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Agalactosyl IgG and β-1,4-galactosyltransferase gene expression in rheumatoid arthritis patients and in the arthritis-prone MRL *lpr/lpr* mouse

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SUMMARY

Reduced galactosylation of immunoglobulin G (IgG) is well documented in rheumatoid arthritis (RA), but the reason for this defect is still unknown. There is some evidence supporting a defect in the biosynthetic pathway, and a reduction in the level of β-1,4-galactosyltransferase (β-1,4-GalTase) enzyme activity. Since glycosyltransferases are, in general, regulated at the level of transcription, we have measured the level of β-1,4-GalTase gene expression in B cells from patients with RA and normal control individuals. We found no significant difference in mRNA levels for the transferase in these two groups ($P > 0{\cdot}7$). MRL/Mp-*lpr/lpr* (MRL-*lpr*) mice develop a spontaneous arthritis with increased levels of agalactosyl IgG (G0). In spite of a significant reduction in the level of β-1,4-GalTase mRNA in total spleen lymphocytes from MRL-*lpr* mice compared with the congenic MRL/Mp-+/+ (MRL-+/+) mice and with CBA/Ca mice, we found comparable levels of the β-1,4-GalTase mRNA in purified B cells from both spleen and lymph nodes of the three strains. Amongst the lymphoid compartments examined, the spleen and peripheral blood were found to be the major contributors of G0 in MRL-*lpr* mice. These data indicate that in neither human RA, nor in an animal model of this disease, is reduced IgG galactosylation caused by impaired expression of the β-1,4-GalTase gene in B lymphocytes. Furthermore, splenic B cells, which have normal levels of β-1,4-GalTase mRNA, appear to be a major source of G0 in MRL-*lpr* mice.

INTRODUCTION

Rheumatoid arthritis (RA) is associated with defective galactosylation of Immunoglobulin G (IgG). When compared with age-matched controls, patients have reduced galactose levels of the oligosaccharide chains linked at asp 297 of the Cγ2 domain.[1,2] Agalactosyl IgG (G0) shows significantly reduced binding to C1q and to Fc receptors.[3] Multiple presentation of IgG G0 to mannose-binding protein (MBP) has been shown recently to result in activation of complement.[4] In addition agalactosyl IgG is associated with pathogenicity in murine type II collagen-induced arthritis,[5] and an elevated percentage of serum agalactosyl IgG is an important prognostic marker for human RA.[6,7]

It has been suggested that defective galactosylation of IgG may be due to aberrant control of lymphocytic β-1,4-galactosyltransferase (β-1,4-GalTase) activity.[8–10] This enzyme exerts several biological functions. First, as a Golgi-membrane bound enzyme, β-1,4-GalTase participates in the synthesis of oligosaccharide chains by specifically transferring galactose from an activated UDP-galactose donor to acceptor sugars containing terminal non-reducing N-acetylglucosamine (GlcNac). Second, during pregnancy, the specificity of β-1,4-GalTase in lactating mammary gland is altered by binding to a modifier subunit, α-lactalbumin, to catalyse the synthesis of lactose. Finally, on the cell surface, β-1,4-GalTase is thought to function as a recognition molecule during a variety of cell–cell and cell–matrix interactions by recognizing specific glycoconjugates on the cell surface and in the extracellular matrix. Thus, the role of β-1,4-GalTase has been documented in fertilization, differentiation and proliferation.[11]

A single gene encoding β-1,4-GalTase has been localized to human chromosome 9 at band p13 and to mouse chromosome

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Abbreviations: β-1,4-GalTase, β-1,4-galactosyltransferase; RA, rheumatoid arthritis; IgG, immunoglobulin G; RPA, ribonuclease protection assay; MRL/Mp-*lpr/lpr*, MRL-*lpr*; G0, agalactosyl IgG.

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4.[12,13] The gene locus spans > 50 Kb of genomic DNA and is comprised of six exons.[14] The β-1,4-GalTase locus has been shown to have two transcription initiation sites and therefore to specify two sets of mRNA transcripts encoding two forms of the protein which have a 13 amino acid difference in the length of the amino terminal domain.[13,15,16] The gene is under the control of multiple promoters with the promoter controlling the first initiation site lacking in the smaller transcript. There appears to be separate promoters for housekeeping, mammary cell-specific, and germ cell-specific expression of β-1,4-GalTase.[17]

