at least one oligosaccharide chain in each Fc. Fab *N*-glycosylation is characterised by a high incidence of di- and monosialylated structures, and of cores with the 'bisecting' GlcNAc residue. The large number of different structures associated with IgG is not the result of studying a polyclonal population, since a similar heterogeneity is found upon analysis of myeloma and hybridoma IgG [49]. This heterogeneity, therefore, creates a very large number of variants (glycoforms) of each unique IgG polypeptide causing further structural, and perhaps functional, diversification.

Altered *N*-glycosylation of IgG in Rheumatoid Arthritis

Studies of the *N*-glycosylation pattern of serum IgG (Fig. 3) have shown that a major difference exists between normal individuals and those with rheumatoid arthritis [36, 41, 43]. Structural analysis indicates that serum IgG in patients with rheumatoid arthritis is not associated with any novel oligosaccharide structures, but rather with an increased number of oligosaccharide moieties whose outer arms lack galactose and terminate in *N*-acetylglucosamine [41]. There appears, therefore, to be a shift in the population of IgG glycoforms towards those with a higher content of agalactosyl oligosaccharides. This change in *N*-glycosylation of IgG is generally measured using one of the following three methods: (1) the use of hydrazine to release IgG-associated oligosaccharides, followed by digestion of these oligosaccharides with exoglycosidases of defined specificities [41, 45]. This method yields important structural information, and also allows the degree of outer arm galactosylation to be probed exactly. That is, the relative incidence of di-galactosyl, G(2), mono-galactosyl, G(1), and agalactosyl, G(0) oligosaccharides associated with an IgG sample can be accurately determined. (2) The use of anti-GlcNAc monoclonal antibodies. This provides information only on the relative incidence of agalactosyl oligosaccharides. (3) The use of chemical methods to determine the relative monosaccharide composition of a given IgG sample. This method provides no structural information. Since the relative content of di-, mono-, and agalactosyl structures fluctuates with age and disease activity (see later), this latter method may be occasionally misleading. Of the various methods, therefore, exoglycosidase analysis of hydrazine-released oligosaccharides is the most informative, and remains the method of choice for probing IgG *N*-glycosylation.

The relative incidence of agalactosyl oligosaccharides, G(0), is an age-related parameter [45], as shown in Fig. 4. Interestingly, G(1) does not vary with age, and G(2) varies inversely to G(0) (data not shown). The relation between G(0) and age determined for unaffected individuals establishes the abnormality of this parameter in patients with adult rheumatoid arthritis (Fig. 5a), and allows an

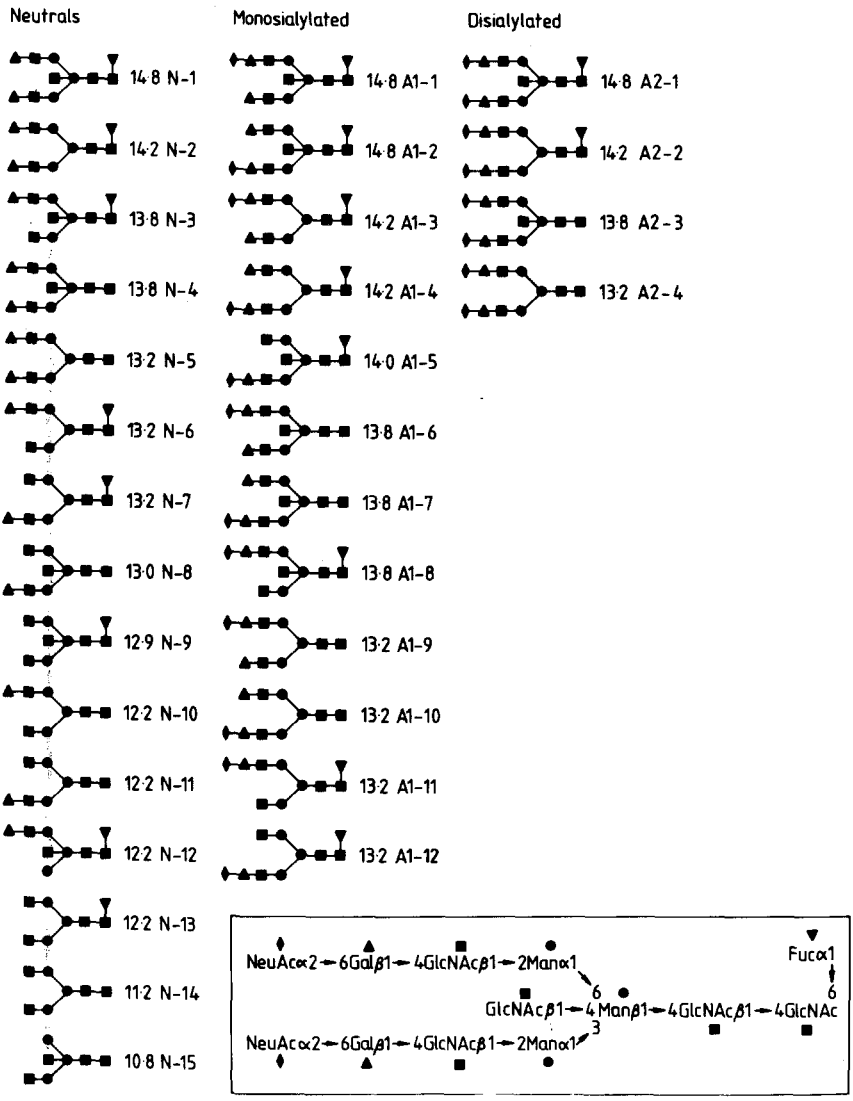

Fig. 2. Primary monosaccharide sequences of the *N*-linked oligosaccharides associated with human IgG. The hydrodynamic volume (as measured in glucose units) of each structure (or of its neutral derivative in the case of those sialylated) is indicated, and was determined by comparison with $\alpha(1\rightarrow6)$ linked glucose oligomer standards [3]

