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Changes in the glycosylation of IgG in the collagen-induced model of arthritis

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It is now well established that rheumatoid arthritis patients have reduced levels of galactose on their immunoglobulin G (IgG) molecules compared with normal individuals. We have investigated whether, in an experimentally induced model of arthritis, similar glycosylation changes on IgG are to be found. Serum IgG was isolated from collagen-induced arthritic DBA/1 mice and a control group, and the glycosylation of the IgG in these preparations was compared using lectin blotting. The glycosylation of IgG in immune complexes was also analysed. Arthritic mice exhibited similar glycosylation changes on their IgG as observed for rheumatoid arthritis patients. On average, there was less galactose on the IgG from arthritic mice than from the control group, but this difference was of borderline significance. However, the *N*-acetylglucosamine content of IgG was significantly elevated in arthritic mice. There was no difference in the sialic acid content of IgG in the two groups. The results for immune complexes were similar to those obtained for serum IgG, but the data were limited by insufficient numbers. The similarity in glycosylation changes in collagen-induced arthritis and in patients with rheumatoid arthritis suggests that common pathogenic mechanisms may be involved.

Keywords: arthritis, collagen, glycosylation

Introduction

The immune response to type II collagen has been used to establish an experimental model of arthritis in mice [1]. The histological and clinical manifestations of the disease bear a close resemblance to human rheumatoid arthritis [2]. Both humoral and cell-mediated immune responses to collagen are involved in the arthritic process. Anti-collagen antibodies are able to transfer the disease, and the development of arthritis is suppressed by anti-IgM treatment [3]. T cells specific for collagen type II are able to transfer the arthritis to both irradiated and normal syngeneic recipient mice [4]. Treatment with anti-CD4 antibody reduces the severity of the arthritis in the collagen-induced experimental model [5, 6].

Biochemical differences have been observed on the IgG molecules in patients with rheumatoid arthritis. The oligosaccharides found on the IgG molecule are different in rheumatoid patients when compared with normal individuals. Glycosylation changes in the immunoglobulin G (IgG) molecule were first reported by Mullinax [7], who showed that the total galactose content of serum IgG is significantly decreased in patients with rheumatoid arthritis. Oligosaccharides on the Fc region of IgG usually terminate in either sialic acid or galactose. Sequential studies on the oligosaccharides present in the Fc region of IgG revealed that the terminal galactose is frequently missing in patients with rheumatoid arthritis, thus exposing an *N*-acetylglucosamine [8]. Hymes *et al.* [9] made a series of interesting observations on antiglobulin purified from a patient with rheumatoid arthritis and Hodgkin's disease in remission. They found that the light chains on the antiglobulin contained 17 times

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more sialic acid than normal IgG and that cleavage of the sialic acid destroyed the antiglobulin activity completely. They thus concluded that the $F(ab')_2$ sugars were crucial for forming self-associated complexes. It has been suggested that in rheumatoid arthritis patients the self-associating immune complexes may arise from the sugars on $F(ab')_2$ of IgG fitting into the pocket left vacant by the missing galactose on the Fc region [10].

In this study we investigated whether mice with collagen-induced arthritis parallel rheumatoid arthritis patients in having glycosylation defects on their IgG molecules. We compared the levels of sialic acid, galactose and *N*-acetylglucosamine on IgG molecules from collagen-immunized arthritic mice and control mice immunized with Freund's complete adjuvant.

Materials and methods

Collagen-induced arthritis

Male DBA/1 mice were purchased from Olac (Bicester, UK). Native rat collagen type II was obtained from a rat chondrosarcoma by pepsin digestion followed by salt precipitation (a gift from Dr Brian Champion). The collagen was dissolved in 0.05 M acetic acid at 2 mg/ml and emulsified in an equal volume of complete Freund's adjuvant (Difco) at 4°C. Animals were injected with 100 μl of this emulsion subcutaneously at the base of the tail and with 100 μl of BCG (Evans) intraperitoneally on day 0. The mice were boosted on day 21 with 100 μg of rat collagen type II and 100 μl of BCG intraperitoneally. The arthritis developed by day 28 and the mice were bled out at the peak of their arthritis.

