

Alterations in carbohydrate composition of serum IgG from patients with rheumatoid arthritis and from pregnant women

J M PEKELHARING,¹ ELLEN HEPP,² J P KAMERLING,³ G J GERWIG,³ AND B LEIJNSE⁴

*From the*¹*Department of Clinical Chemistry, Stichting Samenwerking Delftse Ziekenhuizen, PO Box 5010, 2600 GA Delft, The Netherlands; the*²*Department of Biochemistry, Dr Daniel den Hoed Cancer Center, Rotterdam, The Netherlands; the*³*Department of Bio-Organic Chemistry, Utrecht University, Utrecht, The Netherlands; and the*⁴*Department of Chemical Pathology, Erasmus University, Rotterdam, The Netherlands*

SUMMARY The carbohydrate composition of IgG purified from serum of patients with rheumatoid arthritis (RA), pregnant women, and blood donors has been determined by gas-liquid chromatography. Comparison of the results indicates that IgG from patients with RA contains significantly less galactose but more *N*-acetylglucosamine than normal IgG, whereas the fucose and sialic acid contents are not changed. The carbohydrate content of IgG in RA is reduced. IgG in pregnancy contains more galactose and more sialic acid than normal IgG, whereas fucose, *N*-acetylglucosamine, and the total carbohydrate content are not changed. These data suggest a temporal compensation of the RA associated undergalactosylation of IgG in female patients with RA during pregnancy, a period during which remission of the disease is often observed.

Key words: glycosylation, pregnancy.

Immunoglobulin G is a major plasma glycoprotein containing, on average, 2.8 *N*-linked carbohydrate chains per molecule.¹ The carbohydrate chains consist of a variety of diantennary complex-type structures, some containing an extra 'intersecting' *N*-acetylglucosamine or a fucose residue.^{1,2}

The plasma of patients with rheumatoid arthritis (RA) contains aggregated immunoglobulins.² These so-called 'immune complexes' differ from antigen-antibody complexes in several ways. Because of the absence of antigen it is suggested that the aggregates consist of certain immunoglobulins with affinity for other immunoglobulins (autoantibodies). The strength of the interaction between the immunoglobulins ($K_a=10^5$ l/mol) is less than that observed for normal antigen-antibody interactions, indicating that a different type of interaction may be involved.³

The recent finding of reduced galactosylation of

the diantennary carbohydrate chains of total IgG in patients with RA has led to a new hypothesis concerning the aggregation of IgG molecules.⁴ Less sugar constituents in the *N*-linked glycans of IgG could make IgG more 'sticky' owing to a lectin-like activity.

If incomplete glycosylation of IgG causes the formation of harmful IgG aggregates, then conditions inducing an increased plasma glycoprotein glycosylation, such as pregnancy,⁵ could reverse this aggregation. A decrease in C1q binding activity has indeed been found in patients with RA during pregnancy,⁶ and a remission of RA during pregnancy is commonly observed,^{7,8} indicating that the finding of an increased glycosylation of IgG during pregnancy would support the suggestion of a key role for the glycosylation of IgG in the pathogenesis of immune aggregates in rheumatoid arthritis.

The carbohydrate composition of IgG samples from the serum of pregnant women, patients with RA, and healthy blood donors was compared by detailed quantitative monosaccharide analysis with gas-liquid chromatography.

Accepted for publication 31 July 1987.

Correspondence to Dr J M Pekelharing, Department of Clinical Chemistry, Stichting Samenwerking Delftse Ziekenhuizen, PO Box 5010, 2600 GA Delft, The Netherlands.

Materials and methods

ISOLATION OF IgG

Blood samples were obtained from pregnant women, healthy blood donors, and from patients with active RA visiting the rheumatology polyclinics. Serum was extracted with an equal volume of n-hexane to remove lipids, and dialysed for 24 hours against 0.0175 M phosphate buffer, pH 6.3 (buffer A). After filtration, 2 ml serum samples were applied to a column (35×2 cm) of diethylaminoethylcellulose (DEAE) (Pharmacia, Uppsala, Sweden) equilibrated with buffer A. IgG, the unbound fraction, was eluted with buffer A. The bound fraction was eluted with 0.4 M phosphate buffer, pH 5.2, containing 2 M NaCl, and discarded. The column was regenerated by washing with 0.25 M phosphate buffer, pH 6.3, followed by buffer A.

