The Carbohydrate Content of IgG from Patients with Cystic Fibrosis

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Summary

We compare the complete carbohydrate composition of IgG purified from the serum of patients with cystic fibrosis (CF) with that of control subjects. Our results indicate that IgG from cystic fibrosis patients is underglycosylated with respect to galactose and sialic acid, the two terminal sugars on the antennae of the complex-type oligosaccharide chains found on IgG. The galactose content, as determined by gas liquid chromatography, was 2.81 ± 0.86 (S.D.) mole/mole of IgG for normal subjects versus 1.5 ± 0.39 for CF subjects ($P < 0.025$). Sialic acid content, as determined by the Warren procedure, was 1.33 ± 0.39 for normals versus 0.47 ± 0.10 for CF subjects ($P < 0.001$). Neither galactose nor sialic acid values for the two groups overlapped. The contents of the core sugars, mannose and glucosamine, and of fucose were not significantly different. When the data are expressed as residues per 3 moles mannose, similar results are obtained. We suggest that immune complex formation, which has been documented in many CF patients, exposes sugar chains of IgG molecules to hydrolytic activity of serum glucosidases, resulting in partial removal of the more peripheral sugars. Because serum glycoproteins missing sialic acid and galactose are not readily cleared from the circulation, the observed changes may contribute to elevated levels of IgG and immune complexes in older people with CF.

Abbreviations

CF, cystic fibrosis
SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis

Several reports suggest that glycoproteins with *N*-glycosidically linked oligosaccharides from CF patients have altered carbohydrate content (1-3, 5, 6, 16, 22, 32). Pursuing the suggestion of Pearson and Lubin (30) that fucose, sialic acid, and hexose content of heparin-precipitated glycoproteins from serum of patients with CF are altered, we noted that IgG is the major serum component precipitated (24). In light of this finding, we have purified IgG from serum samples taken from healthy individuals, from patients with non-CF related pulmonary disease, and from CF patients and compared their carbohydrate contents.

Previous studies of the carbohydrate content of human IgGs have revealed considerable heterogeneity (11). This is particularly true when monoclonal antibodies are compared (19). IgG heavy chain has one or two *N*-linked oligosaccharides of the complex type, which contain fucose, galactose, and sialic acid as terminal sugars in addition to the mannose and *N*-acetylglucosamine found in the core region. The light chain is most likely unglycosylated; however, oligosaccharide structures have been elucidated in the light chains of myeloma IgG (9). Our results, in general, reflect the previously published compositional data for IgG in serum and show that IgGs purified from the serum of patients with CF are underglycosylated with respect to galactose and sialic

acid. The core sugars and fucose show no significant differences. The implications of these results are discussed.

MATERIALS AND METHODS

Specimens. Nonfasting venous blood samples were allowed to clot at 4°C. After centrifugation, the serum was removed, aliquoted into plastic tubes (Falcon 2054), and stored for varying periods at -70°C. Samples were obtained with informed consent and with approval from the Human Experimentation Committee of University Hospitals of Cleveland.

Subjects. A diagnosis of cystic fibrosis was made by clinical criteria and elevated sweat chloride concentrations. Control subjects consisted of two normal, healthy volunteers and three patients with non-CF related chronic pulmonary diseases (Table 1). Clinical scores were assigned to CF subjects according to the criteria of Doershuk *et al.* (13).

Isolation of serum IgG. Serum was dialyzed against an equal volume of 3.5 M ammonium sulfate overnight, then against an excess of 1.75 M ammonium sulfate. The precipitate was collected by centrifugation. The pellet was washed three times with 1.75 M ammonium sulfate, resuspended in phosphate buffered saline and dialyzed extensively against 0.02 M potassium phosphate buffer, pH 8. The retentate was then applied to a column of DEAE Affi-Gel Blue (BioRad, 14-ml column volume) and eluted with starting buffer. Protein was monitored by absorption at 280 nm and the first eight tubes containing protein were pooled, lyophilized, and dialyzed against distilled water. The samples were rehydrated, and then brought to their original volume with distilled water.