assessment of the normality or otherwise of this parameter in other diseases. From a study of other autoimmune rheumatological conditions, chronic inflammatory disorders, and diseases with known infectious etiology, it emerges that juvenile rheumatoid arthritis, tuberculosis, Crohn's disease, and systemic lupus erythematosus (SLE) with Sjogren's syndrome (Figs. 5, 6) are also associated with an elevated G(0), whereas the other diseases listed in Table 2 are not [44, 46].

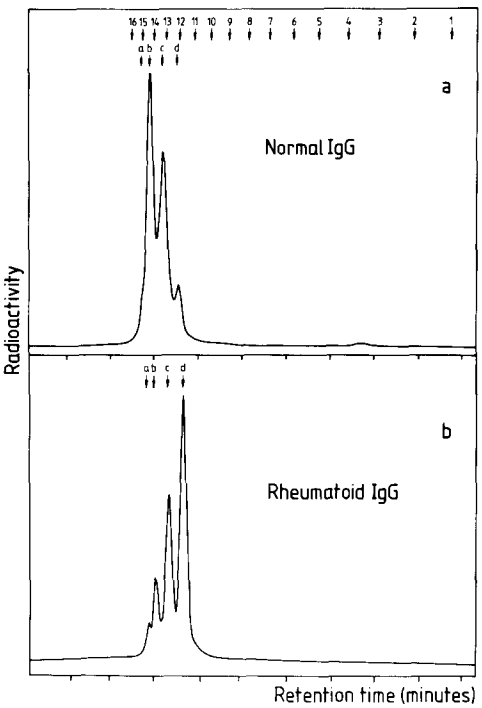

Fig. 3a, b. Representative Bio Gel P-4 (−400 mesh) gel-permeation chromatograms of the asialo oligosaccharides of total serum IgG from a healthy individual (**a**), and from a patient with rheumatoid arthritis (**b**) [41]

Further, G(0) correlates well with clinical score in patients with adult rheumatoid arthritis (Fig. 7), and also with disease activity (Fig. 6). It is also interesting that during pregnancy, a state commonly associated with remission of rheumatoid arthritis, a significant increase in the galactose content of IgG is observed [48].

A comparative analysis of IgG from serum and synovial fluid (Table 1) showed that in four of five patients with rheumatoid arthritis, the level of G(0) in the synovial fluid was higher than in serum. These data are consistent with an intra-articular synthesis of agalactosyl IgG. A comparison of the *N*-glycosylation of Fab and Fc fragments derived from total serum IgG of patients with rheumatoid arthritis or from a control group shows that the decreased galactosylation found upon analysis of total serum IgG is largely due to changes in the *N*-linked oligosaccharides of the Fc [43]. There are also quantitatively minor, but potentially significant differences in Fab glycosylation, and these may be restricted to heavy chain *N*-glycosylation [43, 49]. At present, the relation between the percentage incidence of G(0) and the percentage incidence of agalactosyl IgG [i. e., IgG(0), an IgG molecule containing two *paired* Fc-associated oligosaccharides both completely lacking galactose] can only be estimated by assuming random pairing of the two heavy chains [41].

It seems likely that these changes in IgG *N*-glycosylation can be attributed to a reduction in the β-galactosyltransferase activity in the B lymphocytes of patients with rheumatoid arthritis [2]. A specific β-galactosyltransferase has been reported to be present in B lymphocytes, which transfers UDP-Gal to an asialo-agalactosyl