MRL/Mp-*lpr*/*lpr* (MRL-*lpr*) mice spontaneously develop an autoimmune disease with some features that resemble human RA including the decreased IgG galactose level.[18,19] Reduced B-lymphocyte β-1,4-GalTase activity has been reported in this arthritis-prone strain of mouse.[20] We have shown previously that the level of β-1,4-GalTase mRNA is reduced in spleen cells from MRL-*lpr* mice compared with that from the normally galactosylated CBA/Ca strain mice, suggesting an association between the IgG galactose levels and β-1,4-GalTase gene expression.[21] Now, we have extended our studies to the purified B cells from MRL-*lpr* mice and from patients with rheumatoid arthritis in order to define more closely the basis of the decreased IgG galactosylation and have also looked at the distribution of *de novo* IgG G0 secretion amongst the lymphoid compartments in MRL-*lpr* mice to see which ones are contributing to the high level of agalactosyl IgG found in the sera of these mice.

MATERIALS AND METHODS

Study subjects

Blood samples were obtained from patients with RA, each of whom fulfilled the American Rheumatism Association (ARA) revised criteria for the disease.[22] The control population was composed of healthy volunteers.

Mice

Mice were purchased at 7 weeks of age from Harlan Olac Ltd (Bicester, UK) and then maintained in our local animal facility. All the mice used for mRNA analysis were 12-week-old females. Cell suspensions were prepared from spleen and lymph nodes, from pools of seven mice. The mice used to study the distribution of G0-secreting cells comprised pools of MRL-*lpr* mice (mean age 4 months, range 3–5 months), and CBA/Ca mice (mean age 5 months, range 3–7 months).

Serum IgG galactosylation

Serum IgG G0 in the human and murine individuals was measured as follows using a modified version of previously published assays.[23,24] Ninety-six well maxisorb-immunoplates (Nunc, Roskilde, Denmark) were coated overnight with 50 μl/well of recombinant truncated protein G′ [Sigma (Poole, UK), P-4689] at 5·0 μg/ml in phosphate-buffered saline (PBS) at 4°. The wells were aspirated and blocked with 100 μl 0·05% Tween 20, 1% BSA in PBS (PBS-T-BSA) for 1 hr at 37° followed by three washes with 0·05% Tween 20 in PBS. Standards with known G0 levels and sera diluted 1 : 100 (1 : 50 for murine) in 0·1 M glycine, 0·16 M NaCl, pH 7·0 were added in triplicate (50 μl/well) to two identical plates and incubated for 2 hr at 37°. After washing, 50 μl/well PBS was added and the plates floated on a waterbath at 85° for 15 min to partially denature the IgG molecules and thus expose the oligosaccharides. The biotinylated lectin *Bandeiraea simplicifolia* II (BSII, Vector Laboratories Inc., Cambridgeshire, UK) at 0·4 μg/ml in PBS-T-BSA containing 0·1 mM calcium chloride, or biotinylated goat $F(ab')_2$ anti-human IgG [or sheep $F(ab')_2$ anti-mouse IgG] (Sigma) at 4 pg/ml in PBS-T-BSA, were added at 50 μl/well to the cooled plates and incubated at 4° overnight. After three washes, 50 μl/well of Streptavidin-horseradish-peroxidase (DAKO Ltd., Buckinghamshire, UK) was added and incubated at 37° for 1 hr. A colour reaction was produced using 50 μl/well 0·1 M citrate phosphate buffer pH 4·1 containing 0·5 mg/ml 2,2-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) (Sigma) and 1 : 2000 hydrogen peroxide. After 15 min, the reaction was stopped with 50 μl/well sodium fluoride (2 mg/ml) and the plates were read on an automated enzyme-linked immunosorbent assay (ELISA) reader (Dynatech Laboratories Ltd, Billinghurst, West Sussex, UK) at 410 nm. The results were expressed as the ratio of BSII : anti-IgG binding and quantified against the standard curve of known G0 samples. The G0 standards used in this study were human (both RA and control subjects) and murine (BALB/c, CBA/Ca, DBA/1 and MRL-*lpr*) IgG molecules whose G0 values were defined by comparison in both dot-blot and ELISA systems with human and murine IgG whose G0, G1 and G2 values had been determined by Dr T. Rademacher and colleagues at the Department of Biochemistry, University of Oxford, using the hydrazinolysis method.[1]

