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Effect of Infliximab on the Glycosylation of IgG of Patients With Rheumatoid Arthritis

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In patients with rheumatoid arthritis (RA) a decrease in the terminal galactose content of N-linked glycans of the Fc region of agalactosyl immunoglobulin G (IgG) (G0) occurs. The aim of this study was to evaluate, for the first time, the effect of infliximab, a new monoclonal antibody for the treatment of RA, on this phenomenon. A total of 19 patients with active RA were treated with intravenous infliximab (3 mg/kg) in combination with methotrexate (MTX) (10–20 mg). IgG was purified from their serum by caprylic acid. Analysis of IgG glycosylation was performed by lectin blotting/immunoblotting and enzyme linked lectin assay (ELLA)/enzyme linked immunosorbent assay (ELISA) using the *Griffonia (bandeiraea) simplicifolia* lectin II and protein-A/alkaline phosphatase. The purity of IgG samples obtained was higher than 90%. The sensitivity of the lectin/immunoblotting method was of about 0.25 μg of IgG. The inter- and intraassay coefficients of variation (CV) were 1.3% and 9.0% for lectin blotting, and 4.6% and 8.3% for immunoblotting, respectively. The sensitivity of the ELLA/ELISA approach was 0.025 μg/μL and the inter- and intraassay CV were 6.2% and 7.7% for ELLA, and 5.1% and 14.1% for ELISA, respectively. A good linear correlation ($r^2 = 0.18$, $P < 0.05$) was obtained between the two different experimental approaches. A decrease of G0 was observed in patients who clinically improved (according to the American College of Rheumatology criteria) following the pharmacological treatment. Our data indicate that infliximab can reduce the concentration of G0 in patients with active RA. J. Clin. Lab. Anal. 21:303–314, 2007.

Key words: infliximab; rheumatoid arthritis; IgG glycosylation; lectin; immunoblotting; enzyme-linked immunosorbent assay (ELISA)

INTRODUCTION

It is well known that protein glycosylation plays a central role in many functions of the immune system (1). A number of diseases are characterized by abnormal protein glycosylation (2), including rheumatoid arthritis (RA), which is known to be associated with changes of immunoglobulin G (IgG) glycosylation (3,4). In particular, a decrease in the terminal galactose content of N-linked glycans of the fragment crystallizible (Fc) region of agalactosyl IgG (G0) was described, a phenomenon responsible of the induction of autoantigenicity and production of rheumatoid factor (RF), autoantibodies directed against the Fc region of G0 (5).

Different drugs are known to affect protein glycosylation, such as: 1) tunicamycin, streptovirudin, mycospocidin, 2-deoxyglucose, 2-fluoro-2-deoxy-D-glucose,

Abbreviations: BS II, *Griffonia (bandeiraea) simplicifolia* lectin II; ELISA, enzyme-linked immunosorbent assay; ELLA, enzyme-linked lectin assay; G0, agalactosyl IgG; MTX, methotrexate; QC, quality control; RA, rheumatoid arthritis; RF, rheumatoid factor(s); TNF, tumor necrosis factor.

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fluoroglucose, fluoromannose, glucosamine, mannosamine, amphomycin, tsushimycin, bacitracin, showdomycin, and diumycin, which are inhibitors of the lipid-linked saccharide (dolichol) pathway (6); 2) bromoconduritol, 1-deoxymannojirimycin, swainsonine, and castanospermine, which are known to interfere with the normal processing of asparagine-linked oligosaccharides of glycoproteins (6); 3) 25-hydroxycholesterol and compactin, inhibitors of dolichol synthesis (6); 4) monensin and nigeracin, inhibitors of glycoprotein transport (6); 5) methotrexate (MTX), which is capable of markedly inhibiting the fucosylation and sialylation of human serum α-acid-glycoprotein in RA patients (7); 6) leflunomide, capable of causing perturbation of protein glycosylation events through depletion of pyrimidine nucleotide sugar (8); 7) chloroquine and ammonium chloride, which can interfere with the sialylation of Ig (9); and 8) bindarit, an inhibitor of abnormal glycosylation of hemopexin in rats with adjuvant arthritis (10,11).

