CHANGES IN GLYCOSYLATION OF IgG DURING FASTING IN PATIENTS WITH RHEUMATOID ARTHRITIS

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SUMMARY

Patients with rheumatoid arthritis (RA) have a higher proportion of agalactosyl IgG than healthy individuals. Glycosylation status was examined in 26 RA patients who fasted for 7-10 days and afterwards followed a vegetarian diet for 3.5 months. The decrease in the proportion of agalactosyl IgG correlated significantly with the clinical improvement after the fasting period, but not after the vegetarian diet period. Although the glycosylation status of IgG may have played a role in the improvement of disease during the fasting period, it did not seem to be associated with, and therefore responsible for, the clinical improvement observed after the vegetarian diet.

KEY WORDS: Agalactosyl IgG, Rheumatoid arthritis, Disease activity, Vegetarian diet, Fasting.

The human IgG heavy chain has an N-glycosylation site, at Asn 297 on the CH₂ domain. This site bears biantennary oligosaccharides, a variable proportion of which bear terminal galactose on one or both of their outer arms [1]. In sera from normal individuals, the proportion of IgG that lacks terminal galactose [%G(0)] varies with age [2]. Patients with rheumatoid arthritis (RA), Crohn's disease and tuberculosis have been shown to have higher %G(0) than age-matched healthy controls [1]. Pregnancy-induced remission of RA and post-partum disease exacerbation have been found to occur simultaneously with changes in %G(0) [3]. As the biological properties of agalactosyl IgG are different from those of IgG with terminal galactose on the oligosaccharide chains, it has been suggested that agalactosyl IgG participates in the pathogenesis of rheumatoid inflammation [1].

Like pregnancy, fasting is known to reduce disease activity in RA patients [4-7]. The influence of fasting on the glycosylation status of IgG has not previously been studied in RA patients. The aim of the present study was to correlate changes in %G(0) with clinical changes during a 7-10 day period of fasting followed by 3.5 months of vegetarian diet.

PATIENTS AND METHODS

Patient population, diet and assessment of disease activity

Twenty-six RA patients were treated with fasting, followed by a vegetarian diet for 3 months as part of a clinical trial [8]. The dietary regimen has been described elsewhere [8]. Briefly, the patients began the trial with a fasting period of 7-10 days duration. After the fasting, the diet was gradually 'built up' by introducing small amounts of a limited number of food items. During the following 3.5 months, the patients were kept on a strict gluten-free vegan diet, i.e. they were not allowed to eat meat, fish, eggs, dairy products and food that contained gluten. Clinical examinations were performed and blood samples were drawn at the time of inclusion, and after 1 and 4 months. Serum samples were stored at −20°C until required for analysis.

In order to assess the clinical improvement, a disease improvement index was constructed that was based on number of swollen joints, Stanford health assessment questionnaire index [9], pain score on a visual analogue scale, number of tender joints, patient's global assessment and erythrocyte sedimentation rate [10]. These variables have recently been assigned as the core measures for clinical trials in rheumatoid arthritis [11]. A two grade improvement in patient's global assessment was considered as a substantial improvement. For the other five variables, a ≥20% improvement compared with the baseline value was defined as a substantial improvement [12]. A disease improvement index, which was the sum of the number of core variables that had improved substantially compared with baseline, was assigned to each patient after 1 and 4 months, respectively.

Assessment of the proportion of agalactosyl IgG

The procedure for measuring %G(0) was a slight modification of the methods previously described by Sumar et al. [13] and Thompson et al. [14]. Briefly, Maxisorb immunoplates were coated with 50 μl/well protein A (Sigma) at 5.0 μg/ml in phosphate-buffered saline (PBS) and incubated overnight at 4°C. The wells were aspirated and non-specific binding was blocked with PBS–Tween (0.05%)–bovine serum albumin (BSA) (1.0%) for 1 h at 37°C, followed by three washes with PBS–Tween. Aliquots of diluted sera were added in duplicate to two identical plates and incubated for 2 h at 37°C. After washing, PBS was added and the plates floated on a water bath at 85°C for 10 min to partially denature the IgG molecules to expose the
oligosaccharides. The biotinylated lectins *Bandeiraea simplicifolia* (BSII) and *Ricinus communis* agglutinin (RCAI) (Vector Laboratories) were added to the cooled duplicate plates and incubated at 4°C overnight. After three washes, streptavidin-peroxidase was added and incubated at 37°C for 1 h. A colour reaction was produced using 2,2′-azino-bis-3-ethylbenzthiazoline-6-sulphonic acid and hydrogen peroxide. After 15 min, the reaction was stopped with NaF (2 mg/ml) and the plates read on an automated ELISA reader (Dynatech Laboratories Ltd, Billingshurst) at 410 nm. The ratios BSII/RCAI were calculated for patient samples and for standards of known %G(0) levels, and the %G(0) of the patients’ samples were deduced from the standard curve.