Murine cell cultures

Cell suspensions were prepared by disaggregation of bone marrow, lymph node and spleen from pools of mice. Erythrocytes were removed from all cell suspensions using 0·16 M ammonium chloride pH 7·2. The cells were washed twice before culture. Peripheral blood mononuclear cells were separated by centrifugation on Lymphopaque (density 1·086 g/ml) at 750 ***g*** for 30 min at room temperature. The interface cells were washed twice prior to use. Cells were cultured for 4 days at 10^6 cells/ml in complete medium consisting of: RPMI-1640 supplemented with 5% γ-globulin free, heat-inactivated fetal calf serum (FCS) (Life Technologies, Renfrewshire, UK), 2 mM L-glutamine, 50 U/ml penicillin-streptomycin, 50 nM 2-mercaptoethanol, 1 mM sodium pyruvate and 1% non-essential amino acids (Life Technologies, Renfrewshire, UK). The percentage of spontaneously secreted G0 was measured as above using BSII and *Ricinus Communis* agglutinin I, and IgG concentration was measured with an ELISA assay according to Shields *et al.*[25]

Preparation of cells for mRNA measurements

Human peripheral blood mononuclear cells (PBMCs) were isolated by centrifugation over Lymphoprep (Nycomed, Oslo, Norway) at 800 ***g*** for 20 min at 18°. Cells were removed from the interface and washed twice in Hanks' balanced salt solution (HBSS). To isolate peripheral blood lymphocytes, PBMCs obtained as above were resuspended at 5×10^6/ml in RPMI-1640 containing 20% FCS, monocytes depleted by plastic adherence at 37° for 1 hr, and the non-adherent cells washed twice in PBS prior to RNA extraction. Purified B cells were obtained by positive selection from PBMCs using anti-CD19 coated Dynabeads M-450 (Dynal, Oslo, Norway) following

the manufacturer's instructions. This population comprised >95% CD19^{+} cells in each experiment.

The B cells from the mouse spleen and lymph node cell suspensions were isolated using magnetic beads coated with anti-mouse IgG antibodies (Dynal), in accordance with the manufacturer's instructions. This population consisted of >95% sIgG^{+} cells in each experiment. The surface–IgG negative population was obtained by two rounds of depletion using anti-mouse IgG-coated Dynabeads. These cells were then placed on plastic at 37° for 1 hr to remove adherent cells, following which the non-adherent cells were washed twice in PBS prior to RNA isolation. This cell population consisted of >70% CD3^{+}, <10% Mac-1^{+} and <1% sIgG^{+} in the case of spleen, and >85% CD3^{+}, <2% Mac-1^{+}, and <1% sIgG^{+} cells in the case of lymph node.

Production of antisense RNA probes

The β-1,4-GalTase probe used for the human studies was prepared by subcloning a 150 bp *Sac*1–*Pst*1 fragment containing nucleotides 58–208 of human β-1,4-GalTase exon 2[14] into pBluescript KS (Stratagene, La Jolla, CA). High specific-activity RNA probe (approximately 10^8 c.p.m./μg) was transcribed with T3 RNA polymerase (Stratagene) after linearization with *Pvu*II. The probe was 377-nucleotides long and the fragment protected by β-1,4-GalTase mRNA was 150-nucleotides long. The human β-tubulin cDNA clone Db-1 was used as an internal control to normalize for the amount of RNA in each sample lane. High specific-activity RNA probe (approximately 10^8 c.p.m./μg) was transcribed with T3 RNA polymerase from a 264 bp *Pst*I fragment (nucleotides 128–392 of Db-1 cDNA clone[26]) subcloned into pBluescript KS (Stratagene) and linearized with *Nco*I. The probe was 232-nucleotides long and the fragment protected by β-tubulin mRNA 172-nucleotides long. pT7 RNA 18S antisense control template (Ambion, Austin, TX), also used as an internal control for the RPA, produced a 109-nucleotide run-off transcript, 80 nucleotides of which were complementary to human 18S ribosomal RNA. An 18S ribosomal RNA probe was made to a purposefully lower specific activity of approximately 10^3 c.p.m./μg. Following transcription and DNAse digestion, samples were extracted with phenol-chloroform and purified by polyacrylamide-urea gel electrophoresis.

The probes used for the studies on mice have been described previously.[21] The probe recognizing murine β-1,4-GalTase (specific activity of approximately 10^7 c.p.m./μg) corresponds to nucleotides 704–1263 of the sequence published by Shaper *et al.*[15] The murine glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe (Ambion, Austin, TX), used as an internal control to normalize for the total amount of RNA, was synthesized to a purposefully lower specific activity of approximately 10^6 c.p.m./μg.