Table 1 *Monosaccharide composition per three mannoses and percentage sugar of IgG from 10 blood donors, nine patients with RA, and six pregnant women*

No	Man	GlcNAc	Gal	NeuAc	Fuc	Sugar (%)
<i>Blood donors</i>						
1	3	3.09	0.79	0.20	0.70	2.9
2	3	3.08	1.03	0.24	0.73	2.1
3	3	2.94	0.74	0.14	0.72	2.1
4	3	3.26	0.83	0.21	0.76	2.2
5	3	3.29	0.87	0.16	0.71	2.4
6	3	3.13	0.75	0.13	0.69	2.2
7	3	2.93	0.82	0.22	0.67	2.5
8	3	3.30	0.93	0.20	0.77	3.0
9	3	2.91	0.71	0.18	0.66	2.3
10	3	3.01	0.88	0.14	0.63	1.9
Mean N	3	3.09	0.84	0.18	0.70	2.36
<i>Patients with RA</i>						
1	3	3.50	0.61	0.14	0.73	2.2
2	3	3.30	0.59	0.20	0.74	2.2
3	3	3.49	0.59	0.14	0.74	2.4
4	3	3.24	0.57	0.12	0.71	2.0
5	3	3.30	0.58	0.14	0.72	2.1
6	3	3.19	0.62	0.11	0.68	1.8
7	3	3.23	0.92	0.28	0.63	1.9
8	3	3.24	0.69	0.13	0.78	2.0
9	3	3.32	0.88	0.21	0.68	1.9
Mean RA	3	3.31	0.67	0.16	0.71	2.06
<i>Pregnant women</i>						
1	3	3.23	0.95	0.26	0.84	2.1
2	3	3.18	0.98	0.21	0.64	3.5
3	3	3.15	0.77	0.23	0.71	2.1
4	3	3.16	0.96	0.22	0.65	2.1
5	3	3.14	1.10	0.24	0.70	2.3
6	3	3.17	0.94	0.23	0.70	2.3
Mean P	3	3.17	0.95	0.23	0.71	2.40

Man=Mannose; GlcNAc=N-acetylglucosamine; Gal=galactose; NeuAc=N-acetylneuraminic acid; Fuc=fucose.

PURITY CHECK

Immunoelectrophoresis of the bound and the unbound fractions was carried out using a rabbit antiserum against human plasma proteins as well as specific rabbit antisera against IgG, IgA and IgM.

SAMPLE PREPARATION

The isolated IgG was dialysed against distilled water for three days at 4°C. The samples were then freeze dried and stored over P₂O₅ for 24 hours.

MONOSACCHARIDE ANALYSIS

Carbohydrate samples were subjected to methanolysis (1.0 M methanolic HCl, 24 hours, 85°C) followed by gas-liquid chromatography of the trimethylsilylated (N-reacetylated) methyl glycosides on a capillary CPsil5 WCOT fused silica column (0.34 mm×25 m; Chrompack, Middelburg, The Netherlands).⁹

Results

Immunoelectrophoresis of the unbound fraction with rabbit antiserum against human IgG showed only one large precipitation arc in the IgG region. With antiserum against human plasma proteins no other proteins were detected, in particular no IgA and IgM were detected. The bound fraction was found to contain all other plasma proteins together with a small amount of IgG (slides not shown).

Table 2 *Mean monosaccharide content (per 3 mannoses) and sugar content (%), standard deviations and significance of the differences of IgG from healthy blood donors (n=10), patients with RA (n=9), and pregnant women (n=6)*

	Blood donors	Patients with RA	p Value
GlcNAc	3.09 (0.15)*	3.31 (0.11)	<0.01
Gal	0.84 (0.10)	0.67 (0.13)	<0.05
NeuAc	0.18 (0.04)	0.16 (0.06)	NS†
Fuc	0.70 (0.04)	0.71 (0.04)	NS
Sugar (%)	2.36 (0.35)	2.06 (0.19)	<0.05
	Blood donors	Pregnant women	
GlcNAc	3.09 (0.15)	3.17 (0.03)	NS
Gal	0.84 (0.10)	0.95 (0.11)	<0.05
NeuAc	0.18 (0.04)	0.23 (0.02)	<0.05
Fuc	0.70 (0.04)	0.71 (0.07)	NS
Sugar (%)	2.36 (0.35)	2.40 (0.55)	NS
	Pregnant women	Patients with RA	
GlcNAc	3.17 (0.03)	3.31 (0.11)	<0.01
Gal	0.95 (0.11)	0.67 (0.13)	<0.01
NeuAc	0.23 (0.02)	0.16 (0.06)	<0.05
Fuc	0.71 (0.07)	0.71 (0.04)	NS
Sugar (%)	2.40 (0.55)	2.06 (0.19)	NS

*Values are mean (SD).