However, up to our best knowledge, the only drug capable of affecting the G0 concentration in patients with RA is sulphasalazine, which is able to reverse the reduction of the lymphocytic galactosyltransferase (Gtase) activity (4).

Recently, new drugs for the treatment of RA have been developed, including biological agents such as infliximab (12), a chimeric monoclonal antibody that binds soluble tumor necrosis factor α (TNF-α), reducing its biological activity and, as a consequence, inflammation (13). Since the effect of infliximab on IgG glycosylation is still unknown, the aim of this study was to measure IgG glycosylation in patients with RA treated with infliximab.

MATERIALS AND METHODS

Reagents

Acrylamide/bis-acrylamide, 40% solution (cat. #A-7168), blotting paper (cat. #P-4681), bovine serum albumin (cat. #A-3294), Bradford reagent (cat. #B-6916), bromophenol blue (cat. #18030), CAPS (3-cyclohexylamino-1-propanesulfonic acid, cat. #C-2632), diethanolamine (cat. #D-8885), DTT (1,4-dithio-DL-trheitol, cat. #43815), human IgG (cat. #I-4506), 2-mercaptoethanol (cat. #M-6250), phosphatase substrate (cat. #S-0942), protein A-alkaline-phosphatase (cat. #P-7488), sodium acetate (cat. #S-8750), sodium azide (cat. #S-2002), sodium hydroxide (cat. #S-0899), sodium phosphate (cat. #S-7907), Tris (cat. #T-1503), and Tween 20 (cat. #P-7949), were obtained from Sigma-Aldrich Co (St. Louis, MO; www.sigma-aldrich.-com). Ammonium persulfate (cat. #161-0700), sodium dodecyl sulfate (SDS, cat. #161-0416), and Temed

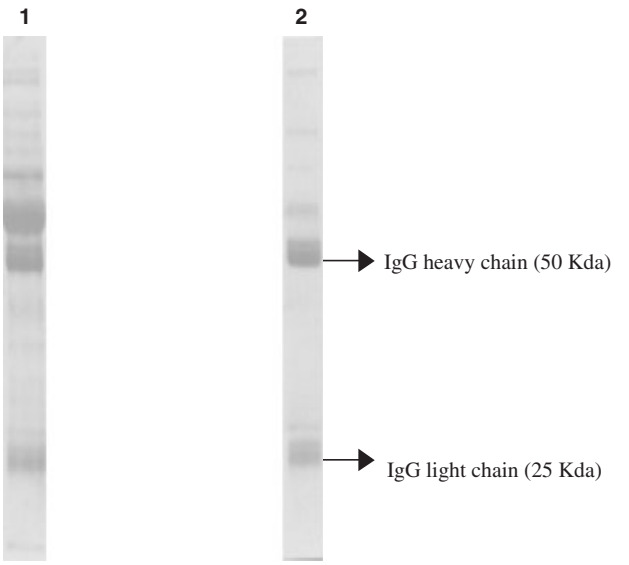

Fig. 1. Purification of IgG from serum samples. SDS-PAGE (10% *T*, Coomassie blue staining) of human serum (5 μg total protein as estimated by the Bradford assay) before (1) and after (2) protein precipitation with caprylic acid.

(N,N,N′,N′-tetramethylethylenediamine, cat. #161-08009) were obtained from Bio-Rad (Hercules, CA; www.discover.bio-rad.com). BCIP (5-bromo-4-chloro-3-indolyl-phosphate, cat. #1383221), NBT (nitro blue-tetrazolium-chloride, cat. #1383213), and streptavidin-AP (cat. #1089161) were obtained from Roche Diagnostics (Indianapolis, IN; www.roche.com). Methanol (cat. #414816), and potassium phosphate (cat. #471786) were obtained from Carlo Erba (Rodano, Milano, Italia; www.carloerbareagenti.com). Glycine (cat. #A1067-1000) was from Applichem (Darmstadt, Germany; www.applichem.de). Hydrogen peroxide (cat. #K13385810) was from Merck (Darmstad, Germany; www.merck.de). polyvinylidene fluoride (PVDF paper, cat. #IPVH00010) was from Millipore (Billerica, MA; www.millipore.com). I-block (cat. #AI300) was from Tropix (Applied Biosystems, Foster City, CA; www.appliedbiosystems.com). Biotinylated *Griffonia (Bandeiraea) Simplicifolia* lectin II (cat. #B-1215) was obtained from Vector Laboratories (Burlingame, CA; www.vectorlabs.com).

