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Abnormal galactosylation of serum IgG in patients with systemic lupus erythematosus and members of families with high frequency of autoimmune diseases

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Summary. Gas chromatographic carbohydrate analyses of IgG from 30 patients with idiopathic systemic lupus erythematosus (SLE) revealed lower content of galactose when compared to that in 36 controls of similar ages (mean $\pm$ SD, 3.18 ± 0.66 vs 3.82 ± 0.41 galactose residues/mole of IgG, $P < 0.001$). Abnormal galactosylation was observed in 60% of SLE patients. Analyses of IgG from 58 members of five families, characterized by a high frequency of SLE and other autoimmune diseases and serological abnormalities, and 51 controls of similar age range revealed that IgG galactose deficiency was detectable not only in some members with clinical and serological abnormalities ($P \leq 0.001$), but also in those without evidence of autoimmune diseases or abnormal serologies ($P \leq 0.001$). These data indicate that abnormal galactosylation of IgG frequently occurs in asymptomatic members of families with a high frequency of SLE and other autoimmune diseases and suggests that this abnormality may be an indicator for the development of these diseases.

Key words: Galactosylation – IgG – Families – Systemic lupus erythematosus – Autoimmune diseases

Introduction

Previous studies have shown that the terminal galactose of the *N*-linked oligosaccharide in the CH2 domain of serum IgG tends to be absent in several autoimmune diseases, including rheumatoid arthritis (RA) [1–5], juvenile RA [4], and Crohn's disease [3, 5]. It has been proposed that this deficiency is associated with a reduced activity of β-1,4-galactosyltransferase [(UDP-galactose: *N*-acetyl-*D*-glucosaminyl-glycopeptide 4-β-*D*-galactosyltransferase, EC 2.4.1.38 (GT)], an enzyme responsible for the addition of galactose to the terminal *N*-acetylglucosamine in plasma cells secreting IgG. This hypothesis is supported by the detection of decreased GT activity in circulating B-cells from patients with RA [6, 7]. Earlier studies of glycosyltransferases have suggested that glycosylation can be genetically controlled [8].

On the basis of these studies, it is important to determine whether the observed deficiency of galactose in IgG is only an epiphenomenon of certain autoimmune diseases or whether it can contribute to the development of these diseases (i.e., whether it precedes the development of the diseases). To investigate the basis of the deficient galactosylation of IgG in these diseases, we assayed the galactose content of IgG preparations from members of five families that have been previously studied [9–12] and that are characterized by a high frequency of autoimmune diseases. The aim was to determine whether the galactose deficiency of IgG is limited to individuals with clinically defined disease or whether it also occurs in those with serological abnormalities alone. Furthermore, since probands in each of these families were patients with systemic lupus erythematosus (SLE), and previous data concerning galactose deficiency in IgG from patients with SLE were inconsistent [2–5], we extended our earlier study [3] by examining IgG galactosylation in a larger number of patients with this disease.

Patients and methods

Patients and controls. Blood samples from 20 patients with SLE were obtained from individuals attending the outpatient rheumatology clinics of the Department of Medicine, The University of Alabama at Birmingham (UAB). An additional 10 patients with SLE were members of families with a high frequency of autoimmune diseases (see below). Sera were obtained from individuals who fulfilled either the preliminary [13] or revised [14] criteria for the classification of SLE. Blood samples from 58 adult members of five families with a high frequency of autoimmune diseases were obtained at the outpatient rheumatology clinic of the Department of Medicine, Johns Hopkins University, Baltimore, Md, USA [9–12]. Control sera were from UAB employees or patients who visited a UAB hypertension clinic. None had a history of autoimmune disease.

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Isolation of IgG. IgG was isolated from sera by ammonium sulfate precipitation and subsequent ion exchange chromatography as has been previously described by our group [3]. Evaluation of the purity by solid-phase immunosorbent assay [15] revealed that these preparations contained no more than 0.07% IgA or IgM.