Control mice were age matched and immunized with 100 μl of Freund's complete adjuvant (Difco) subcutaneously at the base of the tail and 100 μl of BCG intraperitoneally on day 0. The mice were boosted on day 21 with 100 μl of BCG intraperitoneally.

Histology

Histological studies were carried out on the control and collagen-induced arthritic mice to establish the extent of the arthritic process. Paraffin sections of the joints were stained with haematoxylin and eosin.

Purification of IgG

IgG was purified by a combination of salt fractionation and ion-exchange chromatography [11]. Aliquots of 50 μl of sera were mixed with equal volumes of saturated ammonium sulphate solution (BDH) for 30 min at room temperature. Supernatants were removed after centrifugation and the pellets were resuspended in 50 μl of 200 mM potassium phosphate buffer, pH 7.2. Following extensive dialysis against the potassium phosphate buffer, the samples were loaded onto columns filled with DEAE–cellulose DE52 (Whatman), which had been pre-equilibrated with the potassium phosphate buffer. Fractions were collected and the first peak containing IgG retained for protein estimation.

Protein estimation

Proteins were estimated using the Pierce BCA assay reagent (Pierce Europe), which involves the biuret reaction and the detection of Cu^{2+} ion using bicinchoninic acid.

Detection of IgG glycoforms by immunoblotting

Sheets of nitrocellulose (Schleicher & Schull) were soaked in phosphate-buffered saline (PBS) for 5 min and inserted into the Bio-Rad dot-blot apparatus (Bio-Rad Laboratories). Samples (100 μl) of IgG (10 μg/ml PBS) were blotted in triplicate onto the nitrocellulose, air dried by suction, and the blots were removed and denatured by boiling for 5 min in half-strength PBS. The nitrocellulose was blocked with a solution of 1% bovine serum albumin (BSA) in PBS containing 0.05% Tween 20.

Terminal glactose and *N*-acetylglucosamine were detected by our previously developed lectin assay [12]. Sialic acid was detected by digoxigenin-labelled *Sambucus nigra* (SNA) at 1 μg/ml (Boehringer Mannheim). *Ricinus communis* agglutinin (RCA1), a gift from Dr E. Wawrzynczak, was biotinylated and diluted to 5 μg/ml in PBS/BSA. Biotinylated *Bandeiraea simplicifolia* (BSII; Vector Laboratories) was diluted to 5 μg/ml in PBS/BSA. The nitrocellulose-blotted IgG samples were incubated in the lectin solutions for 2 h at room temperature on a rocker. Following three washes in PBS/BSA the blots were incubated for 2 h on a rocker with streptavidin (1:500 dilution in PBS/BSA) for ricin and BSII, and anti-digoxigenin for SNA (1:1000 in PBS/BSA). Following three washes with PBS/BSA the RCA1 and BSII blots were incubated with chloronaphthol and SNA with 4-nitroblue tetrazolium chloride and 5-brom-4-chloro-indolyl phosphate until the colour developed and then read at 405 nm in a video densitometer (Bio-Rad). Samples were compared in the

same assay to avoid variations of colour development. The specificity of the lectins was established using a panel of glycoproteins (carboxypeptidase, transferrin, fetuin and asialofetuin) as supplied in the glycan differentiation kit (Boehringer Mannheim).

Isolation of immune complexes

Complexed IgG was isolated from sera by differential solubility in polyethylene glycol (PEG) [11]. Fifteen microlitres of a 12% PEG 6000 solution was added to 50 μl of serum and then left overnight at 4°C. Following centrifugation the pellets were washed with 2% PEG solution in veronal-buffered saline, and their protein content was determined using the Pierce BCA assay.

Statistical analysis

Significant differences between means were determined using the Mann–Whitney test.

Results

Collagen-induced arthritis

Arthritis developed in collagen-treated mice from day 28. Histological studies confirmed severe arthritic changes in the joints of the collagen-treated mice (Figure 1), although the degree of arthritic changes varied within the group. The control group was found to have no abnormalities in the joints.

