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Structural changes of immunoglobulin G oligosaccharides with age in healthy human serum

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Age-related changes of IgG N-linked oligosaccharides isolated from normal human serum are reported for 403 individuals (male 227 and female 176), varying in age from 0 to 85 years. The IgG N-linked oligosaccharides were released from the protein by digestion with a glycoamidase and reductively aminated with the fluorescent reagent, 2-aminopyridine. The mixture of pyridylaminated oligosaccharides was separated at high resolution by HPLC using a reverse-phase column. From the results of neutral oligosaccharide analysis, agalactosyl glycoform and bisecting GlcNAc-containing glycoform were shown to increase with increasing age. Spearman's correlation coefficients were 0.503 and 0.473, respectively. Thus, in healthy people, an increase of both types of glycoforms correlates weakly with age. In addition, differences were demonstrated between male and female groups in their twenties. The quantity of agalactosyl glycoform was found to be lower in females than in males. No significant differences, however, were observed in the quantity of bisecting GlcNAc-containing glycoforms between males and females.

***Keywords*: age-related change of IgG oligosaccharides, healthy human IgG oligosaccharides**

***Abbreviations*: Gal, D-galactose: GlcNAc, *N*-acetyl-D-glucosamine; Man, D-mannose; Fc, C-terminal half of the heavy chain dimers of IgG; HPLC, high-performance liquid chromatography; IgG, immunoglobulin G; ODS, octadecylsilyl; PA, pyridylamino**

Introduction

Human immunoglobulin G (IgG) contains one asparagine-linked oligosaccharide in each C-terminal half of the heavy chain dimers of the IgG (Fc) region. Takahashi *et al.* reported detailed glycoform profiles of IgG by use of high-performance liquid chromatography (HPLC) and elucidated each structure of 16 neutral [1] and seven sialyl oligosaccharides [2]. It is well established that the IgG oligosaccharide profile in human serum is changed in various diseases. For example, in patients with rheumatoid arthritis, galactose residues of IgG molecules are decreased [3], and similar phenomena have been observed in patients with myotonic dystrophy [4]. By contrast, the digalactosyl IgG glycoform was markedly increased in pregnant women [8]. For such research, information on IgG oligosaccharides of healthy controls is indispensable. The age-related decrease of galactosylated oligosaccharides in 151 healthy individuals has been reported previously [5]; and using *N*-acetylglucosamine-specific lectin binding, normal IgGs from 112 healthy individuals have been classified by age and sex [6]. However, more precise and wider age-matched controls are now required since not only simple galactosylation changes but also more precise changes of other glycoforms in various diseases have been clarified. In this paper, we report age-related IgG oligosaccharide profiles for 403 healthy persons. Changes of bisecting GlcNAc-containing oligosaccharides and differences between sexes are reported for the first time.

Materials and methods

Materials

Serum was obtained from 403 individuals (male 227 and female 176) ranging in age from 0 to 85 years. The donors were all confirmed to be in good health at a medical check-up. The serum samples have been stored at $-80\,^{\circ}C$. Protein G affinity column and Sephadex G-15 were obtained from Pharmacia Biotech AB (Uppsala, Sweden). Glycoamidase A (Glycopeptidase A; EC 3.5.1.52) from almond was purchased from Seikagaku Kogyo (Tokyo, Japan), 2-aminopyridine from Wako Pure Chemicals (Osaka, Japan), and Sodium cyanoborohydride from Aldrich Chemicals (Milwaukee, WI, USA).

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Methods

Preparation and derivatization of IgG N-linked oligosaccharides

Each IgG sample of about 500 μg was purified from each 50 μl of serum using protein G affinity column as described previously [6]. Oligosaccharide analyses of IgG were performed as previously described [1]. Briefly the oligosaccharide moieties were released from the IgG glycopeptide fraction by glycoamidase A digestion. The reducing ends of the oligosaccharides were reductively aminated with 2-aminopyridine by use of sodium cyanoborohydride [7]. The Pyridylamino (PA)-oligosaccharides were purified by gel filtration on a Sephadex G-15 column.