Alternatively, serum IgG was isolated using immobilized Staph Protein A. The Staph Protein A, covalently coupled to Sepharose 4B (Pharmacia), was equilibrated in 0.1 M phosphate buffer, pH 7.0 at 4°C. Columns containing 1 ml of settled gel were poured and washed extensively with phosphate buffer. Serum samples were centrifuged at 3000 rpm for 10 min to remove particulate matter and a 0.5-ml aliquot applied to each column. The columns were then washed with 30 ml of phosphate buffer, at which point the eluant no longer absorbed at 280 nm. The IgG fraction was then eluted with 6 ml of 1 M acetic acid. Both fractions were immediately dialyzed against glass distilled water and lyophilized. The fractions were resuspended in a volume of phosphate buffer equivalent to that of the original serum. All procedures were carried out at 4°C.

SDS-PAGE. SDS, 10% polyacrylamide slab gels were prepared according to the method of Laemmli and Favre (20). Samples in 5% 2-mercaptoethanol and 0.5% SDS were electrophoresed for approximately 3 h at 4°C and at a current of 35 mA per gel. Proteins were visualized using Coomassie Blue R-250 stain.

Immunoanalytical methods. IgG and transferrin were quantitated by radial immunodiffusion (Kallestad). Double immunodiffusion analysis was carried out by the method of Ouchterlony and Nilson (28). Antisera to IgM, IgG, and IgA were obtained from Meloy. Antisera to C3 and transferrin were obtained from Kallestad.

Table 1. Profile of serum donors

Donor	Age	Disease state or clinical score	Serum IgG (mg/ml)
Control population			
1N	19	Normal	8.65
7N	25	Normal	10.11
163	5	Recurrent pneumonia	8.20
174	5	Persistent pneumonia and asthma	8.20
211	13	Allergic	9.10
Cystic fibrosis			
930	18	66—moderately severe	16.5
964	19	81—healthy	16.2
971	17	58—moderately severe	8.2
978	16	85—healthy	11.7
987	22	88—healthy	10.2

Carbohydrate analysis. Sialic acid was measured by the method of Warren as modified by Sarris and Palade (31). Bound sialic acid was hydrolyzed by mild acid treatment (0.05 M H₂SO₄ at 80°C for 1.5 h). This assay is linear in the range of 2–25 nmoles.

Fucose was hydrolyzed using 0.125 M H₂SO₄ at 100°C for 1 h followed by immediate neutralization with 0.1 N NaOH. Fucose was quantitated using the fucose dehydrogenase method of Finch *et al.* (14). The formation of NADH was followed using an Aminco SPF-125 Spectrofluorometer (excitation wavelength, 340 nm; emission wavelength, 460 nm). This assay was linear over the range 1–20 nmole/500 μ l sample volume.

Galactose and mannose were analyzed as trimethylsilylated derivatives of methylglycosides by gas-liquid chromatography on a Varian Model 3700 (flame ionization detector) with inositol as internal standard. Methanolysis was carried out in 1.5 N methanolic HCl at 85°C for 14 h. Separations were performed on a stainless steel column (1/4 in. x 6 ft) packed with 3% OV 17. The column temperature was programmed from 110–250°C at a rate of 4°C/min. Hexosamines were determined on a Beckman model 119 CL amino acid analyzer by the method of Cheng and Boat (10).

RESULTS

Purification of IgG. The recoveries of IgG purified by ammonium sulfate precipitation and DEAE Affi-Gel Blue ranged from 40–83% with an average of 61.8%. By SDS-PAGE analysis of 20 μ g of protein, two major bands corresponding to heavy and light chains of IgG and three to four faint bands were seen in each lane (Fig. 1). Double immunodiffusion analysis revealed a major IgG precipitation line and faint precipitation bands with antisera to IgA and transferrin, but none with anti-IgM and anti-C3. Transferrin is the most common contaminant of IgG prepared by this procedure; however, none of our IgG samples had a concentration of transferrin above the level of the sensitivity of the radial immunodiffusion assay, 0.06 mg transferrin/ml, which would represent a maximum of 1.7% contamination.

A representative sample of IgG purified on immobilized Staph Protein A had a recovery of 81% and contained 3% IgA and 5% IgM by weight. Only one spurious band was seen on SDS-PAGE when each lane contained 10 μ g protein; this band corresponded in molecular weight to either IgM heavy chain or albumin.

