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Evaluation of β1,4-galactosyltransferase in rheumatoid arthritis and its role in the glycosylation network associated with this disease

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Evidence indicating an important link between glycosylation changes and autoimmune rheumatic disease is presented. Attention is especially focused on the interrelationship between reduced galactosylation of the oligosaccharides of IgG, auto-sensitization which is thought to be of central importance in the pathogenesis of rheumatoid arthritis (RA), and the enzyme β1,4-galactosyltransferase (GTase) that catalyses the addition of galactose to the oligosaccharide chains on this molecule. Data are presented to indicate that GTase undergoes a variety of normal and disease associated changes. These variations are believed to contribute to the pathological processes in rheumatoid disease, and a hypothesis is suggested, whereby disease is associated with the dysregulation of an integrated glycosylation network, comprising IgG galactosylation, lymphocytic GTase and anti-GTase antibodies, that is a component of the normal immune system.

Keywords: β1,4-galactosyltransferase, rheumatoid arthritis, glycosylation network

Introduction

Glycoproteins are ubiquitous in nature [1] and come in a variety of shapes and forms [2]. The majority of molecules involved in the immune system are glycosylated, for example the HLA Class II molecules and the T cell receptor [3, 4], and tend to exhibit normal variation in the extent of their glycosylation [5]. Glycosylation changes can have profound effects on glycoprotein function [6] and the relevance of immunoglobulin G (IgG) glycosylation changes to the pathogenesis of rheumatoid arthritis (RA) has been the subject of much speculation [7].

Abnormalities of IgG glycosylation are recognized in individuals with RA [8], where a shift in the population of IgG glycoforms towards those with a lower content of galactose is observed in both serum and synovial fluid, when compared to age-matched controls.

Oligosaccharide side chains may play an important role in the integrity of IgG as their absence alters not only the conformation of the molecule [9], but also its functional properties. For example, binding to monocyte and macrophage Fc receptors [10, 11], complement component C1q [12], and the ability to induce cellular cytotoxicity [10], all rely on a normal degree of glycosylation. Immune complexes containing aglycosylated immunoglobulin fail to be eliminated rapidly from circulation [13], and feedback immunosuppression is lost [14].

The glycoprotein UDP β1,4-galactosyltransferase (GTase) is an intracellular membrane-bound enzyme that can be localized to the Golgi apparatus [15], but may also be found on the cell surface [16] and in a soluble form in milk, amniotic fluid, cerebrospinal fluid, saliva, urine and serum [17].

The molecular role of GTase is to catalyse the transfer of galactose (Gal) from UDP-Gal to an *N*-acetylglucosamine (GlcNAc) acceptor during oligosaccharide elongation, for example, in IgG and IgA glycosylation [18]. The gene encoding human GTase is thought to be located on chromosome 9 [19], and it may specify more than one mRNA transcript [20].

GTase seems to play a multifunctional role in normal cell physiology and has been associated with sperm-egg binding [21], cell-cell recognition [22], embryonic maturation [23], and cell development [24]. GTase activity has been shown to be reduced in peripheral lymphocytes of patients with RA [25, 26].

Control of β1,4-galactosyltransferase activity [26]

To investigate the potential mechanisms controlling protein glycosylation we analysed concomitant lymphocytic galacto-

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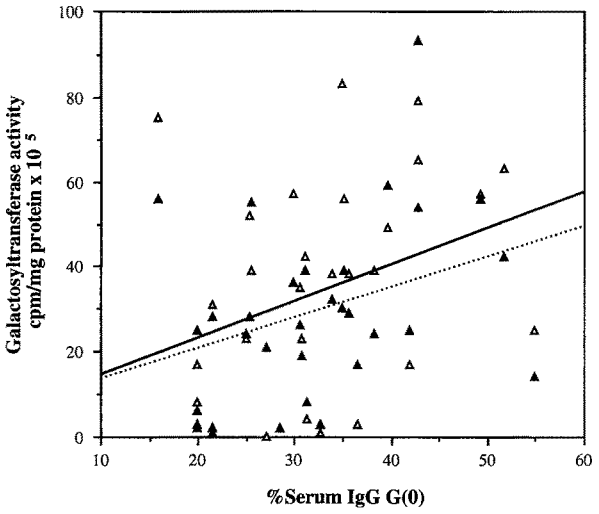