### RESULTS

Patient characteristics are given in Table I. There was no significant difference in the mean %G(0) between baseline samples and samples collected either after the fasting period or after 3.5 months of vegetarian diet (Wilcoxon signed-rank test). However, the difference between %G(0) at baseline and after the fasting period correlated significantly with the disease improvement index after the fasting period (Kendall’s $\tau = 0.29, P = 0.04$) (Fig. 1a). After 3.5 months on a vegetarian diet, the correlation between the change in %G(0) and disease improvement index was no longer significant (Fig. 1b).

### DISCUSSION

Several studies have convincingly demonstrated that fasting can ameliorate clinical manifestations of RA [4–7], and several features associated with the inflammatory process have been shown to change during energy deprivation. For example, fasting modifies lymphocyte responses to mitogens [6], it reduces the generation of chemoattractants in plasma [5, 7], it alters the fatty acid composition of cell membranes [7] and influences several neutrophil functions [5, 7]. In agreement with these reports, it was previously shown that the clinical and laboratory disease activity variables of the RA patients in the present study changed significantly during the fasting period, denoting reduced disease activity [8]. Furthermore, this improvement was sustained during the following 3.5 months in which the patients followed a vegetarian diet.

In the present study, we have examined the relationship between changes in IgG glycosylation and clinical improvement after fasting and vegetarian diet, respectively. As no single clinical or biochemical disease activity variable is considered suitable to assess disease activity in RA patients, we constructed a disease improvement index based on six core variables [10]. After the fasting period, but not after the vegetarian diet, there was a significant positive correlation between the clinical improvement and the reduction in %G(0).

The interpretation of positive correlations is always difficult with regard to the relationship between cause and effect. Is the clinical improvement observed after fasting (in part) due to a normalization of IgG glycosylation, or is the reduction in %G(0) merely an epiphenomenon secondary to reduction in disease activity? In favour of the first proposal is the fact that the glycosylation status of IgG has a profound influence on effector functions that are dependent on interactions with cell-bound receptors. For example, IgG lacking terminal galactose on the oligosaccharide side chains has been shown to have reduced binding to monocyte or macrophage Fc receptors and the Clq component.

**TABLE I**

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Median</th>
<th>Range</th>
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<tbody>
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<td>26–63</td>
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<table>
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<th>Female</th>
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<table>
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<th>Disease duration (yr)</th>
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<td>14</td>
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<th>Corticosteroids</th>
<th>Antimalarial drugs</th>
<th>Penicillamine</th>
<th>Sulphasalazine</th>
<th>Cytostatic drugs</th>
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</tr>
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<td></td>
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<td>5</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

*Waxler’s test was carried out with human red cells sensitized with rabbit IgG. Titres $\geq 32$ were considered as positive.
of complement [15, 16]. It has also been suggested that agalactosyl IgG may play a role in the formation of immune complexes and interaction with RF [17, 18].

However, since the correlation between the change in \( \%G(0) \) and the disease improvement index was not significant after the period in which the patients followed a vegetarian diet, it is likely that the improvement observed after 3.5 months was due to other factors than the IgG glycosylation. This lack of correlation between the change in \( \%G(0) \) and disease improvement index may be explained by the disease duration of the patients, which has been shown to be an important factor when looking at correlations between \( \%G(0) \) and clinical variables [19–21]. The \( \%G(0) \) can be predictive of disease outcome in patients with < 2 yr disease duration [19], and differences in \( \%G(0) \) in serum and synovial fluid from RA patients are also related to disease duration [20]. In the present study, the median disease duration was 6 yr; therefore, by looking at patients with such a wide range (from 1 to 38 yr), we may be obscuring any further correlations. Although it is possible that changes in glycosylation of IgG during the fasting period may have contributed to the clinical improvement, our results do not indicate that the glycosylation status of IgG plays an important role in the long-term diet-mediated amelioration of RA.

REFERENCES