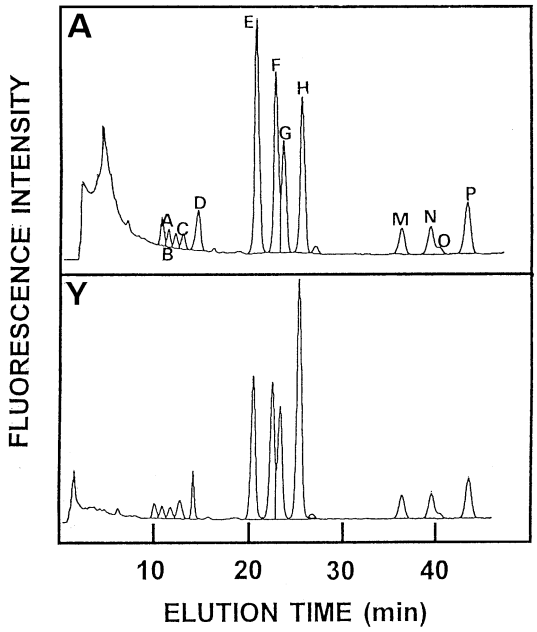
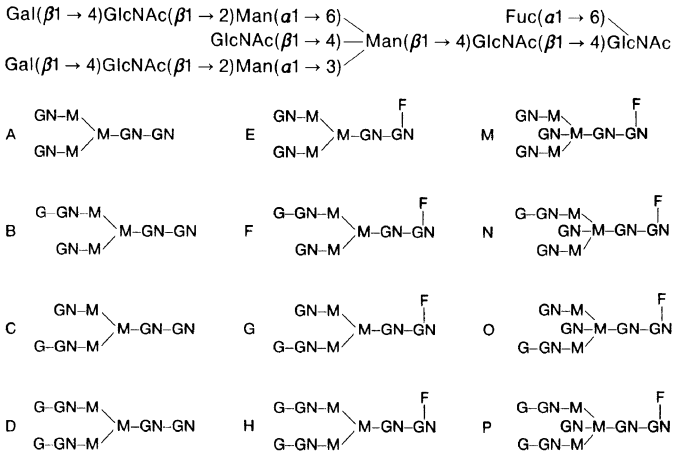


Figure 1. Comparison of HPLC profiles of PA-oligosaccharides of IgG purified from an aged male (81 years old) (A) and a young male (29 years old) (Y). Structures of oligosaccharides are as follows. Symbols: G, galactose; M, mannose; F, fucose; GN, *N*-acetylglucosamine. Peak P, for example, represents:



HPLC profiles of PA-oligosaccharides

After desialylation by sialidase digestion, the PA-derivatized neutral oligosaccharide mixture thus obtained was analysed by HPLC on a Shim-pack CLC ODS (octadecylsilyl) column (6 mm × 150 mm, Shimadzu, Kyoto) with fluorescence detection. The structure of each peak separated on the column was identified as described previously [1] (see the legend to Figure 1). Each neutral oligosaccharide was characterized as being devoid of terminal galactose residues, G0 (peaks A, E, and M), one terminal galactose residue, G1 (B, C, F, G, N and O) or having two terminal galactose residues, G2 (D, H and P) (Figure 1). The area of each peak reflects the mole number (relative quantity, %) of the oligosaccharides (M + N + O + P).

G0% represents % of agalactosyl oligosaccharides (A + E + M). M–P% represents percentage of bisecting GlcNAc-containing oligosaccharides.

Statistics

r_s = Spearman's correlation coefficient,
p = level of significance.

Results and discussion

Characteristic galactosylation profiles of IgG neutral oligosaccharides related to age

Typical age-related oligosaccharide profiles of healthy males' (81 and 29 years old) IgG are shown using HPLC (Figure 1). In an aged person (A), peak E (agalactosyl, G0) is predominant. By contrast, in a young person (Y), a predominant peak H (digalactosyl, G2) and lower content of G0 and G1 peaks are characteristic. Figure 2a shows the correlation between the increase of G0% and ages (0–85 years) for 227 males and 176 females. Spearman's correlation coefficient (r_s) is 0.503. Figure 2b shows a comparison between males and females of the change of G0% with age. The values for Spearman's correlation coefficient in males and females are 0.327, and 0.666, respectively. This means that the value of G0% correlates with age better in females than in males. The age-related changes of G0% reported here (Figure 2a) are similar but much less apparent than those reported by Parekh *et al.* [5], in which an age-related parabolic change in G0 glycoform was shown. However, we have found significant differences between the sexes (Figures 2bM and 2bF). In Table 1, G0% in serum IgG from normal individuals was classified by age and sex. The difference of G0% values between sexes is large up to 30 years of age. The difference, however, is not clear over 30 years of age. Although the number of samples in the 20–29 category was sufficient, the 0–19 category lacked sufficient numbers for statistical significance. Tsuchiya *et al.* reported that G0 values for males were slightly higher than those for females in all ages (20–70 years) [6]. To determine G0%, Tsuchiya *et al.* used the indirect method of *N*-acetyl