Figure 1. Regression analysis of paired B (open triangles and unbroken line) and T (closed triangles and dotted line) lymphocytic galactosyltransferase activities and serum agalacto–immunoglobulin (percentage serum IgG G(O)) in the control population ($r = 0.263$ and 0.211 for B and T cells, respectively, $p = <0.05$). (Axford *et al.* (1992) *J Clin Invest* **89**: 102–31.)

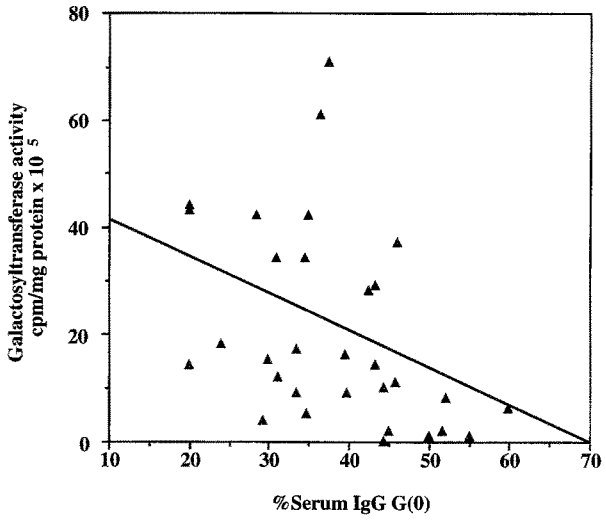

Figure 2. Regression analysis of paired B lymphocytic galactosyltransferase activity and serum agalacto–immunoglobulin G values (percentage serum IgG G(O) in the RA population ($r = 0.338$, $p < 0.004$). There ia a significant difference ($p < 0.01$) when this value is compared with the control regression value (r = 0.263). (Axford *et al.* (1992) *J Clin Invest* **89**: 1021–31.)

syltransferase (GTase) activity and serum agalactosylated-IgG (G(0)) levels, in healthy individuals ($n = 32$: mean age was 54.5 yr [range 32–78 yr]; 22 females) and patients with RA ($n = 32$: mean age was 59.9 yr [range 28–88 yr]; 27 females).

GTase activity was determined using a well characterized biological assay in which radio-labelled galactose was transferred to terminal GlcNAc sites on an ovalbumin acceptor molecule [27] and agalactosylated-immunoglobulin G (GO) levels were detected by immunoblotting with a panel of lectins [28].

Comparative analysis of the data revealed a significant decrease in lymphocytic GTase activity (B Cell – 40%, $p < 0.01$ and T cell – 29%, $p < 0.05$), together with a significant increase in G(0) (1.1 ± 0.2 SD above the age-related population mean, $p < 0.01$) compared to the age-matched control population.

The relationship between GTase and G(0) was found to be positive and linear for the peripheral lymphocytes (B Cell $r = 0.263$ and T Cell $r = 0.211$; $p < 0.05$) of the control population (Fig. 1) and negative and linear for the (B Cell $r = -0.338$, $p < 0.004$ and T Cell $r = -0.16$, $p = 0.1$) lymphocytes of the RA population (Fig. 2). The difference between the control and RA correlations were found to be significant (B cell $p < 0.001$ and T cell $p < 0.05$).

Collectively these data describe a defect in RA lymphocytic GTase which is concomitant with increased agalactosylated-IgG levels, and outline the possibility of an RA associated disruption of a positive feedback mechanism controlling GTase levels and thus IgG-galactosylation.