†NS=not significant, p>0.05; Mann-Whitney test.

The sugar analysis data of IgG obtained from 10 blood donors, nine patients with RA, and six pregnant women are given in Table 1.

In Table 2 the significance of the differences of the mean monosaccharide and sugar contents is given, calculated with the Mann-Whitney test.

The IgG from patients with RA contained 20% less galactose than IgG from healthy subjects. The mean *N*-acetylglucosamine content was increased by 20%. The mean fucose and sialic acid contents were not different from normal. In pregnancy the mean galactose content was increased by 13% and the mean sialic acid content by 44%, whereas *N*-acetylglucosamine and fucose contents were not different from normal.

Discussion

The monosaccharide analysis of IgG from patients with RA shows a significantly lower amount of galactose as compared with IgG from healthy individuals, whereas the *N*-acetylglucosamine content is significantly higher (Table 1). Although the analysis of carbohydrate content does not give information about the glycan structure, it is reasonable to assume that the major difference between IgG from patients with RA and from normals is the reduced galactosylation of the diantennary glycans, with a concomitant increase in *N*-acetylglucosamine content. In an earlier study only the undergalactosylation was indicated.⁴ It was stated that there are no apparent changes in the levels of the β -*N*-acetylglucosaminyltransferase enzymes GnT I, GnT II, GnT III, and $\alpha(1\rightarrow6)$ -fucosyltransferase. As only diantennary structures were detected⁴ the observed increase of *N*-acetylglucosamine in this study has to be explained as a change in GnT III activity, responsible for the attachment of the intersecting *N*-acetylglucosamine. The presence of this sugar residue reduces the galactosylation of the mannose $\alpha(1\rightarrow3)$ arm by about 78%.¹⁰ Thus if the 20% increase is of *N*-acetylglucosamine residues of the intersecting type, this would result in a decrease of galactose residues of about 16%, comparable with the 20% that we found. Taken together, our results suggest a 20% increase in intersecting *N*-acetylglucosamine residues, resulting in undergalactosylation of IgG in patients with RA.

Using a solid phase lectin binding assay, others found that IgG from patients with RA bound significantly more to the immobilised plant lectins peanut agglutinin (PNA) and concanavalin A (con A) than IgG from normals.¹¹

This indicates either an increased content or an increased availability of terminal galactose groups (binding to the PNA lectin) and diantennary sugar

chains (binding to the con A lectin). As our results and those obtained by others⁴ indicate a decreased galactose content and suggest a decreased con A binding owing to the intersecting GlcNAc, a conformational change in the structure of IgG in patients with RA leading to increased lectin binding could explain these results. Indeed, an altered conformation of IgG in RA has recently been suggested.¹²

Our findings could support a viral aetiology of the disease. Viruses may alter the glycosylation of cell glycoproteins.^{13 14} If the IgG producing B cells are infected by a virus, for instance the RA associated Epstein-Barr virus,¹⁵ which has a preference for B lymphocytes,¹⁶ the result could be a modified glycosylation of IgG produced by the infected cells. This mechanism has been shown to occur in lymphoblastoid cells infected by influenza virus, where decreased glycoprotein sialylation takes place and is only partially compensated for by an increased cellular sialyltransferase.¹⁴ Another example is the polyoma virus transformation of baby hamster kidney cells, which induces a twofold increase in the activity of the enzyme GnT V that adds *N*-acetylglucosamine $\beta 1-6$ to the $\alpha 1-6$ linked mannose.¹⁷

If a reduced galactose content of IgG is a prerequisite for the appearance of (some of) the symptoms associated with RA, conditions of increased galactosylation could form a compensation and could be the basis of a therapeutic approach. Such a condition may exist for IgG during pregnancy or oestrogen therapy, two processes where an increased glycosylation of other plasma glycoproteins has been found.⁵

Our results show that a significant increase (13%) of the galactose content of IgG occurs during pregnancy. The galactose content of IgG in pregnancy is 42% higher than in the RA group. Thus a partial compensation during pregnancy of the IgG undergalactosylation in female patients with RA can be imagined. IgG from one female patient with RA during pregnancy was analysed (results not shown). It contained high levels of galactose as found in the pregnancy group, and also high levels of *N*-acetylglucosamine as found in the RA group. Owing to the slight variation in carbohydrate analysis within each group this result has to be confirmed by carbohydrate analysis of IgG obtained from a larger number of pregnant women with RA.