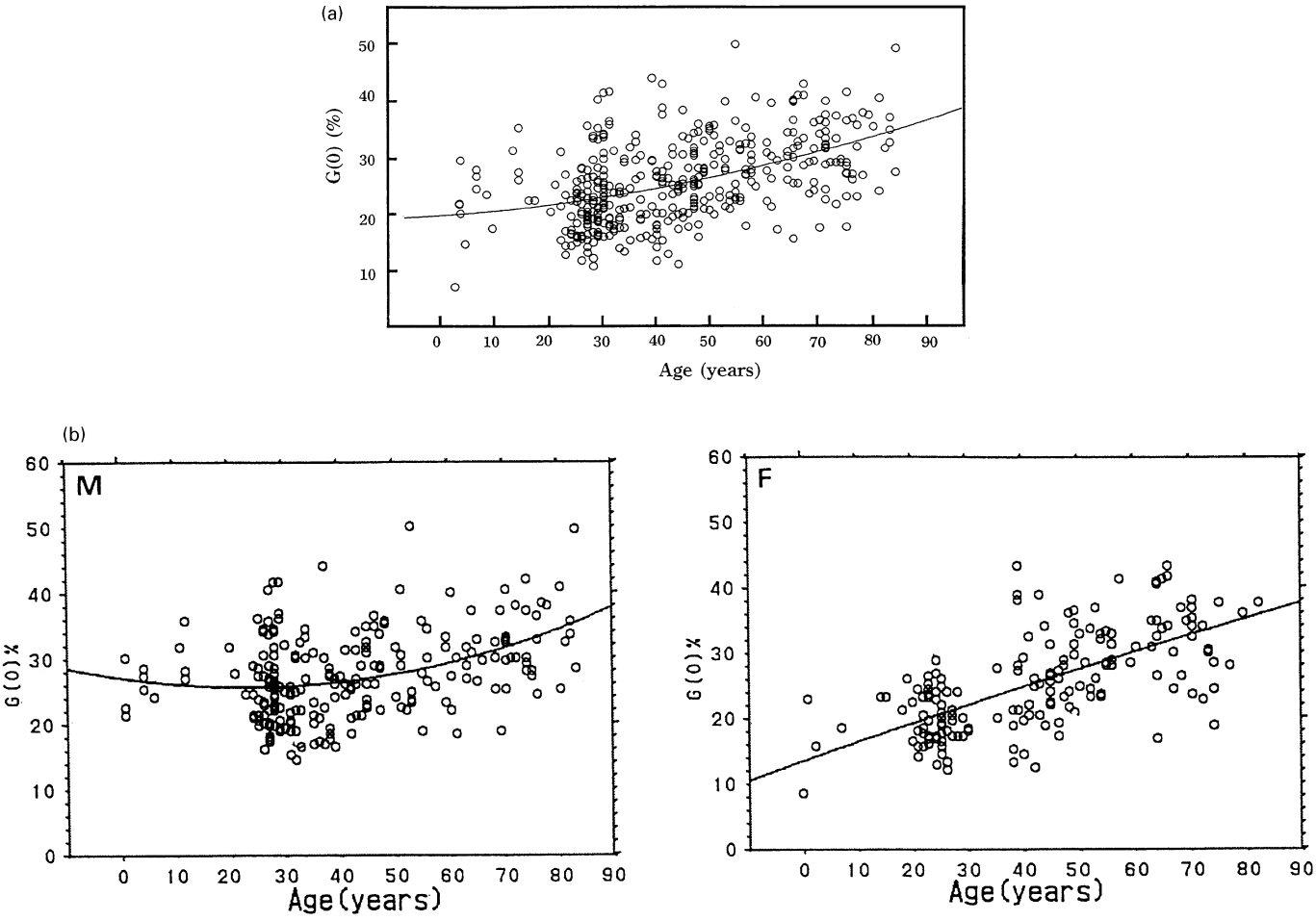


Figure 2. (a) Relation of the percentage of agalactosyl glycoforms (G0%) to age for IgG from 403 normal subjects. The solid curve is given by the equation $y=20.6+7\times10^{-2}x+1.3\times10^{-3}x^2$, and $r_s=0.503$ (b) Correlation of G0% and age for IgG from 227 males (M) and for 176 females (F). The solid curve is given by the equation $y=26.8-1.3\times10^{-1}x+2.7\times10^{-3}x^2$, and $r_s=0.327$, for males; and $y=13.2+3.0\times10^{-1}x+3\times10^{-4}x^2$, and $r_s=0.666$, for females.