Ribonuclease protection assays (RPA)

Total cellular RNA was isolated by acid guanidium thiocyanate-phenol-chloroform extraction.[27] The RPA was performed using the RPA II kit (Ambion) according to the manufacturer's instructions. Briefly, an excess of both the β-1,4-GalTase and control-labelled RNA probes were mixed with sample RNA and incubated at 42° overnight to allow hybridization of the probes to target mRNA contained within the sample RNA. The mixture was then treated with 0·1 U RNAse A and 20 U RNAse T1 for 30 min at 37°. Protected fragments were analysed on a polyacrylamide-urea gel. The level of β-1,4-GalTase mRNA and of steady-state control mRNA was assessed either by scanning densitometry of autoradiographs from pre-flashed Fuji XR film using a Bio-Rad GS-250 Molecular Imager (Bio-Rad, Richmond, CA), or by scanning the gels using a Bio-Rad GS-250 PhosphoImager. Results are expressed as the ratio of β-1,4-GalTase mRNA: control signal.

RESULTS

Levels of β-1,4-GalTase mRNA in B cells from patients with RA and normal controls

The level of PBMC (total cells isolated using lymphoprep) β-1,4-GalTase mRNA was found to be reduced significantly in eight patients with RA compared with eight normal controls ($P < 0{\cdot}005$, data not shown). However, β-1,4-GalTase mRNA levels were not reduced significantly in either peripheral blood lymphocytes (non-adherent PBMC) from six patients with RA compared with six normal individuals ($P > 0{\cdot}9$, data not shown), or in B cells (CD19^{+} cells) from 14 patients with RA compared with 13 normal controls ($P > 0{\cdot}7$, Fig. 1a).

IgG galactosylation levels in patients with RA

The percentage of oligosaccharide chains lacking galactose was significantly higher in the sera from the patients with RA than in the normal subjects in all the studies conducted (Fig. 1b). No relationship was found between the level of IgG galactosylation and the level of CD19^{+} B-cell β-1,4-GalTase mRNA ($r = 0{\cdot}27$, $P > 0{\cdot}2$).

β-1,4-GalTase gene expression in B cells from MRL-*lpr* mice

The arthritis-prone MRL-*lpr* strain mice, the congenic strain

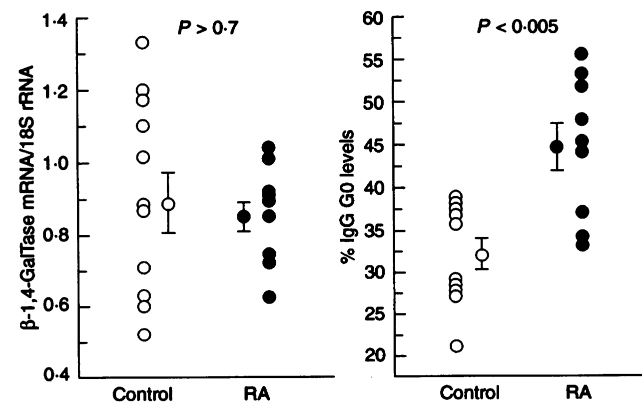

Figure 1. (a) Quantitation of the human peripheral blood B cell (CD19^{+}) GalTase mRNA levels by phospho-imaging. Because of limited amounts of RNA, some B-cell samples were pooled from two different individuals with similar levels of G0. Results are expressed as the ratio of β-1,4-GalTase mRNA signal over 18S ribosomal RNA signal. Mean ± standard error of the mean is indicated. (b) Percentage of agalactosyl IgG in sera of RA patients and of normal individuals used in this study. Mean ± standard error of the mean is indicated.

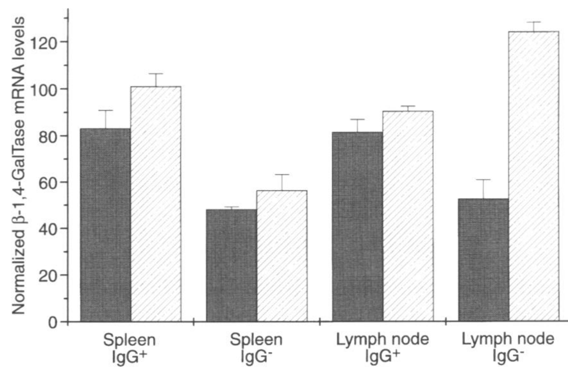

Figure 2. Quantitation of the GalTase mRNA levels for B cells (IgG^+) and IgG-negative non-adherent cells (IgG^-) from spleen and lymph nodes of MRL-*lpr* (solid bars), MRL-+/+ (hatched bars), and CBA/Ca strain mice (arbitrarily assigned a value of 100 for each experiment). Error bars indicate ± standard error of the mean for three separate RPA measurements on each sample. Each sample contains total RNA from pools of seven mice.