Investigations into a possible humoral associated con trol mechanism revealed that antibodies to GTase may be an integral component of the above glycosylation network [29]. The presence of antibodies to GTase has been shown in a variety of diseases, and the data suggests that naturally occurring IgM antibodies may be part of a normal physiological immune regulatory mechanism controlling glycosylation, whereas IgG antibodies may be associated with disease and cause a down regulation of GTase activity or expression.

Are there genetic changes associated with reduced GTase activity? [30]

To assess the structural integrity of the gene encoding for GTase in RA patients, we carried out restriction endonuclease digestion of leukocyte DNA. The *GTase* gene was analysed for restriction fragment length polymorphism (RFLP) using 10 different restriction endonucleases, in combination with a 1.28 kb cDNA probe specific for the coding region and a 0.2 kb probe for the 5′ untranslated region containing the transcriptional initiation sites for the two known forms of mRNA that this gene expresses (gifts from M. Fukuda).

No RA specific polymorphism was detected with any of the restriction enzymes used, when DNA samples from 10 patients with RA and 10 healthy controls were analysed. However, the data suggest the presence of a naturally-occurring polymorphism as evidenced by the finding of an identical *Pst* I RFLP using the 1.28kb cDNA (Fig. 3) which was seen in one control and one RA patient. Lack of DNA polymorphism

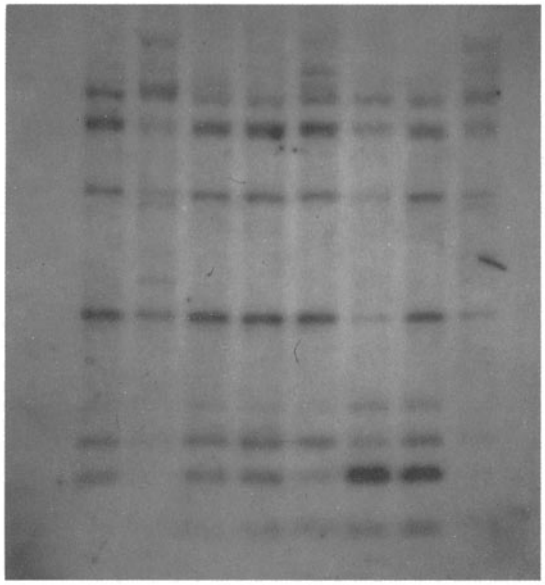

Figure 3. Result of hybridization of 1.28 kb GTase DNA to southern blot containing eight samples of genomic DNA extracted from PBL mononuclear cells (10 μg: separated on 0.7% agarose gel) digested with *Pst* I endonuclease. Lanes 1–4 represent normal healthy controls and lanes 5–8 rheumatoid arthritis patients. Changes in band patterns observed in lanes 2 and 8.

Table 1. The effects of B lymphocyte intracellular components on purified human galactosyltransferase.

	GTase activity		
Strain	*Expected*	*Observed*	*% Increase*
MRL lpr/lpr	38.1 ± 6.1	57.0 ± 6.1	49.6*
MRL +/+	44.4 ± 6.3	56.9 ± 6.6	28.1*
CBA/Ca	74.8 ± 26.5	85.8 ± 29.9	14.7

The mean observed and expected galactosyltransferase (GTase) activity (Mean ± SEM cpm per mg protein × 10^5 of peripheral blood B lymphocytes from MRL lpr, congenic (99.6% genetic homology) MRL +/+ and the histocompatible non-autoimmune CBA/Ca mice. B lymphocyte intracellular components did not inhibit human GTase, conversely there was an increase in overall GTase activity above that expected in all experiments (*$p < 0.05$). (Axford *et al.* (1994) *Autoimmunity* **17**:157–63)

upon reprobing with a cDNA for the 3′ untranslated region suggests that the polymorphism seen is not due to incomplete digestion by the restriction enzyme.