Table 1 Values of G(0)% in serum IgG from normal individuals classified by age and sex[a]

Age	*0–19*	*20–29*	*30–39*	*40–49*	*50–59*	*60–69*	*70–85*
Male	27.1 ± 1.3 (11)[b]	26.0 ± 0.8 (66)	23.8 ± 0.9 (48)	28.0 ± 0.9 (34)	28.5 ± 1.5 (22)	29.0 ± 1.4 (18)	33.0 ± 1.1 (28)
Female	19.7 ± 2.0 (8)	19.5 ± 0.5 (57)	24.1 ± 2.4 (15)	25.4 ± 1.0 (37)	29.5 ± 1.0 (22)	33.6 ± 1.5 (20)	30.9 ± 1.4 (17)

[a] Values are mean ± SEM (Standard Error of Mean).
[b] Number of individuals.

glucosamine-specific lectin binding to IgG. By contrast, our data indicate directly the value of G0% in IgG on the basis of the peak areas in the HPLC profiles.

Characteristic profiles of bisecting GlcNAc-containing oligosaccharides related to age

Figure 1 shows characteristic lower content of peaks M–P (bisecting GlcNAc-containing oligosaccharides) in a young person and higher content of peaks M–P in an aged person. Figure 3 shows the correlation between percentage of M–P and age. Spearman's correlation coefficient (r_s) is 0.473, showing a weak correlation between the two values. The increasing bisecting GlcNAc-containing glycoform in IgG molecules may mean that catabolism of IgG molecules having bisecting GlcNAc-containing oligosaccharides becomes less efficient with age. By contrast, among G1 oligosaccharides, the ratio of peaks F and G does not change with age (data not shown).

Different profiles of IgG oligosaccharides between male and female groups in their twenties

Typical sex-related IgG oligosaccharide profiles of a healthy male (27 years old) and a female (24 years old) are shown using HPLC (Figure 4). The predominant peak H (digalactosyl, G2) in a female contrasted to the relatively lower content of peak H in a male is characteristic. Figure 5a shows that there are significant differences between 66 males and 57 females ($p < 0.001$) for G0%. No significant differences were observed between sexes for M–P%, although a weak correlation was found between M–P% and age (Figure 3). Both peaks F and G have only one terminal galactose residue, but the linking position of the galactose to *N*-acetyl glucosamine is different (see the legend for Figure 1). Since the IgG galactosylation profile is a little different between males and females, we supposed that the ratio of peaks F and G might be different. Figure 5c shows, however, no significant difference in the G/F ratio between sexes.

We previously reported that an extremely high level of galactosylation was observed in the period of pregnancy and among fetus/newborns [8]. The fact that a female has her highest level of galactosylation in her twenties (Table 1) may be associated with preparation for possible pregnancy.

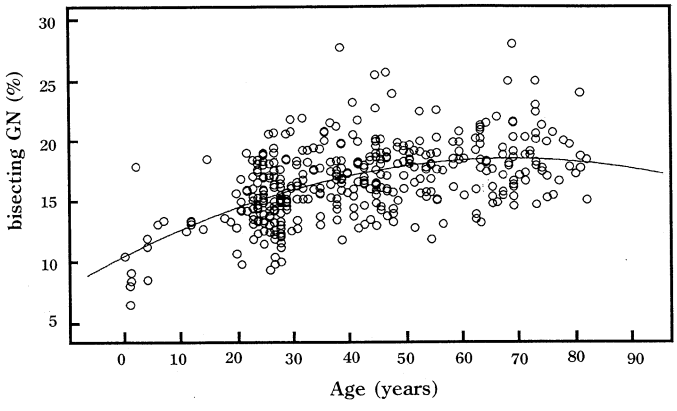


Figure 3. Correlation of M–P% and age for IgG from 403 normal subjects. The solid curve is given by the equation $y = 0.12 + 3 \times 10^{-3}x - 2.2 \times 10^{-5}x^2$, and $r_s = 0.473$.