Carbohydrate content. The carbohydrate content of serum IgG purified by Staph Protein A was determined by gas-liquid chromatography of trimethylsilylated derivatives. The only significant difference between five control samples and five CF samples was a decrease in the galactose content of IgG from CF serum samples, 1.79 ± 0.40 (S.D.) moles of galactose/3 moles of mannose for normal subjects *versus* 1.20 ± 0.21 (S.D.) moles of

galactose/3 moles of mannose for CF subjects. The sialic acid content of CF IgG was lower, *i.e.*, 0.48 ± 0.19 mole/3 moles of mannose *versus* 0.60 ± 0.26 mole/3 moles mannose for normal subjects, although this difference did not achieve statistical significance. The fucose, glucosamine, and mannose contents were nearly identical for both groups.

The carbohydrate content of IgG purified by a second method, *i.e.*, ammonium sulfate precipitation and DEAE Affi-Gel Blue chromatography, is reported in detail. Data are expressed as mole sugar per mole IgG to reflect the total carbohydrate content of the immunoglobulin (Table 2) and as mole sugar/3 moles mannose to reflect the content of the individual oligosaccharide chains (Table 3). Both sialic acid and galactose contents are significantly decreased in CF IgG, with no overlap. The fucose content of these IgGs is not significantly different, whether determined by gas-liquid chromatography (Table 3) or by fucose dehydrogenase (1.15 ± 0.52 for the normal group compared with 0.96 ± 0.08 for the CF group, expressed as residues per 3 mannose residues).

When mean values in Table 2 are examined, it appears as if CF IgG may be generally underglycosylated. Fucose, glucosamine, and mannose fall within the range of normal values for IgG, with the exception of values from CF donor 930. The explanation for the deviation of carbohydrate content of IgG from this individual is unknown at this time.

DISCUSSION

The most striking finding of this study is that the content of the two sugars that have terminal positions on the antennae of complex-type oligosaccharide chains of IgG, namely galactose and sialic acid, is decreased in CF IgG. The two core sugars, *N*-acetylglucosamine and mannose, and fucose are present in the usual, or perhaps slightly diminished, amounts. These findings raise three questions.

1. How do the present data compare with those of others, both with respect to what is known about the carbohy-

Fig. 1. SDS-PAGE analysis of IgG purified by ammonium sulfate precipitation of DEAE Affi-Gel Blue. Lane marked with an arrow, standard molecular weight markers, from top to bottom: 94,000, 67,000, 43,000, 30,000, 20,100, and 14,400. The other lanes contain 20 μ g of purified protein from the 10 serum samples used in this study.

Table 2. Carbohydrate composition of human IgG purified by DEAE Affi-Gel Blue (mole/mole of IgG)

Donor	Fucose ¹	Sialic acid ²	Galactose ³	Glucosamine ⁴	Mannose ³
Normals					
1N	4.00	1.97	4.30	13.74	7.58
7N	2.11	1.42	2.60	11.96	6.07
163	2.69	1.22	2.59	14.33	7.40
174	4.79	1.05	2.04	9.01	5.27
211	<u>4.32</u>	<u>1.00</u>	<u>2.52</u>	<u>12.95</u>	<u>6.32</u>
	3.58 ± 1.11	1.33 ± 0.39	2.81 ± 0.86	12.40 ± 2.09	6.53 ± 0.96
Cystic fibrosis					
930	1.78	0.38	0.95	4.91	3.72
964	2.07	0.37	1.50	13.69	7.29
971	2.48	0.54	N.D.	11.18	N.D.
978	3.33	0.46	1.84	10.86	6.52
987	<u>3.38</u>	<u>0.59</u>	<u>1.73</u>	<u>11.71</u>	<u>5.71</u>
	2.61 ± 0.73 ⁵	0.47 ± 0.10 ⁶	1.50 ± 0.39 ⁷	10.47 ± 3.30 ⁵	5.81 ± 1.53 ⁵

¹ Determined by fucose dehydrogenase (14).² Determined by a modification of the Warren method (31).³ Data obtained from gas-liquid chromatography of trimethylsilylated derivatives.⁴ Determined by the amino acid analyzer method of Cheng and Boat (10).⁵ Not significantly different from controls.⁶ *P* is less than 0.001.⁷ *P* is less than 0.025.