In conclusion, although the data indicate that the RA associated decrease in lymphocytic β1,4 GTase is not as a direct consequence of gross structural changes within the *GTase* gene locus, it does provide evidence for the presence of polymorphic forms of the *GTase* gene which may contribute to the

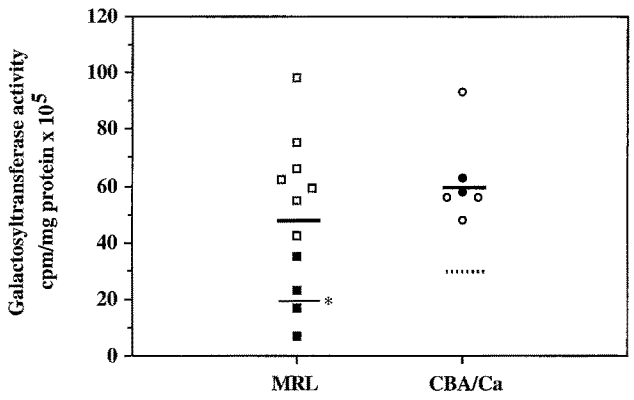

Figure 4. Comparison of lymphocyte GTase activity in MRL and CBA/Ca mice. The thick unbroken bars represent mean activity of each mouse group, the thin unbroken and broken bars represent the mean MRL and CBA/Ca peripheral lymphocyte GTase activity – 2 standard deviations respectively. Closed symbols represent peripheral lymphocytes and open symbols represent splenic lymphocytes. *$p = 0.002$: peripheral MRL vs SP MRL or CBA/Ca. (Axford *et al.* (1994) *Autoimmunity* **17**: 157–63.)

RA phenotype. Present studies have now focused on the expression of this gene locus.

Are there intracellular inhibitors of GTase? [31]

No evidence has been found to suggest the presence of a soluble intracellular GTase inhibitor. This was demonstrated by the results of mixing experiments using lymphocyte lysates derived from the MRL mouse model of RA in the presence of purified human milk GTase (Boehringer, Mannheim). The changes in peripheral B lymphocyte (PBL) GTase activity in this arthritis-prone MRL lpr/lpr (MRL/lpr) mouse are similar to those found in RA.

No inhibition of commercially-purified human milk GTase activity was detected in the presence of pooled PBL cell extracts, irrespective of whether they were derived from the arthritic MRL ($n = 4$) or the histocompatible non-autoimmune control CBA/Ca ($n = 2$) mice. There was, however, an increase in observed GTase activity in both the MRL PBL (30%, $p = 0.001$), and in the CBA/Ca experiments (Table 1), which may be due to differences in intracellular basic proteins and peptides which make up the pool of polycations that are thought to function as physiological activators of GTase [32]. Interestingly, the percentage increase in GTase activity appears to be greater in the MRL/lpr group, as compared to the congenic MRL +/+ and CBA/Ca groups. This observed difference in synergism resulting from these mixing experiments would indicate that intracellular polycations may be components of an additional regulatory mechanism that the cell may employ to control protein glycosylation. The finding that the increase was more pronounced with the MRL/lpr cells

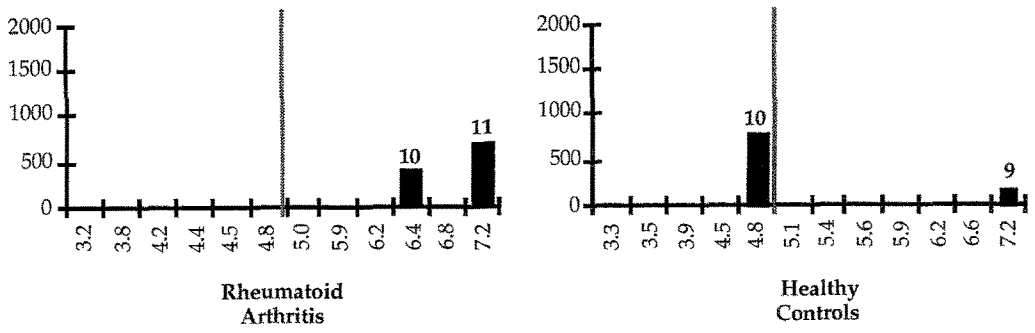

Figure 5. Representative isoenzyme profiles of RA and healthy control sera. Horizontal axis represents pH of fractions. Vertical axis represents cpm of enzyme assay-labelled galactose incorporated into albumin acceptor. Columns indicate fractions that are +1 SD above the mean. The figures above each column represent the % of the total counts for each serum. The data show the mean values from 16 specimens in each group.