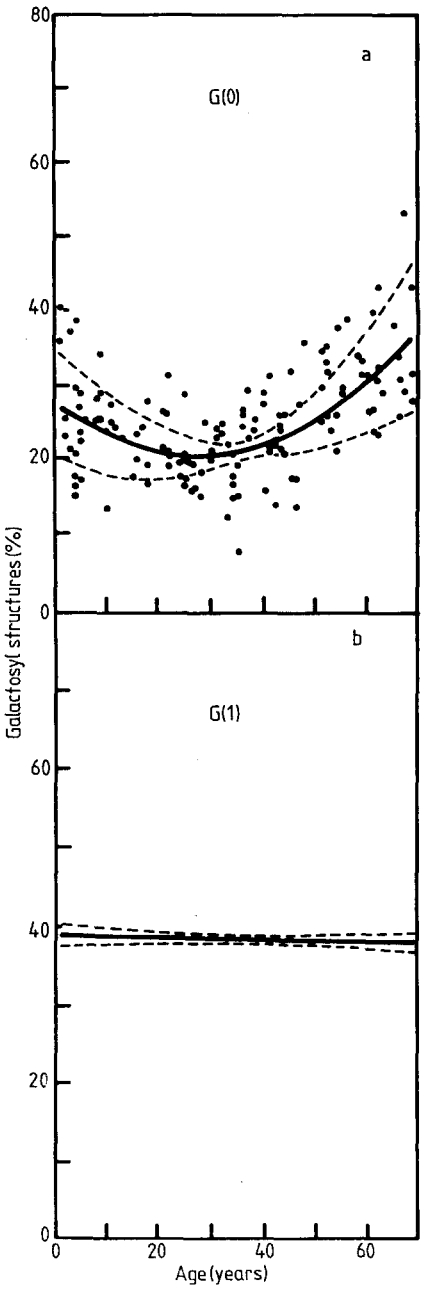

Fig. 4a, b. The variation in the relative incidence of agalactosyl (**a**) and mongalactosyl (**b**) *N*-linked oligosaccharides on total human serum IgG with age. *Dots* (**a**) represent the value of G(0) for each individual. The *solid curve* (**a**) is a quadratic regression line of the data ($n=2$, no other transformations improved the fit), and the *solid curve* (b) is a linear regression line of the data. *Dashed curves* (a, b) represent calculated regression lines with 95% confidence intervals

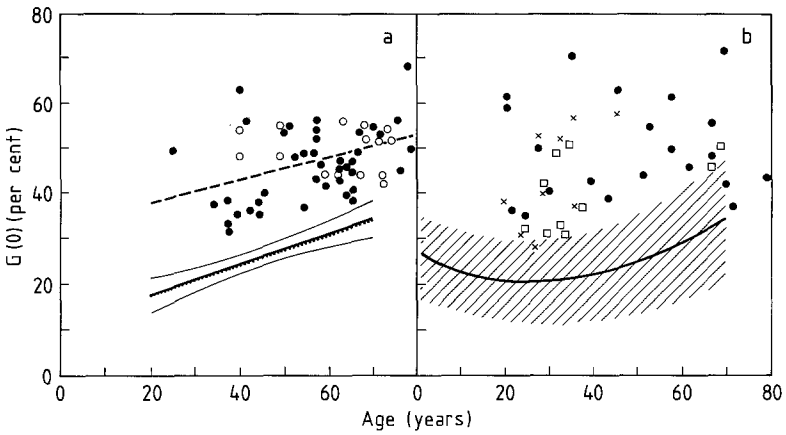

Fig. 5. a Relation of the percentage incidence of agalactosyl monosaccharide sequence to age of IgG from patients with adult onset rheumatoid arthritis. All patients studied had active disease at the time of sampling. The *closed circles*, •, depict values of G(0) from patients reported previously [44] and the *open circles*, ○, from [41]. The regression line (*dashed*) for the patients from both studies is given by the equation $y=31.9+0.264x$, SD of slope 0.079, $n=56$ and is significantly different ($P<0.01$) from a linear approximation (*dots*) [$y=10.2+0.343x$, SD of slope 0.041, $n=111$] of the function for the normal subjects (Fig. 4) between ages 20 and 70, as tested by the analysis of covariance. The 95% confidence limits for the fit of the data from normal subjects are indicated by *solid lines*. **b** Relation of the percentage incidence of agalactosyl monosaccharide sequence G(0) to age of IgG from patients with tuberculosis, ●; Crohn's disease, X; and SLE with Sjogren's, □. All patients were active at the time of sampling. The regression lines for the three diseases were calculated from the following equations; tuberculosis, $y=50+0.007x$, SD of slope 0.132, $n=21$; Crohn's, $y=16.6+0.88x$, SD of slope 0.42, $n=9$ and SLE+Sjogren's, $y=26.8+0.32x$, SD of slope 0.17, $n=10$. All three lines were significantly different ($P<0.01$) from a linear approximation of the G(0) data from normal subjects (Fig. 4). The *solid line* depicts the regression function for normal subjects generated by the least squares method for the means of ages with $n>3$. The outer bounds of the *hatched lines* depict the regression functions for ± 2 SD of the mean for ages with $n>3$

IgG [16]. The affinity of this enzyme for UDP-Gal in the B lymphocytes of patients with rheumatoid arthritis is lower than in B lymphocytes from a control group. Further, the specific activity of the galactosyltransferase from the B lymphocytes of such patients towards asialo-agalactosyl IgG is found to be reduced to 50%–60% of controls [16]. The galactosyltransferase deficiency also appears to be greater in B lymphocytes than in T lymphocytes or monocytes of patients with rheumatoid arthritis [2]. However, a decrease in the level of β-galactosyltransferase activity of *both* B and T lymphocyte has recently been found in patients with tuberculosis (J. Axford, personal communication).