Lectin detection of galactose IgG

Galactose was readily detectable with RCA1 lectin on DBA/1 mouse IgG. The galactose was lower on the IgG samples from the collagen-induced arthritic group ($P > 0.05$ Mann–Whitney; $P < 0.027$ Student's t) compared with the controls (Figure 2).

Lectin detection of N-acetylglucosamine on IgG

N-acetylglucosamine as detected by BSII was found to be significantly elevated on the IgG

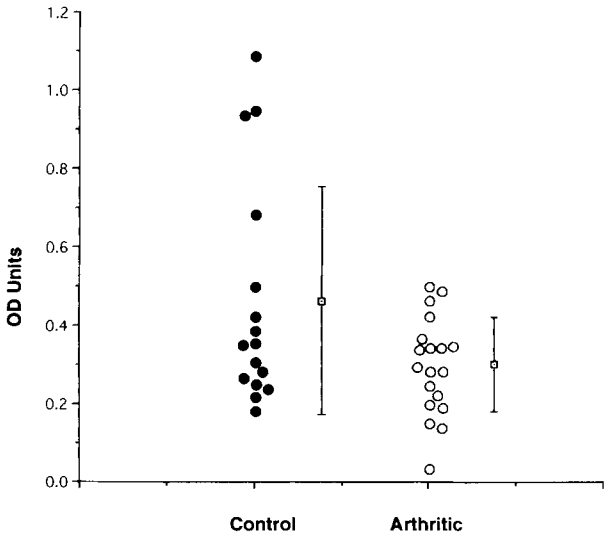

Figure 2. Binding of ricin to galactose on purified IgG from control and arthritic DBA/1 mice. Bars indicate the means and standard deviations.

Figure 1. A joint showing irregularities of the articular surface (A) with a mixed inflammatory infiltrate (I) filling the joint space and extending into the periarticular soft tissue (PA). There is inflammatory damage to the periosteum (PO) resulting in bone destruction and new bone formation.

samples from the collagen-induced arthritic group compared with the control group ($P < 0.01$) (Figure 3). Eleven out of 23 arthritic mice had BSII levels higher than any of the control group. The difference between the arthritic and the control group was more significant with BSII than with RCA1. This is very similar to our human studies, in which BSII is better able to discriminate between normal subjects and patients with rheumatoid arthritis. Furthermore, as with our human studies, the correlation of the inverse pattern of binding between the RCA1 and BSII was not significant.

Lectin detection of sialic acid on IgG

A wide range was observed in the SNA binding on the IgG samples from the control group. No significant differences were observed between the mean values from the collagen-induced arthritic group and the control group (Figure 4).

Arthritic score

There was no correlation in the arthritic score of the mice with the lectin binding on their IgG molecules (data not shown).

Analysis of oligosaccharides in serum enriched for immune complexes

We have analysed the oligosaccharides on IgG molecules from circulating immune complexes. Serum was precipitated with polyethylene glycol to enrich for immune complexes, however monomeric IgG will also be present. The analysis of oligosaccharides on IgG molecules from immune complexes was made difficult as only low levels of circulating immune complexes were found in both the control group and in the collagen-induced arthritic DBA/1 mice. Sufficient quantities of immune complexes for sugar analysis were found in only a few animals, however the limited data obtained would seem to suggest that lectin binding in the serum immune complexes reflected that in the serum IgG for both RCA1, BSII and SNA binding (Figure 5 a–c).

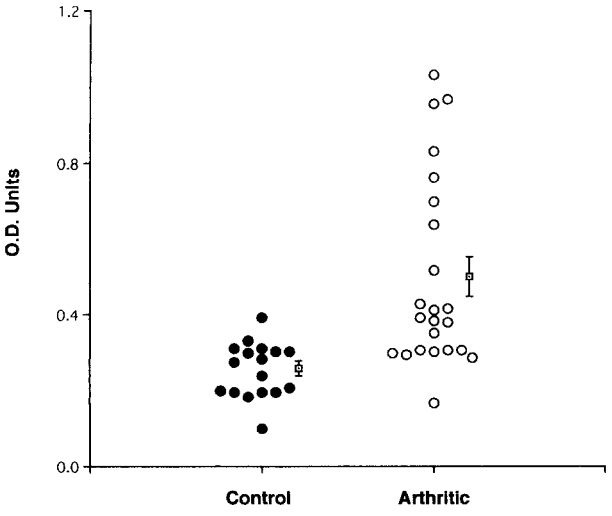

Figure 3. Binding of *Bandeiraea* to *N*-acetylglucosamine on purified IgG from control and arthritic DBA/1 mice ($P < 0.01$). Bars indicate the means and standard deviations.