Table 3. Carbohydrate composition of human IgG purified by DEAE Affi-Gel Blue (mole/3 mole of mannose)

Donor	Fucose ¹	Sialic acid ²	Galactose ³	Glucosamine ⁴	Mannose ³
Normals					
1N	1.58	0.78	1.70	5.44	3
7N	1.04	0.70	1.28	5.91	3
163	1.09	0.49	1.05	5.81	3
174	2.72	0.59	1.16	5.13	3
211	<u>2.04</u>	<u>0.43</u>	<u>1.19</u>	<u>6.13</u>	3
	1.69 ± 0.70	0.60 ± 0.14	1.27 ± 0.25	5.68 ± 0.39	
Cystic fibrosis					
930	1.41	0.30	0.75	3.90	3
964	0.85	0.15	0.62	5.63	3
978	1.53	0.21	0.85	4.99	3
987	<u>1.77</u>	<u>0.31</u>	<u>0.91</u>	<u>6.15</u>	3
	1.39 ± 0.38 ⁵	0.24 ± 0.08 ⁶	0.78 ± 0.13 ⁷	5.17 ± 0.96 ⁵	

¹ Determined by fucose dehydrogenase (14).² Determined by modification of the Warren method (31).³ Data obtained from gas-liquid chromatography of trimethylsilylated derivatives.⁴ Determined by the amino acid analyzer method of Cheng and Boat (10).⁵ Not significantly different from controls.⁶ *P* is less than 0.001.⁷ *P* is less than 0.025.

drate content of IgG in general and the carbohydrate content of other serum-type glycoproteins from CF subjects in particular?

- What may be the reason(s) for the altered carbohydrate content of IgG from CF patients?
- What physiologic significance might these findings have for the CF patient?

When the carbohydrate content of individual myeloma IgGs is examined, the data are highly variable (11). Ranges, expressed in terms of moles per mole of IgG, are as follows (11): fucose, 1.3–3.9; mannose, 5.0–17.0; galactose, 1.5–7.1; *N*-acetylglucosamine, 4.0–13.0; and sialic acid, 0–3.5. These findings apparently

have no relationship to the subclass of myeloma IgG examined (11). Schultze *et al.* (34) examined normal IgG, and their findings are, to the nearest whole residue of protein: fucose, 2; mannose, 8; galactose, 4; *N*-acetylglucosamine, 9; and sialic acid, 1. Others have reported lower mannose and higher *N*-acetylglucosamine contents (11). The carbohydrate compositions reported here are in line with values reported in the literature, especially in view of the fact that different analytical methods were used. It is striking that in the face of variability among the literature reports, we see consistent differences in galactose and sialic acid contents of CF IgG preparations, whether expressed in mole/mole of IgG or in the number of residues per oligosaccharide chain.

The data shown in Table 3 are consistent with the general

structure of complex type *N*-linked oligosaccharides found in IgG (19).

Kornfeld *et al.* (19) and Narisimhan *et al.* (27) have reported that the oligosaccharides found on IgG are variably glycosylated, with the terminal sugars of the antennae, galactose and neuraminic acid, most often absent. Our data support the contention that these terminal sugars are often missing in IgGs from all donors; however, IgG in our CF serum samples has relatively fewer completed oligosaccharide chains.

This is the first time that the complete carbohydrate composition of a known serum-type glycoprotein, *i.e.*, IgG, taken from CF patients has been compared with the carbohydrate composition of a normal glycoprotein. Pearson and Lubin (29) reported that heparin precipitated more protein from CF serum than from normal serum and that the carbohydrate composition of the heparin-precipitated glycoproteins from CF sera contained 27% less sialic acid, 27% more fucose, and 31% less hexose, despite the fact that total serum glycoproteins from both groups had the same carbohydrate content (30). We later showed that the major protein precipitated by heparin from serum is IgG, and that the amount of protein precipitated is directly related to the amount of IgG found in serum samples (24). Altered carbohydrate content probably has no relationship to increased heparin precipitation. Levy *et al.* (21) have shown that this effect is most likely related to the nature of the antigen combining site of the immunoglobulin, rather than to its subclass, isoelectric point, or even its isotype.

Scanlin *et al.* (32) also stated that heparin-precipitated material from serum of CF patients had an altered carbohydrate content; however, no supporting data were given. In the same paper they reported the isolation of a glycoprotein with apparent molecular weight of 220,000 from spent media of CF fibroblasts that has increased fucose content when compared with its normal counterpart. Our data give no evidence for altered fucose content in CF IgG; regardless of the assay method used. Other recent data relating to altered carbohydrate composition of CF glycoproteins is concerned more with altered lectin binding (5, 35). Ben-Yoseph *et al.* (5) report that alpha₂-macroglobulin from CF homozygotes has 64% less sialic acid and displays decreased concanavalin A and wheat germ agglutinin binding. The decreased sialic acid is in accord with our findings, but the basis for the decreased lectin binding is unclear because these investigators did not report a complete carbohydrate analysis. Increased concanavalin A binding to CF IgG, as reported by Shapira and Menendez (35), could be explained by the absence of terminal sugars, which protect mannose and glucosamine binding sites.