Carbohydrate analysis. Monosaccharides from IgG were determined as trifluoroacetates of methyl glycosides by gas-liquid chromatography as described elsewhere [3, 16]. The content of galactose, which was expressed in residues per mole of IgG, was determined relative to the IgG content of mannose. Previous studies have demonstrated that the content of mannose in serum IgG from healthy controls and from patients with RA, SLE, and Crohn's disease is relatively constant at 8.04 ± 0.72 residues per mole [3]. This approach minimizes possible errors in the assay due to errors in determining the concentration of the IgG preparations, errors in pipetting of samples and internal standards, or partial losses of the sample or internal standard during the derivatization procedures.

Statistical analysis. Comparisons between means were performed using Student's *t*-test. Differences between proportions were examined using the chi-square distribution test. In all instances a $P \leq 0.05$ was arbitrarily chosen as the level of statistical significance. Results are expressed as mean ± 1 SD.

Results

The mean galactose content in serum IgG from the 30 patients with SLE was lower than that in 36 controls of similar ages (3.18 ± 0.66 and 3.82 ± 0.41 galactose residues/mole of IgG, respectively) ($P < 0.001$). Decreased IgG galactose (i.e., galactose content greater than 2 SD below the mean of the controls) was apparent in 11 (37%) of the patients. However, decreases in IgG galactose were evident in 18 (60%) of the patients when the SLE patients were compared with the controls grouped according to age by decade (Table 1). The greater frequency of galactose-deficient IgG indicated by the latter comparison reflected the negative correlation between IgG galactose content and age, which has been previously observed in normal individuals as well as in patients with RA [3, 17].

In order to investigate further the relationship between abnormal galactosylation of IgG and autoimmune diseases, we analyzed the serum IgG from adult members of five families characterized by a high frequency of autoimmune diseases [9–12]. At least one member of each family had SLE (Fig. 1). From a total of 58 members studied, 17 had a current diagnosis or history of autoimmune disease (10 had SLE, 2 Sjögren's syndrome, 1 Crohn's disease, 1 ulcerative colitis, 1 Henoch-Schönlein purpura, 1 vasculitis, and 1 Hashimoto's thyroiditis). An additional 23 members had serological abnormalities without clinical signs of disease (10 had antinuclear antibodies, 18 anti-ssDNA, 1 rheumatoid factor, and 1 benign false-positive syphilis test serology) and 18 members had no clinical or serological abnormalities. When compared with an equal number of controls of approximately the same age, the mean IgG galactose content was significantly lower, not only in family members with clinical abnormalities ($P = 0.001$), but also in those with serological abnormalities ($P < 0.001$), and in family members with no clinical or laboratory evidence of autoimmune disease ($P < 0.001$) (Table 2). When family members were subdivided into age groups by decade and compared with 51 controls grouped in the same fashion, IgG with decreased levels of galactose (greater than 2 SD below the mean of appropriate controls) was observed in 3 of 17 members with autoimmune disease (18%), 7 of 23 members with serological abnormalities (30%), and 6 of 18

Table 1. Galactose in serum IgG from SLE patients

	n	Ages mean ± SD range	IgG galactose residues/mole mean ± SD	*P*[a]	No. of patients with decreased IgG galactose[b]
SLE[c]	7	25.0 ± 4.3 19–30	3.44 ± 0.31	0.002	4
	11	34.8 ± 2.6 31–40	3.20 ± 0.65	<0.001	7
	7	45.1 ± 3.3 41–50	2.90 ± 0.88	0.084	4
	5	54.2 ± 2.3 51–57	2.83 ± 0.71	0.005	3
Total	30	38.2 ± 10.5 19–57	3.18 ± 0.66	<0.001	18
Controls	7	25.7 ± 4.0 20–30	4.03 ± 0.31		
	11	35.6 ± 3.4 31–40	3.98 ± 0.39		
	8	46.9 ± 3.4 41–50	3.66 ± 0.49		
	10	54.7 ± 2.8 51–59	3.63 ± 0.33		
Total	36	41.5 ± 11.3 20–59	3.82 ± 0.41		

[a] Student's *t*-test, one-tailed *P* values relative to corresponding control group
[b] Decreased IgG galactose was defined as a galactose content >2 SD below the corresponding mean control value
[c] The SLE data include ten family members with this diagnosis