MRL-+/+, and the CBA/Ca strain with normally galactosylated IgG, all showed similar levels of β-1,4-GalTase mRNA in surface–IgG-positive splenic B cells. However, β-1,4-GalTase mRNA levels were reduced in surface–IgG negative non-adherent spleen cells from both MRL-*lpr* and MRL-+/+ strain mice compared with CBA/Ca mice (Fig. 2). In the lymph node compartment, β-1,4-GalTase mRNA levels were again comparable in surface–IgG positive cells from all three strains of mice. For the surface–IgG negative non-adherent lymph node cells, the mRNA levels for this enzyme were much reduced in MRL-*lpr* mice compared with that in both MRL-+/+ and CBA/Ca strain mice (Fig. 2).

Distribution of G0-secreting B cells in arthritic versus control mice

In MRL-*lpr* mice the G0 values of the secreted IgG in peripheral blood and spleen cell cultures were significantly higher when compared with bone marrow ($P = 0{\cdot}007$ and $P = 0{\cdot}004$ respectively, Fig. 3) but not lymph node ($P > 0{\cdot}2$). We also measured the concentration of total IgG secreted

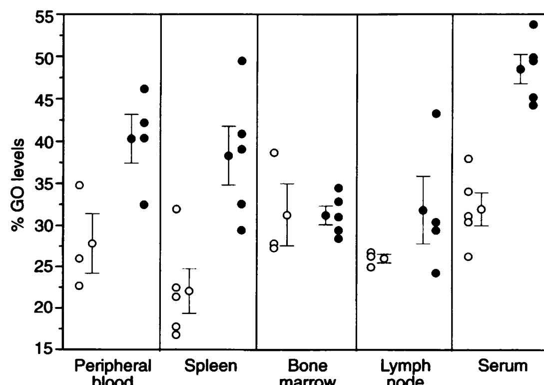

Figure 3. G0 levels for *de novo* secreted IgG from spleen, lymph node, bone marrow, and peripheral blood, and for serum, with mean ± standard error of the mean from MRL-*lpr* (solid circle) and CBA/Ca (open circle) mice.

spontaneously in cell cultures from four different lymphoid compartments. The only significant difference was the increased level of secretion by the spleen suspensions compared with the bone marrow ($P = 0{\cdot}024$, data not shown).

In the control strain CBA/Ca, the amount of IgG secreted and the percentage of IgG G0 were not significantly different in any of the compartments studied.

When comparing the two strains, G0 levels of secreted IgG from peripheral blood and spleen cell cultures (but not from bone marrow or lymph node) were significantly higher in MRL-*lpr* mice ($P = 0{\cdot}041$ and $P = 0{\cdot}006$ respectively) reflecting the increased serum IgG G0 levels in these mice ($P < 0{\cdot}001$). The production of IgG (IgG concentration) was raised significantly only in the MRL-*lpr* splenic cell supernatants when compared with CBA/Ca ($P = 0{\cdot}03$).

DISCUSSION

In this study, we show that peripheral blood and spleen cell cultures from MRL-*lpr* mice secrete agalactosyl IgG *in vitro*. We have also shown previously that immunoglobulins newly secreted from PWM-stimulated lymphocytes from RA patients have reduced levels of galactose.[28] These data imply that the defect in IgG galactosylation is at least in part attributable to the biosynthetic pathways. Galactose is added to the oligosaccharide chains of IgG by the enzyme β-1,4-GalTase in trans-cisternae of the Golgi complex. Not much is known about the control of glycosylation. Although there is extreme microheterogeneity in the structure of the oligosaccharides of IgG, this variation is not completely random. For example, it was shown that the molar ratio of agalactosylated, monogalactosylated, and digalactosylated oligosaccharides of IgG is relatively constant in normal healthy individuals of similar age.[29] Therefore, potent control mechanisms must regulate the oligosaccharide synthesis machinery. Part of this control comes from the specificity and the amount of glycosyltransferases present (cell- and tissue-specific glycosylation), the primary peptide structure, and the constraints imposed by the three-dimensional structure of individual proteins.[30]

The defect in galactosylation in RA seems to be restricted to IgG. Transferrin,[9] IgA,[31] and the Fab of IgG[1,30] have almost fully galactosylated oligosaccharides in patients with RA. Galactosylation at the Fc site of IgG is complicated by the unique structure of IgG in that unlike other glycoproteins that have their carbohydrates exposed, the IgG oligosaccharide moiety is contained within the space between the Fc $C\gamma 2$ polypeptide domains. Therefore, it could be that galactosylation at this site is more sensitive to small fluctuations in the level of enzyme β-1,4-GalTase, or alternatively the enzyme level is not the limiting factor but the rate of H–H chain disulfide bond formation in the Golgi determines the efficiency of galactosylation at this site.[32]