One hypothesis that might fit our findings would be that the biosynthesis of *N*-linked glycoproteins of CF subjects, particularly with respect to the role of sialyl- and galactosyltransferase activities, is incomplete. The best evidence against this hypothesis is the work of Hunt and Summers (17) who infected CF heterozygote and homozygote fibroblasts with vesicular stomatitis virus and showed that the glycosylation of viral glycoproteins, which contain oligosaccharide structures almost identical to those found on serum-type glycoproteins, was identical to the glycosylation of proteins from normal fibroblasts. Margolies (23) investigated levels of fucosyl- and sialyltransferases in CF lymphoid lines and fibroblasts and showed no striking differences in the activities of these enzymes compared with normal cells. This is also in accord with the work of Ben-Yoseph *et al.* (6) who showed that both plasma and fibroblast sialyltransferase activity was normal when asialofetuin was used as acceptor. The same authors also report that sialic acid metabolism, in general, appears normal. Based on these observations, it appears unlikely

that abnormal biosynthetic mechanisms are responsible for our findings; however, extensive studies of glycosyltransferases in CF tissue have not been done.

Another explanation for the decreased sialic acid and galactose content of IgG from CF patients is altered degradation. Many investigators have examined activities of glycosidases in CF and control serum, but the results are confusing. One can support the view that serum glycosidase activities are the same (4, 18), higher (8), or lower (16, 33) in CF patients simply by choosing the appropriate literature references. The work of Day *et al.* (12) and Thornburg *et al.* (36) shows that the carbohydrate moiety on the immunoglobulin molecule is buried in the protein portion of the molecule and is not susceptible to glycosidase activity. Formation of antigen-antibody complexes uncovers the immunoglobulin oligosaccharides. Substantial amounts of circulating IgG in CF may be antigen bound (25). In this case, the terminal sugars may become susceptible to removal by circulating glycosidases. Our findings could reflect an immune reaction in these chronically infected patients and not a primary aberration of biosynthetic or degradative mechanisms.

Diminished glycosylation of the antennae may contribute to the elevated levels of IgG and of circulating antigen-antibody complexes (25) in older subjects with CF. Thornburg *et al.* (36) implicate the carbohydrate moiety of IgG in the clearance of immune complexes via the hepatic galactose receptor described by Morell *et al.* (26); thus any IgG-antigen complex whose oligosaccharides terminate in glucosamine would not be taken up by the receptor and would remain in circulation.

Historically, there has been considerable discussion concerning the carbohydrate content of glycoproteins from CF subjects. Interest in this subject has been spurred by the abnormally viscous mucus found in this condition and the role that mucin-type glycoproteins play in determining the properties of mucus (7). It should be carefully noted here that, although our results do show altered glycosylation of one serum-type glycoprotein, they do not support the hypothesis that mucin-type glycoproteins are abnormal in CF. Serum-type glycoproteins are not only structurally different from mucin-type glycoproteins, but their biosynthesis is entirely different (15). Thus these studies do not shed light directly on reported abnormalities of mucins and mucous secretions in CF (37–39).

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Severe Combined Immunodeficiency in a Child with a Healthy Adenosine Deaminase Deficient Mother

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Summary

We investigated adenosine deaminase (ADA) deficient severe-combined immunodeficiency (SCID) in an 8-month-old child with ADA deficient mother. The ADA deficiency in the child was unusual in that the thymic histology was normal. In addition, the thymocytes formed E-rosettes with sheep erythrocytes and were stimulated by T-cell mitogens. ADA activity could not be detected in the child's thymocytes.

Studies on the family indicated that the father had about one-half of the normal erythrocyte ADA activity. All the family members with detectable ADA activity appeared to have, according to starch gel electrophoresis of erythrocyte lysates, the common ADA-1 phenotype; however, rigorous identification of phenotype was not possible in this study. The mother had less than 1% of normal ADA activity in both erythrocyte and lymphocyte extracts, but her whole peripheral blood lymphocytes demonstrated about 6% of normal activity. Normal concentrations of