Table 2. Galactose in serum IgG from members of families with high incidence of autoimmune diseases

	n	Ages mean ± SD range	IgG galactose residues/mole mean ± SD	*P*[a]
Autoimmune disease	17	37.1 ± 11.4 19–55	3.46 ± 0.36	0.001
Controls	17	37.0 ± 11.2 20–55	3.87 ± 0.37	
Autoantibodies	23	56.6 ± 19.2 22–86	3.04 ± 0.54	<0.001
Controls	23	55.3 ± 17.4 22–78	3.55 ± 0.45	
Normal	18	46.3 ± 14.9 21–69	3.13 ± 0.61	<0.001
Controls	18	46.3 ± 14.7 22–68	3.75 ± 0.43	

[a] Student's *t*-test, one-tailed *P* values relative to appropriate controls. Family members within each group were compared to an equal number of unrelated controls of similar age

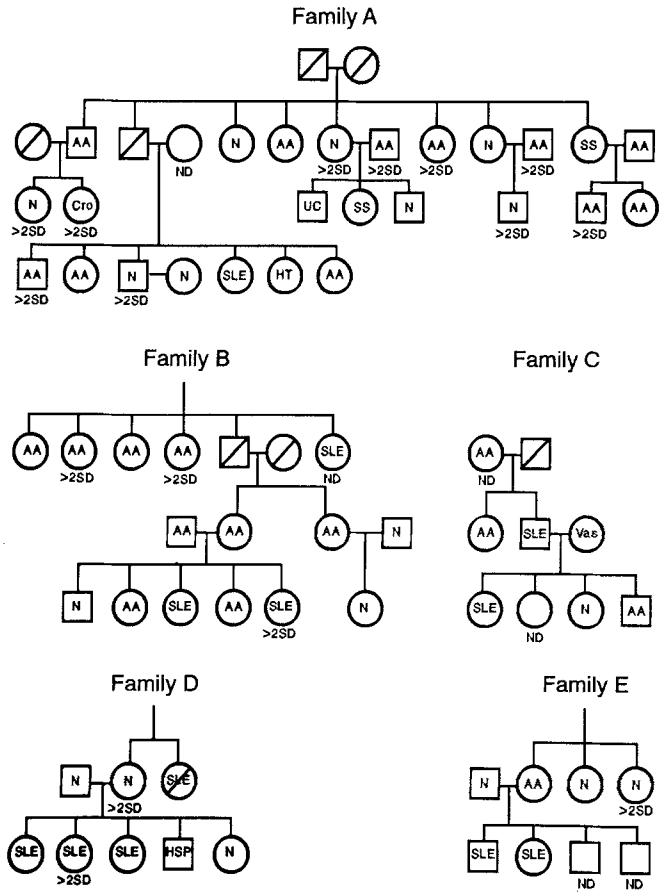

Fig. 1. Occurrence of autoimmune diseases, serological abnormalities and deficiency of galactose in serum IgG from members of five families. >2 SD = IgG galactose levels >2 SD below the mean value for age related controls; AA = autoantibody; Cro = Crohn's disease; HSP = Henoch-Schönlein purpura; HT = Hashimoto's thyroiditis; N = normal; ND = not determined; SS = Sjögren's syndrome; SLE = systemic lupus erythematosus; UC = ulcerative colitis; Vas = vasculitis

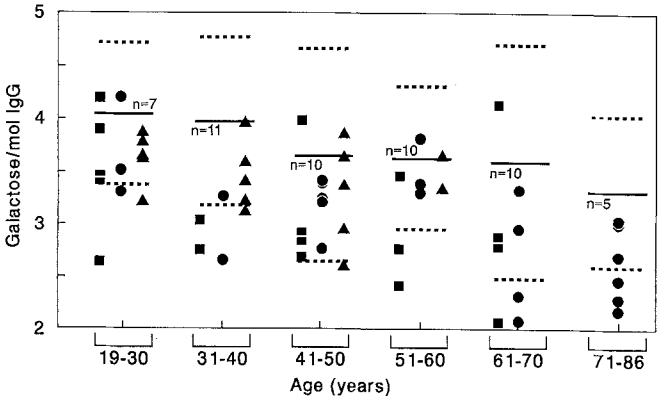

Fig. 2. Galactose content of IgG in members of families with high prevalence of autoantibodies and autoimmune diseases. ● = Members with autoantibodies; ■ = apparently healthy members; ▲ = members with autoimmune diseases. Full horizontal bars indicate mean molar concentration of galactose in control IgG; broken horizontal bars indicate ±2 SD; *n* = number of controls in age groups

apparently healthy members (33%) (Fig. 2). The small differences in the occurrence of decreased levels of IgG galactose among these groups are not statistically significant.