Carbohydrate-dependent Functions of IgG

The pathological consequences of the shift in population of IgG glycoforms towards IgG molecules whose oligosaccharides terminate in *N*-acetylglucosamine in rheumatoid arthritis, tuberculosis and Crohn's disease can be understood by

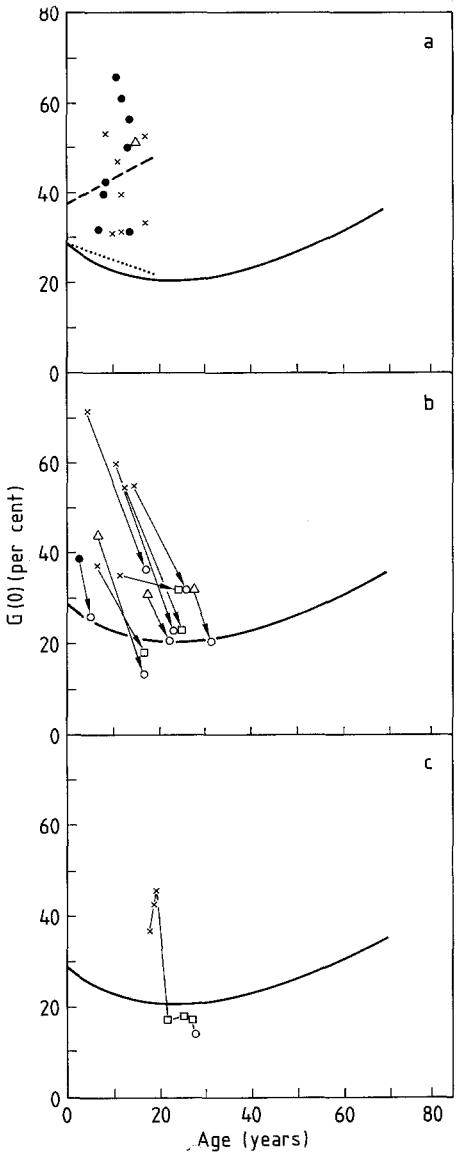

Fig. 6. a Variation in the relative incidence of agalactosyl *N*-linked oligosaccharides on total serum IgG with age in individuals with systemic onset (x), polyarticular onset (△), and pauciarticular onset (•) juvenile rheumatoid arthritis. The *solid line* depicts the regression function for normal subjects (Fig. 4). The values of G(0) for patients were significantly different from age-matched controls ($P<0.01$) as determined using an analysis of covariance between the regression lines for the diseased group (*dashed*) and corresponding age-matched controls (*dotted*) [45]. **b** Retrospective analysis of G(0) in patients a separate set from those reported in **a** with juvenile onset arthritis. Seven of the patients were in remission at the time of the second serum sample. Included are patients with systemic (x), $n=6$; poly (△) $n=3$; and pauciarticular (•), $n=1$; presentation. Most patients progressed to symmetrical polyarthritis, $n=8$. Disease progression in two patients was limited to the pauciarticular type of arthritis, one symmetric, the other asymmetric. The *solid curve* represents the age-dependent function for the normal subjects between ages 1 year and 50. **c** Retrospective analysis of G(0) in a patient presenting with systemic onset juvenile arthritis with progression to symmetric polyarthritis before inactive disease (□) and eventual remission (○)

considering the carbohydrate-dependent effector functions of the Fc and Fab regions of IgG. Carbohydrate-dependent functions of the Fc moiety of IgG all involve interactions with cellular-bound receptors, whereas fluid-phase reactions are independent. For example, aglycosyl and deglycosyl IgG molecules retain the properties of the normal glycosylated molecules in the binding of antigen, protein A, and C1q, and also with respect to C1 activation [30]. However, in such molecules there is complete loss of binding to monocyte and macrophage Fc receptors [30, 39], the ability to induce cellular cytotoxicity is reduced [39],

Table 1. Relative incidence of agalactosyl oligosaccharides in IgG isolated from serum and synovial fluid of patients with rheumatoid arthritis

Disease activity[a]	Age	G(0) percent[b]		
		Serum	Synovial fluid	Control
A	38	33	39	25±4
4[c]				
I	50	24	27	26±8
A	53	48	48	26±8
A	63	43	50	32±7
A	80	49	51	n.d.

[a] A: Active; I: inactive
[b] Values of G(0) are accurate to <1%
[c] Normal values are determined for 5-year intervals from the G(0) vs age data (Fig. 4a)
n.d. = Not determined

complexes with antigen fail to be eliminated rapidly from circulation [39], and feedback immunosuppression is lost [20]. Serial lectin chromatography of native, normally glycosylated IgG has confirmed that there exists within the total IgG population a range of affinities for monocyte Fc receptors which is independent of subclass, but correlates with Fc *N*-glycosylation (i. e., different glycoforms) [32]. This indicates clearly that not only the degree but also the nature of Fc *N*-glycosylation is of biological relevance.

The *N*-linked oligosaccharides of the Fab moiety of IgG tend to influence solubility/aggregation phenomena. Naturally occurring monovalent antibodies (i. e., non-precipitating) are formed by *N*-glycosylation of only one of the Fab arms (asymmetric glycosylation). These endo-β-*N*-acetylglucosaminidase- H sensitive oligosaccharides (and, therefore, putatively of the oligomannose or hybrid classes) act as combining site 'blockers' to render the molecule functionally univalent [12, 29, 31]. Non-precipitating univalent antibodies are known to have enhanced cytotoxic properties as compared to normal bivalent precipitating antibodies [10]. The presence of an oligosaccharide with non-reducing terminal mannose on the non-antigen binding Fab arm may have an additional role in acting as a ligand for