Together with an increase of IgG galactose during pregnancy an increase of sialic acid is also found. This suggests a more complete glycosylation of the diantennary glycans of IgG during pregnancy. The *N*-acetylglucosamine content of IgG during pregnancy is not different from normal.

Given the fact that the half life of IgG in the

circulation is approximately three weeks, the recurrence of symptoms in female patients with RA a few weeks after delivery can also be explained, because the more galactosylated and sialylated IgG produced during pregnancy will be gradually replaced by the RA associated undergalactosylated IgG with possible aggregating properties produced thereafter. Alternatively, the increased *N*-acetylglucosamine content of RA IgG could also have a role, but no decrease has been found to occur during pregnancy.

The changes in IgG glycosylation in pregnancy may be due to the altered hormonal state. Oestrogens^{5 18} and prolactin¹⁹ do influence glycoprotein glycosylation. The association between autoimmune diseases such as RA and steroid hormones has been well described.^{20 21} Oral contraceptives^{22 23} and the number of pregnancies²⁴ have an influence as well. It is tempting to suggest the glycosylation of IgG as a key mediator in the occurrence of symptoms in RA.

Studies with asialylated IgG prepared in vitro underline the relation between the IgG glycosylation and rheumatoid factors.^{25 26} It would be of interest, in view of our results mentioned above, to extend these experiments with asialo-agalacto IgG prepared in vitro. Other studies have shown that differences in glycosylation of one Fab arm exist between precipitating and non-precipitating antibodies.^{27 28} Thus the characteristics of IgG are delicately dependent on the glycosylation of the molecule.

The fact that remission of the symptoms often occurs in women with RA during pregnancy, together with our finding of an increase in IgG galactosylation in healthy pregnant women, supports the view⁴ that IgG glycosylation could have an important role in the disease. The carbohydrate analysis of IgG of female patients with RA during pregnancy and the investigation of aggregating characteristics of asialo-agalacto IgG prepared in vitro could clarify this point.

The support from Dr A J G Swaak, Dr J L Molenaar, Dr P Herbrink, and Mr G van der Knaap is gratefully acknowledged.