Discussion

The detection of decreased levels of galactose in serum IgG of patients with SLE confirmed our previous data, obtained by analyzing a limited number of specimens [3] and was consistent with data previously reported by Mullinax et al. [2]. According to Parekh et al., the galactose deficiency of IgG occurs only in SLE complicated by Sjögren's syndrome [4]. Since patients in the present study were not systematically evaluated for the presence of Sjögren's syndrome, we were unable to confirm the latter observation. In this regard, only 4 of 22 patients evaluated exhibited anti-SSA or SSB autoantibodies, which are ordinarily found in higher frequency in this disorder [18].

As has been previously shown [3, 17], the IgG deficiency of galactose in RA, as well as in normal controls, increase with age. A similar age dependency was noted among the SLE patients in the present study, although the relationship was not statistically significant (data not shown). Thus, critical assessment of true changes in the galactose content in IgG of individuals with SLE requires matching with controls of a similar age range.

Several possible mechanisms have been proposed to explain the galactose deficiency in chronic inflammatory diseases. One suggestion is that this abnormality occurs post-synthetically as an effect of oxygen-free radicals generated by activated phagocytic cells at inflammatory sites [19]. Radiolytically generated peroxy- or hydroxyl-radicals have been shown to destroy galactose on IgG. This hypothesis is not consistent with age-related galactosylation changes in healthy individuals and does not explain the galactose deficiency in some members from families with a high incidence of autoimmune diseases in whom evidence of autoimmune disease is lacking. Studies with pokeweed mitogen-induced peripheral blood mononuclear cells from rheumatoid patients have indicated that agalactosylated IgG is produced in vitro and can occur as a pre-secretory event [20]. It has also been suggested that this structural abnormality is a result of a defect during the synthesis of IgG caused by a deficiency of galactosyltransferase (GT) in plasma cells [6]. This hypothesis has been supported by the demonstration of a deficiency of GT in circulating B-cells from RA patients. The weak point of this proposed mechanism is that it cannot explain why this abnormality is limited to IgG and does not affect other immunoglobulins, such as serum IgA [3]. Furukawa et al. [7] have hypothesized that the deficiency in galactosylation of IgG in RA patients is associated with an abnormality in the function of GT. In kinetic studies they have shown that GT from homogenates of EBV-infected B-cells from RA patients exhibit lowered GT activity toward asialo-agalacto-IgG and that this reduced activity can be ascribed to lowered activity of GT for the activated donor, UDP-galactose.

This abnormality is apparently specific for IgG since the in vitro galactosylation of other glycoproteins, such as asialo-agalacto-transferrin by this enzyme, is not affected.

If the deficiency of galactosylation is associated with changes during the biosynthesis of the IgG caused by an abnormal function of GT, this process may be genetically controlled. Indeed, the gene for GT has been cloned and sequenced [21].

The results of this study demonstrate that in families that exhibit a high frequency of autoimmune diseases and abnormal serotypes, IgG galactose deficiency occurs not only in family members with clinically diagnosed disease, but also in those with serological abnormalities and in apparently healthy family members. The latter finding is consistent with the observation of decreased IgG galactose in unaffected spouses of RA patients reported by Sumar et al. [22]. Overall, our data support the view that IgG galactose deficiency may be an indicator for the development of autoimmune diseases and that it can occur in the absence of overt autoimmune disease. Further studies should yield insights concerning the role of genetic and/or environmental factors in the expression of abnormal galactosylation of IgG. In addition, longitudinal studies are required to determine whether galactose deficiency can occur in individuals with autoimmune diseases prior to the onset of disease. It is of interest in this regard that longitudinal studies on patients presenting with early synovitis have indicated the relevance of decreased IgG galactose for the development of RA [23].

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