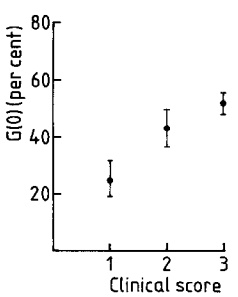

Fig. 7. Relation of the percentage incidence of agalactosyl monosaccharide sequences from IgG of patients with adult onset rheumatoid arthritis to clinical score. The clinical score (mean±SD), 1=inactive/mild, n=3; 2=moderately active, n=7; and 3, severely active, n=4, was determined prospectively for the patients as reported elsewhere [44]

Table 2. Diseases in which the relative content of agalactosyl oligosaccharides in total serum IgG has been determined

Diseases associated with normal IgG (0)	Diseases associated with increased IgG (0)
Primary SLE	Rheumatoid arthritis
Primary Sjogren's	Juvenile-onset RA
Myositis	Tuberculosis
Scleroderma	Crohn's disease
Psoriatic arthropathy	SLE plus Sjogren's
Ankylosing Spondylitis	
Gouty Arthritis	
Osteoarthritis	
Reactive arthritis (yersinia)	
Ulcerative colitis	
Sarcoidosis	
Multiple Sclerosis	
Klebsiella infection	
Leprosy	
AIDS	
Rubella	
Parvovirus infection	
Mumps	
Glandular fever	

the serum mannose-binding protein which activates complement through the classical pathway [24].

The aggregation of human IgG into complexes of various sizes is also critically influenced by the *N*-glycosylation of the Fab moiety of IgG. For example, the cryoglobulin and cold agglutinin properties of certain monoclonal IgM and IgG molecules have been shown to arise from sialylated *N*-linked oligosaccharides located on the Fab [34, 61]. A quantitative analysis of naturally occurring aggregated or self-associated IgG and monomeric IgG showed that aggregated IgG carries an average of 3.5 *N*-linked oligosaccharides per molecule (2 chains on the Fc and 1–2 chains on the Fab), but the monomeric IgG carries an average of 2.2 chains. Comparative analysis of the Fab associated *N*-linked oligosaccharides of aggregated and monomeric IgG showed a 'selection' for particular disialylated oligosaccharides in polymeric IgG [47].

In principle, therefore, one would expect the observed changes in the degree of outer arm galactosylation of IgG oligosaccharides characteristic of rheumatoid arthritis, tuberculosis, Crohn's disease, and SLE with Sjogren's syndrome to cause pathological effects through any one or more of the following mechanisms: first, through altered interactions with monocyte Fc receptors. Interaction with any such receptor that requires Fc oligosaccharides to retain galactose would be diminished, while any interaction requiring exposed non-reducing terminal GlcNAc would be dramatically increased, particularly as a consequence of the pairing of two agalactosyl oligosaccharides in the Fc. Such pairing would create an Fc moiety possessing four, possibly unrestrained, non-reducing terminal *N*-acetylglucosamine residues (see later), which could interact with a variety of cellular receptors against

GlcNAc. Second, a knowledge of the Fc crystal structure [54] suggests that the loss of outer arm galactose would have significant structural consequences, which may lead to immune-complex formation through one of several mechanisms. These mechanisms and the possible consequences of the increased exposure of non-reducing terminal *N*-acetylglucosamine residues are discussed in detail below.

Immune-complexes and Glycosylation

In rheumatoid arthritis it has long been postulated that auto-sensitisation to IgG may play a pivotal role in the pathogenesis of the disease, and immune complexes are formed through the binding of the IgM, IgG, or IgA rheumatoid factors to the constant region domains of IgG molecules. The immunogenic site on IgG has been localised to the Fc moiety [38], but there is no evidence for amino acid changes in the Fc of this IgG. Data on the carbohydrate composition of IgG present in the intermediate complexes isolated from the serum of patients with rheumatoid arthritis invariably show an increased level of sialic acid (usually Fab-associated) as compared to normal serum IgG [19, 23], and decreased content of Fc-associated galactose [23]. In one case, this sialic acid was shown to occur on light chains, and was crucial for IgG self-association, since its removal by neuraminidase treatment abolished complex formation [23]. In a recent study [48] it was found that serum IgG from patients with rheumatoid arthritis had a lower carbohydrate content and therefore presumably lower Fab *N*-glycosylation, consistent with the sequestration into complexes of IgG enriched in Fab *N*-glycosylation. Together, these observations suggest that immune-complex formation in rheumatoid arthritis could involve both Fab *N*-glycosylation and agalactosyl structures in the Fc [43]. The molecular mechanism whereby these two factors might contribute to IgG autoantigenicity or self-association are not known, but a study of the crystal structure of Fc provides some insight into this. The crystal structure [54] clearly indicates that each *N*-linked oligosaccharide in the Fc can interact with the protein surface of the C_H2 domain, principally via the NeuNAcα2$\rightarrow$6 Galβ1$\rightarrow$4 segment of the Man α1$\rightarrow$6 arm (Fig. 1). This is the principle non-covalent protein-oligosaccharide interaction in Fc, and serves to restrain the Fc oligosaccharides, and also to mask certain underlying Fc polypeptide determinants (Fig. 1). It is, therefore, suggested that the change in the degree of occupancy of the Fc carbohydrate-binding site, secondary to decreased outer arm galactosylation of Fc oligosaccharides, could lead to IgG self-association through one of the following mechanisms: first, through the insertion into this vacant site of an appropriate Fab-linked oligosaccharide from another IgG molecule; second, through the interaction of the affected IgG with either naturally occurring or induced anti-GlcNAc antibodies; or third, through interaction of the affected IgG with antibodies induced against the peptide (or peptide-oligosaccharide) epitopes previously largely masked by the native oligosacchaide. Self-association of IgG could occur by any of these mechanisms (Fig. 8). Further, occupation of the vacant oligosaccharide-binding site by other serum or synovial fluid glycoprotein or cartilage components can also be envisaged.