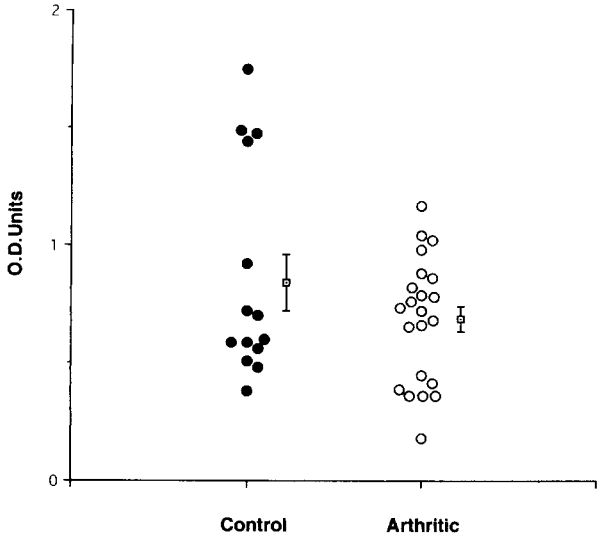

Figure 4. Binding of *Sambucus nigra* to sialic acid on purified IgG from control and arthritic DBA/1 mice. Bars indicate the means and standard deviations.

Discussion

Collagen-induced arthritis in mice closely resembles rheumatoid arthritis in both clinical manifestations and microscopical appearance. We now report that collagen-immunized arthritic mice have glycosylation defects in their IgG similar to rheumatoid arthritis patients.

Collagen-immunized arthritic mice have slightly lower levels of lectin-detectable galactose on their IgG and a significantly increased level of lectin-detectable *N*-acetylglucosamine. This inverse binding pattern is similar to the human rheumatoid arthritis patients, although there was no significant correlation. Interestingly, in this experimental model for arthritis the lectin binding pattern on IgG is different to that in spontaneous systemic lupus erythematosus (SLE)-like autoimmune mice, MRL/lpr [13], in which more *N*-acetylglucosamine