References

- Mizuochi T, Taniguchi T, Shimizu A, Kobata A. Structural and numerical variations of the carbohydrate moiety of immunoglobulin G. *J Immunol* 1982; **129**: 2016-20.
- Rademacher T W, Dwek R A. Structural, functional and conformational analysis of immunoglobulin G-derived asparagine-linked oligosaccharides. *Progress in Immunology* 1983; **5**: 95-112.
- Mannik M, Nardella F A. IgG rheumatoid factors and self-association of these antibodies. *Clin Rheum Dis* 1985; **11**: 551-72.
- Parekh R B, Dwek R A, Sutton B J, et al. Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. *Nature* 1985; **316**: 452-7.
- Raynes J. Variations in the relative proportions of microheterogeneous forms of plasma glycoproteins in pregnancy and disease. *Biomed Pharmacother* 1982; **36**: 77-86.
- Pope R M, Yoshinoya S, Rutstein J, Persellin R H. Effect of pregnancy on immune complex and rheumatoid factors in patients with rheumatoid arthritis. *Am J Med* 1983; **74**: 973-9.
- Ostensen M, Aune B, Husby G. Effect of pregnancy and hormonal changes on the activity of rheumatoid arthritis. *Scand J Rheumatol* 1983; **12**: 69-72.
- Persellin R H, Cerere F. The interaction of pregnancy and the rheumatoid diseases. *Clin Rheum Dis* 1981; **7**: 747-68.
- Kamerling J P, Vliegthart J F G. Gas-liquid chromatography and mass spectrometry of sialic acids. *Cell Biology Monographs* 1982; **10**: 95-125.
- Narasimhan S, Freed J C, Schachter H. Control of glycoprotein synthesis. Bovine milk UDPgalactose: *N*-acetylglucosamine β -4-galactosyltransferase catalyzes the preferential transfer of galactose to the GlcNAc β 1.2 Man α 1.3-branch of both bisected and nonbisected complex biantennary asparagine-linked oligosaccharides. *Biochemistry* 1985; **24**: 1694-700.
- Malaise M G, Franchimont P, Bouillene C, Houssier C, Mahien P R. Increased concanavalin A-binding capacity of immunoglobulin G purified from sera of patients with rheumatoid arthritis. *Clin Exp Immunol* 1987; **68**: 543-51.
- Hanson U B, Ohlin M, Lindstrom F D. IgG with a deviant conformation in serum and synovial fluid from rheumatoid arthritis patients. *Scand J Immunol* 1985; **22**: 27-32.
- Groen T P. Cell surface carbohydrate changes in rat kidney cell lines transformed by adenovirus type 5. Utrecht, Netherlands: Utrecht University, 1984. (Thesis.)
- Duc Dodon M, Cecchelli R, Cacan R, Gazzolo L, Verbert A. Viral neuraminidase and cellular ectosialyltransferase in human lymphoblastoid cells infected with influenza virus. *Biochimie* 1984; **66**: 493-6.
- Yao Q Y, Rickinson A B, Gaston J S H, Epstein M A. Disturbance of the Epstein-Barr virus-host balance in rheumatoid arthritis patients: a quantitative study. *Clin Exp Immunol* 1986; **64**: 302-10.
- Frade R, Barel M, Ehlin-Henriksson B, Klein G. gp 140, the C3d receptor of human B lymphocytes, is also the Epstein-Barr virus receptor. *Proc Natl Acad Sci USA* 1985; **82**: 1490-3.
- Yamashita K, Tachibana Y, Kobata A. Enzymatic basis for the structural changes of asparagine-linked sugar chains of membrane glycoproteins of baby hamster kidney cells induced by polyoma transformation. *J Biol Chem* 1985; **260**: 3963-9.
- Starr C M, Lucas J J. Regulation of dolichyl phosphate-mediated protein glycosylation: estrogen effects on glycosyl transfers in oviduct membranes. *Arch Biochem Biophys* 1985; **237**: 261-70.
- Bradshaw J P, Hatton J, White D A. The hormonal control of protein N-glycosylation in the developing rabbit mammary gland and its effect upon transferrin synthesis and secretion. *Biochim Biophys Acta* 1985; **847**: 344-51.
- Ahmed S A, Penhale W J, Talal N. Sex hormones, immune responses, and autoimmune diseases. Mechanism of sex hormone action. *Am J Pathol* 1985; **121**: 531-51.
- Masi A T, Josipovic D B, Jefferson W E. Low adrenal androgenic-anabolic steroids in women with rheumatoid arthritis: gas-liquid chromatographic studies of RA patients and matched normal control women indicating decreased 11-deoxy-17 ketosteroid excretion. *Semin Arthritis Rheum* 1984; **14**: 1-23.
- Vandenbroucke J P, Valkenburg H A, Boersma J W, et al. Oral contraceptives and rheumatoid arthritis: further evidence for a preventive effect. *Lancet* 1982; **ii**: 839-42.
- Vandenbroucke J P, Witteman J C M, Valkenburg H A, et al. Non-contraceptive hormones and rheumatoid arthritis in perimenopausal and postmenopausal women. *JAMA* 1986; **255**: 1299-303.
- Silman A J. Is pregnancy a risk factor in the causation of rheumatoid arthritis? *Ann Rheum Dis* 1986; **45**: 1031-4.

- 25 Duc Dodon M, Quash G A. The antigenicity of asialylated IgG: its relationship to rheumatoid factor. *Immunology* 1981; **42**: 401-8.
- 26 Galloway G, Leung A Y T, Hunneyball I M, Stanworth D R. The successful use of asialylated IgG as an immunogen and arthritogen in the rabbit. *Immunology* 1983; **49**: 511-8.
- 27 Leoni J, Labeta M, Margni R A. The asymmetric IgG non-precipitating antibody, localisation of the oligosaccharide involved, by concanavalin A interaction. *Mol Immunol* 1986; **23**: 1397-400.
- 28 Labeta M O, Margni R A, Leoni J, Binaghi R A. Structure of asymmetric non-precipitating antibody: presence of a carbohydrate residue in only one Fab region of the molecule. *Immunology* 1986; **57**: 311-7.