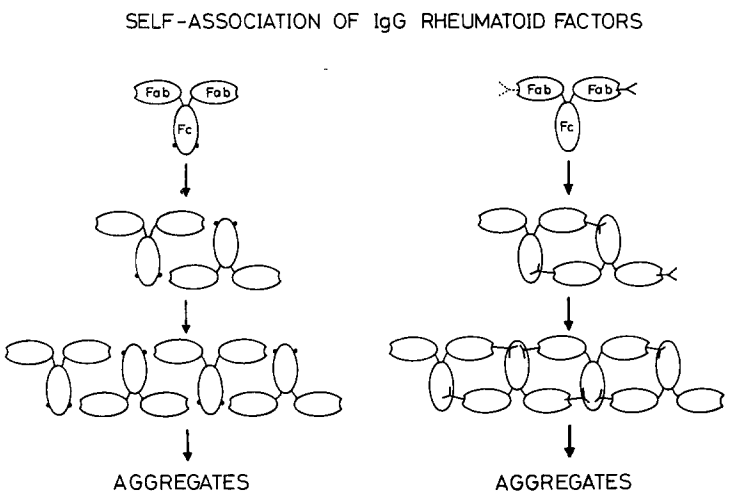

Fig. 8. A model for the self-association of IgG rheumatoid factors. Two mechanisms for this self-association are envisaged. One involves the recognition by the Fab-combining site of carbohydrate, polypeptide or polypeptide-carbohydrate epitope exposed in the CH2 domains of agalactosyl Fc (*left*), while the other involves insertion of Fab *N*-linked oligosaccharides (γ,γ) into the vacant carbohydrate-binding site of agalactosyl Fc (*right*)

Cellular Receptors to GlcNAc

IgG molecules presenting an abnormally high concentration of exposed non-reducing terminal GlcNAc residues may provoke immunopathological changes as a consequence of interaction with cells bearing receptors for the GlcNAc epitope [18]. Such receptors may be functionally important in the recognition of peptidoglycan structures of bacterial cell walls [37], in tumor surveillance by natural killer (NK) cells [1], and in macrophage recognition of cells undergoing programmed cell death (apoptosis) [13]. Further, the membrane CR3 receptor which is expressed on phagocytic and NK cells, has been shown to bind GlcNAc-containing glycans [52]. This receptor also binds endotoxin, possibly via the *N*-acylated glucosamine-containing (lipid x) moiety [62]. In addition, certain macrophage functions have recently been shown to be activated by *N*-acetyl-chito-oligosaccharides (GlyNAc homopolymers) [55].

Antibodies to cross-reactive Glc-NAc-containing epitopes present in the environment, particularly the adjuvant bacterial components (GlcNAc containing peptidoglycans), would have interesting auto-immune effects. For example, immunisation of rabbits with *Mycobacterium smegmatis* results in antisera which recognise highly branched *N*-linked oligosaccharides terminating in *N*-acetyl-glucosamine residues and containing a bisecting GlcNAc residue [8]. These anti-GlcNAc antibodies have been shown to bind to a cell-surface antigen associated with intrathymic and intrabursal maturation of chicken lymphocytes [7]. Moreover, patients with tuberculosis have varied levels of antibody to GlcNAc [4].

Antibody to terminal GlcNAc may also have some of the properties of rheumatoid factor (see above). It has recently been reported that mice immunised with the peptidoglycan/polysaccharide complex of Group A streptococci can be used as a source for production of monoclonal antibodies which bind to the terminal GlcNAc residues situated in the C_H2 domain of serum IgG isolated from patients with rheumatoid arthritis or tuberculosis [51]. This study is important, since Group A streptococci, which are associated with rheumatic fever, appear to be able to evoke formation of antibodies which will bind to an epitope on the agalactosyl IgG present in rheumatoid arthritis. The Group A streptococci polysaccharide/peptidoglycan complex is rich in GlcNAc and patients with rheumatic fever are known to have raised levels of antibody to GlcNAc [33], and patients with rheumatoid arthritis have also recently been shown to have raised levels of antibodies which bind to the Group A streptococci [27].

Rheumatoid Arthritis and Tuberculosis

The presence of agalactosyl IgG in patients with tuberculosis is consistent with the suggestion that mycobacteria, or auto-antigens which cross-react with them might be aetiological agents in rheumatoid arthritis [58]. Immune-complexes containing self-associated IgG have been identified in the serum of tuberculosis patients [25], and clearance studies have shown that IgG from these patients behaves similarly to the 'aged IgG' present in rheumatoid arthritis serum [60]. In addition, several studies have demonstrated that the serum of patients with tuberculosis contains rheumatoid factor which may be of the IgG and IgA isotypes as well as the classical IgM antibodies [26].