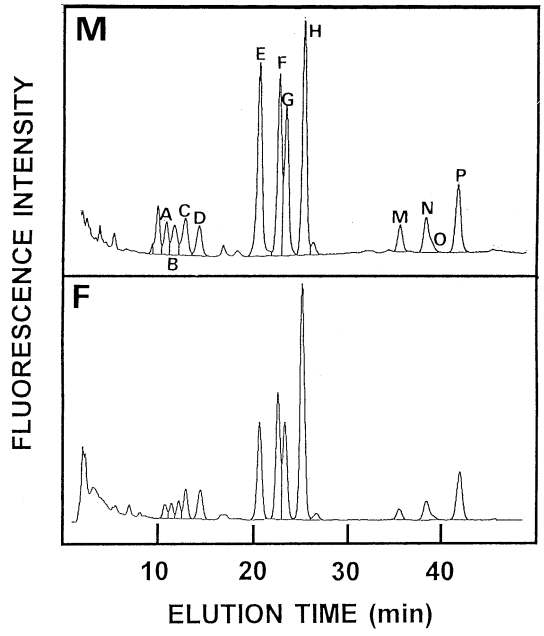


Figure 4. Comparison of HPLC profiles of PA-oligosaccharides of IgG between a young male (27 years old) (M) and female (24 years old) (F). Structures of oligosaccharides are the same as those of Figure 1.

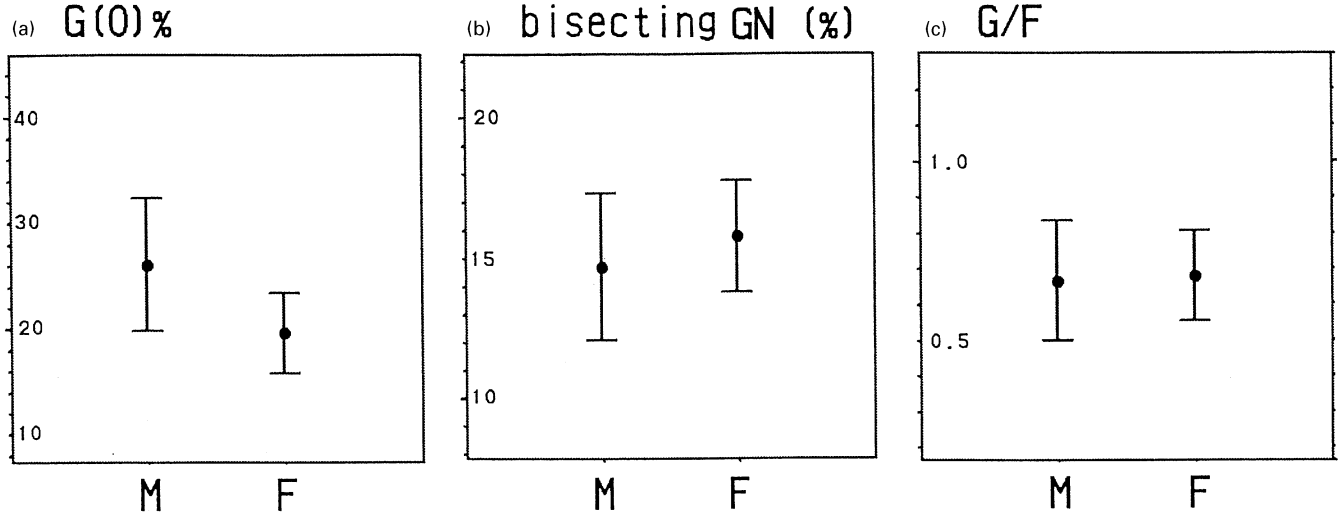


Figure 5. (a) Relation of G0% in twenties to sexes for IgG from 66 males and 57 females. (b) Relation of bisecting GlcNAc-containing oligosaccharide % in twenties to sexes. (c) Relation of the ratio of peak area G/F to sexes. M, 66 males; and F, 57 females.

In conclusion, in healthy individuals, the mean value of both G0% and bisecting GlcNAc% of IgG oligosaccharides increases generally related to age. Although each person has an individual oligosaccharide profile, the mean values reported here should provide valuable age-matched controls for various diseases.

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