Although glycosyltransferases, in general, are thought to be regulated at the transcriptional level, there is some evidence for post-translational regulation of the β-1,4-GalTase enzyme. This enzyme has been shown to be phosphorylated at serine residues[33] and there is some evidence that a cdc-related protein kinase, p58 galactosyltransferase-associated protein kinase, may be involved in the regulation of β-1,4-GalTase activity through phosphorylation.[34]

The suggestion that β-1,4-GalTase enzyme activity is

reduced in RA is controversial. Data on β-1,4-GalTase enzyme activity[8–10,35,36] suggest that the glycoprotein acceptor and/or the method of B-cell preparation used in each study contribute to the observed activity of the β-1,4-GalTase enzyme. Our observation of similar levels of β-1,4-GalTase mRNA in peripheral $CD19^+$ B cells from patients with RA and normal controls, and also in both splenic and lymph node $sIgG^+$ B cells from arthritis-prone and control mice suggest that the B-cell β-1,4-GalTase activity, if reduced in RA, must be post-transcriptionally regulated.

We cannot exclude the possibility that mRNA levels for the enzyme in circulating peripheral blood B cells, the majority of which are resting virgin and memory cells, might not reflect the levels of the message in the plasma cells which are the source of the defectively glycosylated serum IgG. It is not possible to directly isolate sufficient numbers of plasma cells to provide enough RNA for the RPA measurements, and the use of *in vitro* techniques such as Epstein–Barr virus (EBV) transformation has been shown to increase β-1,4-GalTase activity in RA B cells leading to the conclusion that such procedures can not be used in these types of study.[10] An alternative approach might be to use reverse transcriptase–polymerase chain reaction (RT–PCR) but such assays are accurately described as 'semi-quantitative' and are unlikely to provide as accurate a quantitation of mRNA levels as the RPA. All previous studies on β-1,4-GalTase activity in RA have examined B lymphocytes rather than plasma cells and the aim of the present study was to establish if the reported decrease in β-1,4-GalTase activity in this cell type in RA can be explained by a decreased production (or alternatively increased turn-over) of the specific mRNA species. We clearly show this not to be the case.

Our finding of comparable levels of β-1,4-GalTase mRNA in IgG-positive B cells from spleen and lymph nodes of MRL-*lpr* mice support the observations of Axford and co-workers[20] who have reported a reduction in peripheral but not splenic B-lymphocyte β-1,4-GalTase activity in MRL-*lpr* mice compared with CBA/Ca mice, and no significant difference in B-cell GalTase activity when comparing MRL-*lpr* mice with MRL-+/+.

Previously we have reported that there are reduced levels of β-1,4-GalTase mRNA in splenic lymphocytes (spleen cell suspension depleted of plastic-adherent cells) from MRL-*lpr* mice.[21] This has been confirmed and reflected here in decreased levels of the message in the IgG-negative population (cell suspension depleted of plastic-adherent cells and $sIgG^+$ cells) from spleen and lymph nodes, suggesting a further abnormality in MRL-*lpr* T cells. Our findings would correspond with those of Imai and colleagues[37] who report reduced binding to *lpr* lymph node T cells of the lectins RCA and allo A, which bind primarily to a Galβ1–4GlcNAc structure. Contrary to this observation, the same authors reported an increase in the activity of β-1,4-GalTase in MRL-*lpr* lymph node T cells using asialo-agalacto-transferrin as the acceptor.[37] Whether this and other glycosylation abnormalities in MRL-*lpr* T cells[38–40] are associated with the defect in fas-mediated apoptosis in $B220^+$, $CD4^-$, $CD8^-$ (DN) T cells[41] remains to be established.

We have found no association between β-1,4-GalTase mRNA levels or enzyme-activity levels[36] and IgG galactosylation in RA B cells. Recent evidence obtained using lymphoblastoid cell lines also points to a lack of correlation between β-1,4-GalTase enzyme-activity levels and the extent of galactosylation.[42] It may be that β-1,4-GalTase enzyme levels are not directly related to the galactosylation defect of IgG, and that some other abnormality in IgG biosynthesis or regulation of glycosylation may be causing the defective IgG galactosylation seen in RA.