Linkages between HLA-DR phenotypes and the ability to respond to skin tests with mycobacterial antigens have also hinted at an association between rheumatoid arthritis and mycobacterial infections. Patients with rheumatoid arthritis respond relatively poorly to the common mycobacterial (group i) antigen, as do patients with leprosy or tuberculosis [3]. Skin-testing of 86 leprosy patients with four types of mycobacteria demonstrated that HLA-DR4 (a risk factor for rheumatoid arthritis) was associated with high responsiveness to antigens specific for *M. tuberculosis* [40]. Skin-testing of patients with rheumatoid arthritis also showed an increased responsiveness to tuberculin in those patients with the HLA-DR4 haplotype [3]. In contrast, rheumatoid arthritis patients with HLA-DR7 (a protective haplotype) showed low skin-test responsiveness to mycobacteria [3].

Further support for a connection between mycobacteria, or auto-antigens which cross-react with them, and rheumatoid arthritis comes from the finding of decreased levels of antibody binding to mycobacteria in those patients with rheumatoid arthritis who carry the relatively protective DR2 or DR7 haplotype [3, 4]. This class II MHC-associated regulation of antibody levels is an isotype-specific effect resulting in reduced IgM binding to mycobacteria in those patients with rheumatoid arthritis who are HLA-DR7, and reduced IgA binding to mycobacteria in those patients who are HLA-DR2. More recently, two groups (G. M. Bahr, personal communication; G. Tsoulfa, personal communication) have found preliminary evidence that patients with rheumatoid arthritis, have increased levels

of antibody against a 65-kDa protein from *M. tuberculosis* [59] (supplied by Dr. Jan van Embden) relative to matched controls. There is also evidence that this particular mycobacterial antigen (i. e., 65-kDa protein) is relevant at the cellular level. A strongly arthritogenic T cell clone has recently been established from rats with an adjuvant-induced arthritis. This clone recognises both *M. tuberculosis*, and antigens present in human cartilage and synovial fluid [58]. More recently it has been shown that the relevant mycobacterial antigen is the 65-kDa protein discussed above, and that the peptide which it recognises shows some sequence homology with the link protein from cartilage.

Studies with T cells from patients with rheumatoid arthritis are at present incomplete. It is not yet clear whether the increased proliferative response of lymphocytes from such patients to an acetone-precipitated fraction of *M. tuberculosis* is related to the disease, or to the presence of an excess of DR4 individuals in the patient group [21]. If it was related to the disease itself, it will be important to know whether the observation was due to the presence of the 65-kDa protein in this preparation.

Finally, arthritic symptoms have been described in patients who were treated with *M. bovis* BCG immunotherapy for cancer [57]. These patients frequently experience early morning stiffness, finger and wrist swelling with signs of inflammation, and show a response to non-steroidal anti-inflammatory drugs. It is also interesting to note that the introduction of gold therapy for the treatment of rheumatoid arthritis followed the observation that the chronic disease process of tuberculosis resembled the chronic synovitis found in rheumatoid arthritis [14].

Differences in pathology between rheumatoid arthritis and tuberculosis (or Crohn's disease) secondary to the production in each of IgG with agalactosyl Fc may arise either through differences in the anatomical sites of production of such IgG, or through additional differences in Fab *N*-glycosylation. For example, the site of production of IgG with agalactosyl Fc (i. e., intra-articular or otherwise) would be expected to affect both the concentration-dependent self-association of such IgG (see earlier) as well as the clearance of the resulting complexes. Tentative schemes for the origin and consequences of increased IgG(0) in rheumatoid arthritis and tuberculosis are shown in Fig. 9.

Mycobacteria and Crohn's Disease

The presence of the agalactosyl IgG in Crohn's disease may also be a clue to its aetiology. For many years Crohn's disease was not considered to be distinct from intestinal tuberculosis, since the granulomas seen in Crohn's disease are similar in histology to tuberculoid granulomas. In 1932 Crohn, Oppenheimer, and Ginsberg defined "regional ileitis" as a distinct clinical and pathological entity but they did not rule out a role for mycobacteria in its pathogenesis [11]. It is now recognised that Crohn's disease can affect any part of the gastrointestinal tract. Food allergy, autoimmunity and infection due to an unknown transmissible agent, which could be an unusual type of mycobacterium, have been suggested as the cause. Homogenates of Crohn's disease tissue have been shown to induce unusual tissue reactions and granuloma formation in the guts of rabbits [6, 53] and in the