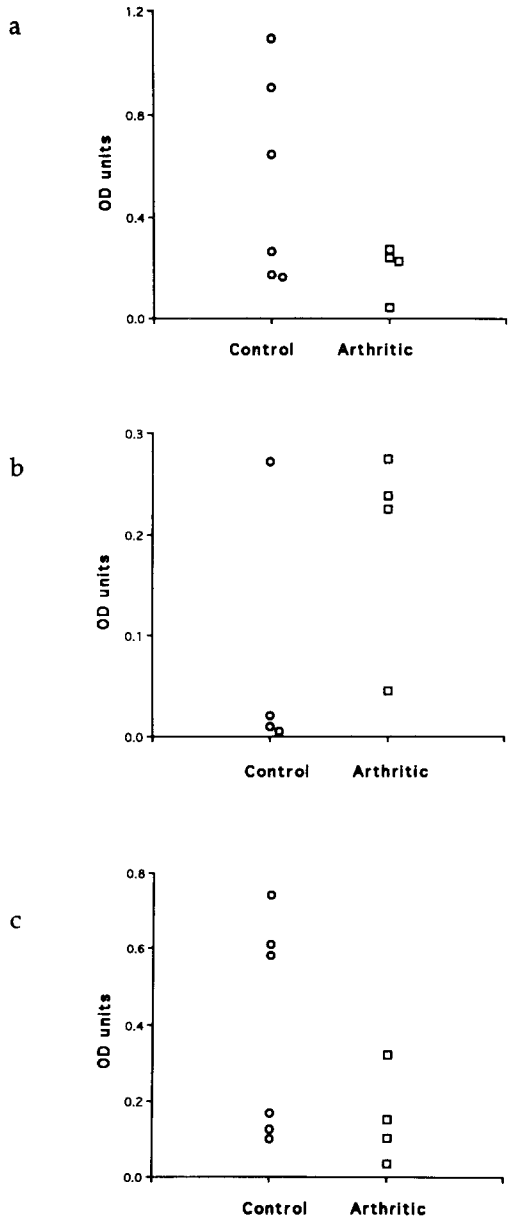

Figure 5. Binding of (a) ricin to galactose, (b) *Bandeiraea* to *N*-acetylglucosamine and (c) *Sambucus nigra* to sialic acid on polyethylene glycol-preciptated Ig from control and arthritic DBA/1 mice.

and galactose are seen at the same time on the IgG molecules.

The pathogenic significance of these oligosaccharides changes in rheumatoid arthritis is uncertain. Pregnancy reveals that there is a close parallel between the degree of glycosylation on the IgG molecule and the clinical severity of the disease [14]. Rook *et al.* (personal communication) have proposed that the glycosylation of IgG may be an important factor in the transfer of autoantibodies to the fetus in an autoimmune mother. Early synovitis patients who have a defective glycosylation on their IgG are also the most likely to develop rheumatoid arthritis [15]. We have no data to determine whether glycosylation defects are necessary for the onset of arthritis or whether they are a consequence of the inflammatory changes, but we are now examining our experimental mice to see if the glycosylation changes precede the development of inflammation.

Cytokines interleukin 1 (IL-1) and IL-6 released during inflammation have been implicated in the regulation of glycosylation of acute-phase proteins in rheumatoid arthritis [16]. Both collagen-immunized arthritic mice and rheumatoid arthritis patients have raised IL-6 levels [17, 18]. Interestingly, there was a striking decrease in the glycosylation of IgG in IL-6 transgenic mice [19], which suggests that cytokines may have an important role in the regulation on the glycosylation of molecules.

We have established that there are differences in the glycosylation of IgG molecules in the arthritic group compared with the control group. It is unlikely that the glycosylation defect is restricted to only the IgG molecule in the arthritic group. Glycosylation differences have been found in other glycosylated proteins such as α_1-acid glycoprotein (AGP) in rheumatoid arthritis [20]. Carbohydrates are now considered to be very important in cell-to-cell recognition and in homing of lymphocytes to certain sites [21, 22]. Defects of IgG glycosylation may reflect a general defect in the glycosylation of molecules in arthritic individuals, which may have far-reaching implications as to the trafficking of lymphocytes. Changes in cell-surface and high endothelial venule glycosylation may lead to the lymphocytes migrating to the synovium [23], resulting in pathogenic chronic inflammation. Glycosylation changes on IgG have also been shown to have profound effects on antigen–antibody interaction, and those slight changes in the position of carbohydrate can effect changes of substantial magnitude in binding specificity and affinity [24]. Furthermore, variations in the glycosylation of IgG can affect complement activation and binding to phagocytic cells [25] that may have pronounced effects on the inflammatory properties of the immune complexes.