may be an indication that possible differences in B cell GTase may exist within the MRL sub-strains. This would parallel the differential MRL/lpr and MRL +/+ GTase activities previously reported in MRL T cells [33], as well as the observation that there is an overall change in IgG glycosylation in MRL/lpr when compared to MRL +/+ mice [34].

Is there a generalized reduction in GTase activity? [31]

To determine whether the reduction in GTase activity is restricted to peripheral lymphocytes, we have measured and compared GTase activity in PBL and paired splenic (SP) B lymphocytes in the MRL model of RA.

A significant reduction in PBL GTase activity (mean ± SEM cpm per mg protein × 10^5; 20.5 ± 5.9) was observed in the autoimmune MRL mice when compared to their paired SP GTase activity (65.3 ± 6.7; $p = 0.002$) and with the non-autoimmune histocompatible CBA/Ca PBL and SP lymphocyte activities ($p = 0.002$). In fact, the MRL PBL formed a distinct group whose activities uniquely fell below 36.0 cpm per mg protein × 10^5, which is equivalent to – 1.7 SD below the mean CBA/Ca level (Fig. 4).

These results would indicate that glycosylation changes in these animals and perhaps humans, are tissue specific and are likely to be associated with the autoimmune disease process, as the mice used were all subjected to similar environmental conditions and were of a similar age.

Are there RA specific GTase isoenzyme changes? [35]

To investigate whether changes in GTase isoenzyme may be of relevance to RA. We have utilized liquid phase isoelectric focusing to identify GTase isoenzymes in RA and healthy control sera. Serum from active RA patients and healthy controls were individually run and separated in a focusing cell in a pH gradient of 3–10. The fractions were then assayed for GTase activity using a well characterized assay in which ^{3}H galactose is transferred to an ovalbumin acceptor molecule. GTase activity peaks thought to represent isoenzymes were defined by subtracting the mean value + 1 SD from all the fractions obtained.

Analysis of these preliminary studies indicates that in healthy control sera, GTase activity peaks occur between pH 4.0–5.0, whereas in RA there is a significant shift to higher pH values. There was an absence ($p = < 0.05$) of enzyme activity below pH 5.0 (Fig. 5). These results suggest that the galactosylation defect as seen in RA IgG may be associated with a change in the properties of GTase isoenzymes.

Conclusions

β1,4-GTase:

(a) is disproportionately reduced in the peripheral B cells in relation to IgG-galactose levels in RA patients, indicating a breakdown in normal homeostatic control;
(b) exhibits a *Pst* I polymorphism, which is not restricted to RA, but is also present in healthy individuals, indicating that RA associated variation in GTase activity is not as a direct result of gross structural polymorphism within the *GTase* gene locus;
(c) is not reduced in peripheral B cells as a consequence of intracellular biochemical inhibition, but its activity may be partially up-regulated by intracellular polycations;
(d) is reduced in a tissue specific manner and the reduction is confined to circulating peripheral lymphocytes;
(e) possibly changes its isoenzyme pattern in RA which may account for the observed differential incorporation of galactose and thus be pathologically relevant to this disease.

Collectively, the results presented here support our speculation that GTase undergoes a variety of normal and disease associated changes, that may be both isoenzyme and tissue specific in origin. These changes may occur at the molecular level or result from post-translational modifications and may be part of a glycosylation network responsible for varying

the carbohydrate content of proteins, and hence altering their function in response to homeostatic feedback mechanisms. Disruption of this network and the resulting glycosylation changes could be immunologically pertinent and may play a pivotal role in the development of pathological processes in RA.

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