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REFERENCES

1. Parekh R.B., Dwek R.A., Sutton B.J. *et al.* (1985) Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. *Nature* **316,** 452.
2. Parekh R.B., Isenberg D.A., Ansell B.M., Roitt I.M., Dwek R.A. & Rademacher T.W. (1988) Galactosylation of IgG association oligosaccharides: reduction in patients with adult and juvenile onset rheumatoid arthritis and relation to disease activity. *Lancet* **i,** 966.
3. Tsuchiya N., Endo T., Matsuta K. *et al.* (1989) Effects of galactose depletion from oligosaccharide chains on immunological activities of human IgG. *J Rheumatol* **16,** 285.
4. Malhotra R., Wormald M.R., Rudd P.M., Fischer P.B., Dwek R.A. & Sim R.B. (1995) Glycosylation changes of IgG associated with rheumatoid arthritis can activate complement via the mannose-binding protein. *Nature Medicine* **1,** 237.
5. Rademacher T.W., Williams P. & Dwek R.A. (1994) Agalactosyl glycoforms of IgG autoantibodies are pathogenic. *Proc Natl Acad Sci USA* **91,** 6123.
6. Young A., Sumar N., Bodman K.B. *et al.* (1991) Agalactosyl IgG: an aid to differential diagnosis in early synovitis. *Arthritis Rheum* **34,** 1425.
7. Van Zeben D., Hazes J.M.W., Zwinderman A.H., Vandenbroucke J.P. & Breedveld F.C. (1993) Factors predicting outcome of rheumatoid arthritis: results of a follow-up study. *J. Rheumatol* **20,** 1288.
8. Axford J.S., Lydyard P.M., Isenberg D.A., Mackenzie L.E., Hay F.C. & Roitt I.M. (1987) Reduced B-cell galactosyltransferase activity in rheumatoid arthritis. *Lancet* **ii,** 1486.
9. Furukawa K., Matsuta K., Takeuchi F., Kosuge E., Miyamoto T. & Kobata A. (1990) Kinetic study of a galactosyltransferase in the B cells of patients with rheumatoid arthritis. *Int Immunol* **2,** 105.
10. Wilson I.B.H., Platt F.M., Isenberg D.A. & Rademacher T.W. (1993) Aberrant control of galactosyltransferase in peripheral B lymphocytes and Epstein–Barr virus transformed B lymphoblasts from patients with rheumatoid arthritis. *J Rheumatol* **20,** 1282.
11. Shur B.D. (1993) Glycosyltransferases as cell adhesion molecules. *Curr Opin Cell Biol* **5,** 854.
12. Shaper N.L., Shaper J.H., Bertness V., Chang H., Kirsch I.R. & Hollis G.F. (1986) The human galactosyltransferase gene is on chromosome 9 at band p13. *Somat cell Mol Genet* **12,** 633.
13. Hollis G.F., Douglas J.G., Shaper N.L. *et al.* (1989) Genomic structure of murine β-1,4-galactosyltransferase. *Biochem Biophys Res Comm* **162,** 1069.
14. Mengle-Gaw L., McCoy-Haman M.F. & Tiemeier D.C. (1991) Genomic structure and expression of human β-1,4-galactosyltransferase. *Biochem Biophys Res Comm* **176,** 1269.
15. Shaper N.L., Hollis G.F., Douglas J.G., Kirsch I.R. & Shaper J.H. (1988) Characterization of the full length cDNA for murine β-1,4-galactosyltransferase. *J Biol Chem* **263,** 10420.