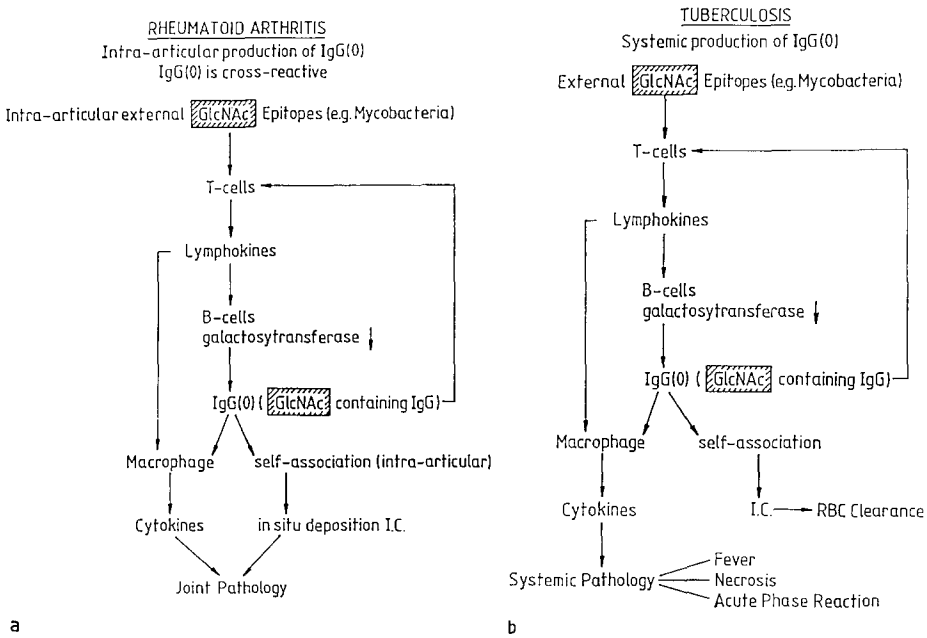

Fig. 9a, b. Tentative models for the production of IgG with agalactosyl Fc [IgG(0)] in response to external GlcNAc epitopes in rheumatoid arthritis (**a**) and tuberculosis (**b**). It is suggested that the decreased activity of β-galactosyltransferase in B lymphocytes is a response to lymphokines released by T lymphocytes. The anatomical site of production of IgG(0) may influence the subsequent pathological damage caused by IgG (0), and may account for differences between diseases that are associated with increased IgG (0). *IL-1:* Interleukin 1; *TNF:* tumour necrosis factor; *I.C.:* immune-complexes

footpads of mice [35, 56]. Slow growing cell-wall defective organisms have been isolated from mesenteric lymph nodes draining the lesions of 22 out of 27 patients with Crohn's disease, and only 1 of 11 controls [5]. However, the only evidence that these are unusual mycobacteria is their positive staining by the Ziehl-Neelsen technique. Recent research has confirmed the presence of such organisms in tissue from inflammatory bowel disease, but not from normal tissue; unfortunately these organisms could not be grown in sufficient quantity for further characterisation, although several groups have cultured an assortment of conventional mycobacterial species from Crohn's disease tissue [5, 9, 17]. However, various mycobacterial species are common in biopsies of normal bowel wall [17] and there is no justification for linking these with Crohn's disease. Present attention remains focussed on the cell wall defective forms, which have the added attraction of being potentially filterable like the granulomagenic entity which was passaged in mouse footpads [35].

Agalactosyl IgG, Mycobacteria, and Tumour Necrosis Factor (TNF) Release

Agalactosyl IgG may be a regulator of TNF release from activated macrophages (G. Rook, unpublished). While this may be a consequence of agalactosyl IgG bound to the macrophage Fc receptor also binding via its exposed non-reducing terminal GlcNAc residues, either to some macrophage GlcNAc-binding receptor or to the GlcNAc-binding CR3 receptor, it is possible that agalactosyl oligosaccharides confer a sufficient affinity for the carbohydrate-dependent interaction between IgG and the Fc receptor to cause direct activation of macrophages and subsequent TNF release [32]. High release of TNF could indeed account for several clinical features of disease states which are characterised by IgG molecules with exposed non-reducing terminal *N*-acetylglucosamine residues. It has been demonstrated that live virulent *M. tuberculosis* can substitute for endotoxin in triggering TNF release and much of the necrosis in tuberculosis can be attributed to endothelial cell damage and microcapillary thrombi [50]. TNF is readily detected in the synovial fluid of patients with rheumatoid arthritis [22]. Further, established synovitis in patients with rheumatoid arthritis is associated with the proliferation of small blood vessels. Recent data have demonstrated that in vivo TNF stimulates neovascularisation which is accompanied by a leukocyte infiltration (i. e., inflammation) [15]. Other well-established angiogenesis factors induce capillary vessel formation in the absence of an inflammatory response. It is interesting to note that chronic inflammatory diseases not associated with raised agalactosyl IgG levels (i. e., sarcoidosis, leprosy) are characterised by granulomas and massive macrophage activation, but with limited weight loss and fever, or necrosis, suggesting the absence of TNF release.

Conclusions

In conclusion, there is a shift in the population of IgG glycoforms towards those with a higher content of agalactosyl biantennary *N*-linked oligosaccharides in active rheumatoid arthritis (both juvenile and adult), tuberculosis, and Crohn's disease, but not in a variety of other rheumatological, inflammatory, or infectious conditions. This shift may contribute to disease pathogenesis both through immune-complex formation and through disturbance of a cellular network directed against the non-reducing terminal GlcNAc epitope. The precise pathology would in each case be modulated by the anatomical site(s) of production of such IgG, and also by the precise mechanism inducing this change in IgG glycosylation. Important amongst such mechanisms may be cross-reactivity between environmental and endogenous carbohydrate epitopes. It will be interesting to see if future research supports the idea that groups of diseases (e. g., rheumatoid arthritis, tuberculosis, Crohn's) are indeed related by a common aetiopathogenesis [i. e., G(0)].

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