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References

1. Courtenay JS, Dallman MJ, Dayan AD, Martin A, Mosedale B. Immunisation against heterologous type II collagen induces arthritis in mice. *Nature* 1980: **283**; 666–8.
2. Trentham DE. Collagen arthritis as a relevant model for rheumatoid arthritis. *Arthritis Rheum* 1982: **25**; 911–16.
3. Helfgottt SM, Bazin H, Dessein A, Trentham DE. Suppressive effect of anti-μ serum on the development of collagen arthritis in rats. *Clin Immunol Immunopathol* 1984: **31**; 403–11.
4. Holmdahl R, Klareskog I, Rubin K, Larsson E, Wigzell H. T lymphocytes in collagen II-induced arthritis in mice. *Scand J Immunol* 1985: **22**; 295–306.
5. Ranges GE, Subramaniam S, Cooper SM. Prevention of type II collagen-induced arthritis by *in vivo* treatment with anti-L3T4. *J Exp Med* 1985: **162**; 1105–10.
6. Hom JT, Butler LD, Riedl PE, Bendele AM. The progression of the inflammation. *Immunology* 1988: **18**; 881–8.
7. Mullinax F. Abnormality of IgG structure in rheumatoid arthritis and systemic lupus erythematosus. *Arthritis Rheum* 1975: **18**; 417–18.
8. Parekh RB, Dwek RA, Sutton BJ, *et al.* Association of rheumatoid arthritis and primary osteoarthritis with changes in the glycosylation pattern of total serum IgG. *Nature* 1985: **316**; 452–7.
9. Hymes AJ, Mullinax GL, Mullinax F. Immunoglobulin carbohydrate requirement for formation of an IgG–IgG complex. *J Biol Chem* 1979: **254**; 3148–51.
10. Roitt IM, Dwek RA, Parekh RB, *et al.* The role of antigen in autoimmune responses with special references to changes in carbohydrate structure of IgG in rheumatoid arthritis. *J Autoimmunity* 1989: **1**; 499–506.
11. Hudson L, Hay FC. *Practical Immunology*, 2nd edn. Oxford: Blackwell Scientific Publications, 1980.
12. Sumar N, Bodman KB, Rademacher TW, *et al.* Analysis of glycosylation changes in IgG using lectins. *J Immunol Methods* 1990: **131**; 127–36.
13. Bond A, Cooke A, Hay FC. Glycosylation of IgG, immune complexes and IgG subclasses in the MRL-lpr/lpr mouse model of rheumatoid arthritis. *Eur J Immunol* 1990: **20**; 2229–33.
14. Pekelharing JM, Hepp E, Kamerlin JP, Gerwig GJ, Leijnse B. Alterations in carbohydrate composition of serum IgG from patients with rheumatoid arthritis and from pregnant women. *Ann Rheum Dis* 1988: **47**; 91–5.
15. Young A, Sumar N, Bodman KB, *et al.* An aid to differential diagnosis in early synovitis. *Br J Rheumatol* 1990: **29**; 47.
16. Pos O, Moshge HJ, Yap SH, *et al.* Effects of monocytic products, recombinant interleukin-1, and recombinant interleukin-6 on glycosylation of alpha-1-acid glycoprotein: studies with primary human hepatocyte cultures and rat: *Inflammation* 1989: **13**; 415.
17. Takai Y, Seki N, Senoh H, *et al.* Enhanced production of interleukin-6 in mice with type II collagen-induced arthritis. *Arthritis Rheum* 1989: **32**; 594–600.
18. Kishimoto T, Hirano T. Molecular regulation of B lymphocyte response. *Annu Rev Immunol* 1988: **6**; 485–512.
19. Rook G, Thompson S, Buckley M, *et al.* The role of oil and agalactosyl IgG in the induction of arthritis in rodent models. *Eur J Immunol* 1990: **21**; 1027–32.
20. Mackiewicz A, Pawlowski AT, Mackiewicz A, Pawlowska A, Wiktorowicz K, Machiewicz S. Microheterogeneity forms of alpha-1-acid glycoprotein as indicators of rheumatoid arthritis activity. *Clin Chim Acta* 1987: **163**; 185–90.
21. True DD, Singer MS, Lasky LA, Rosen SD. Requirement for sialic acid on the endothelial ligand of a lymphocyte homing receptor. *J Cell Biol* 1990: **111**; 2757–64.
22. Yednock TA, Rosen SD. Lymphocyte homing. *Advances Immunol* 1989: **44**; 313–78.
23. Ziff M. Role of the endothelium in chronic inflammatory synovitis. *Arthritis Rheum* 1991: **34**; 1345–52.
24. Wright A, Tao M, Kabat A, Morrison SL. Antibody variable region glycosylation: position effects on antigen binding and carbohydrates structure: *EMBO J* 1991: **10**; 2717–23.
25. Duncan AR, Winter G. The binding site for C1q on IgG. *Nature* 1988: **332**; 738–40.

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