16. Russo R.N., Shaper N.L. & Shaper J.H. (1990) Bovine β1-4-galactosyltransferase: two sets of mRNA transcripts encode two forms of the protein with different amino terminal domains. *J Biol Chem* **265**, 3324.
17. Harduin-Lepers A., Shaper J.H. & Shaper N.L. (1993) Characterization of two *cis*-regulatory regions in the murine β 1,4-galactosyltransferase gene. *J Biol Chem* **268**, 14 348.
18. Bond A., Cooke A. & Hay F.C. (1990) Glycosylation of IgG, immune complexes and IgG subclasses in the MRL-*lpr*/*lpr* mouse model of rheumatoid arthritis. *Eur J Immunol* **20**, 2229.
19. Bodman K.B., Hutchings P.R., Jeddi P.A. *et al.* (1994) IgG glycosylation in autoimmune-prone strains of mice. *Clin Exp Immunol* **95**, 103.
20. Axford J.S., Alavi A., Bond A. & Hay F. (1994) Differential B lymphocyte galactosyltransferase activity in the MRL mouse model of rheumatoid arthritis. *Autoimmunity* **17**, 157.
21. Jeddi P.A., Lund T., Bodman K.B. *et al.* (1994) Reduced galactosyltransferase mRNA levels are associated with the agalactosyl IgG found in arthritis-prone MRL-*lpr*/*lpr* strain mice. *Immunology* **83**, 484.
22. Arnett F.C., Edworthy S.M., Block D.A. *et al.* (1987) The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* **31**, 315.
23. Sumar N., Bodman K.B., Rademacher T.W., Dwek R.A., Williams P. & Parekh R.B. (1990) Analysis of glycosylation changes in IgG using lectins. *J Immunol Methods* **131**, 127.
24. Thompson S.J., Hitsumoto Y., Zhang Y.W., Rook G.A.W. & Elson C.J. (1992) Agalactosyl IgG in pristane-induced arthritis. Pregnancy affects the incidence and severity of arthritis and the glycosylation status of IgG. *Clin Exp Immunol* **89**, 434.
25. Shields J.G. & Turner M.W. (1986) The importance of antibody quality in sandwich ELISA systems. Evaluation of selected commercial reagents. *J Immunol Methods* **87**, 29.
26. Mullis P.E., Lund T., Patel M.S., Brook C.G.D. & Brickell P.M. (1991) Regulation of human growth hormone receptor gene expression by human growth hormone in a human hepatoma cell line. *Mol Cell Endocrin* **76**, 125.
27. Chomczynski P. & Sacchi N. (1987) Single-step method of RNA isolation by acid guanidium thiocyanate-phenol-chloroform extraction. *Anal Biochem* **162**, 156.
28. Bodman K.B., Sumar N., Mackenzie L.E. *et al.* (1992) Lymphocytes from patients with rheumatoid arthritis produce agalactosylated IgG *in vitro*. *Clin Exp Immunol* **88**, 420.
29. Furukawa K. (1991) IgG galactosylation—Its biological significance and pathology. *Mol Immunol* **28**, 1333.
30. Dwek R.A. (1995) Glycobiology: 'towards understanding the function of sugars'. *Biochem Soc Trans* **23**, 1.
31. Field M.C., Amatayakul-Chantler S., Rademacher T.W., Rudd P.M. & Dwek R.A. (1994) Structural analysis of the N-glycans from human immunoglobulin A1; comparison of normal human serum immunoglobulin A1 with that isolated from patients with rheumatoid arthritis. *Biochem J* **299**, 261.
32. Rademacher T.W., Wilson I.B.H. & Isenberg D.A. (1994) Aberrant control of galactosyltransferase, Dr Rademacher, reply. *J Rheumatol* **21**, 2169.
33. Strous G.J., van Kerkhof P., Fallon R.J. & Schartz A.L. (1987) Golgi galactosyltransferase contains serine-linked phosphate. *Int J Biochem* **109**, 307.
34. Bunnell B.A., Adams D.E. & Kidd V.J. (1990) Transient expression of a p58 protein kinase cDNA enhances mammalian glycosyltransferase activity. *Biochem Biophys Res Comm* **171**, 196.
35. Axford J.S. (1992) Changes in normal glycosylation mechanisms in autoimmune rheumatic disease. *J Clin Invest* **89**, 1021.
36. Keusch J., Lydyard P.M., Isenberg D.A. & Delves P.J. (1995) Beta-1,4-galactosyltransferase activity in B-cells detected using a simple ELISA-based assay. *Glycobiology* **5**, 365.
37. Imai Y., Yamashita Y. & Osawa T. (1988) Enhancement of the activities of glycosyltransferases involved in the biosynthesis of mucin-type sugar chains in autoimmune MRL *lpr*/*lpr* mouse T cells. *Mol Immunol* **25**, 419.
38. Katagiri T., Mori T., Nakano T., Ueno K. & Kano K. (1984) Aberrant expression of Forssman and Paul–Bunnel antigens on lymph node cells of MRL/Mp-*lpr*/*lpr* mice. *J Immunol* **133**, 3143.
39. Yamashita Y., Imai Y. & Osawa T. (1989) Poly(N-acetyllactosamine)-type sugar chains in CD45 antigens of abnormal T cells of *lpr* mice are different from those of normal T cells and B cells. *Mol Immunol* **26**, 905.
40. Ishii N. & Watanabe K. (1992) Aberrant expression of GM1 on lymph node cells of MRL/Mp-*lpr*/*lpr* mice: influences on the autoreactivities of anti-asialo GM1 antibodies. *Autoimmunity* **13**, 107.
41. Watanabe-Fukunaga R., Brannan C.I., Copeland N.G., Jenkins N.A. & Nagata S. (1992) Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. *Nature* **356**, 314.
42. Kumpel B.M., Rademacher T.W., Rook G.A.W., Williams P.J. & Wilson I.B.H. (1994) Galactosylation of human IgG monoclonal anti-D produced by EBV-transformed B lymphoblastoid cell lines is dependent on culture method and affects Fc receptor-mediated functional activity. *Hum Antibod Hybridomas* **5**, 143.