Patients

The study included 11 normal subjects and 19 patients with active RA. All patients had a history of failed treatment with at least one disease modifying antirheumatic drug and each (17 females and two males, mean age 58.2 years, mean disease duration 144.2 months) received intravenous infliximab at the dose of 3 mg/kg at baseline and after 2 and 6 weeks and subsequently once

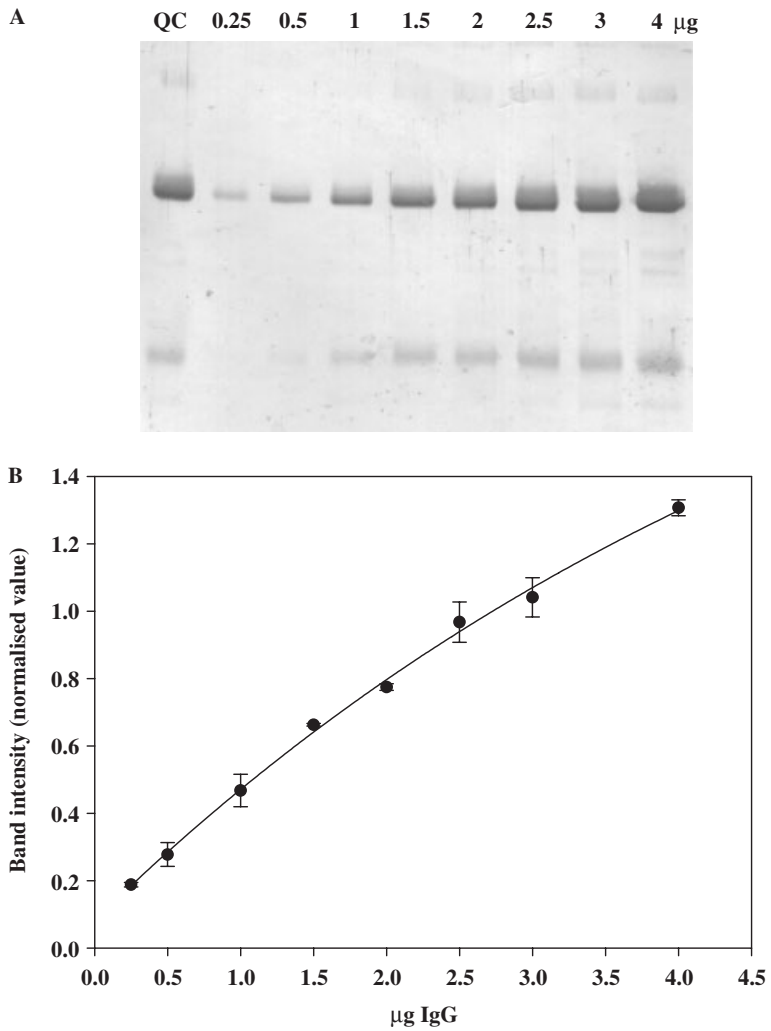

Fig. 2. Analysis of IgG glycosylation: Lectin blotting. **A:** Increasing amounts of standard IgG (0.25–4 μg) were analyzed as described in Materials and Methods. Briefly, the proteins were fractionated by SDS-PAGE on a 10% *T* gel, transferred onto PVDF membrane and stained using *Griffonia (bandeiraea) simplicifolia* lectin II (BS II). Quality control (QC) corresponded to 5 μg of standard human IgG. **B:** Exponential regression curve ($r^2 = 0.99$) was obtained following the densitometric analysis by plotting the band intensity vs. μg of IgG. Error bars are standard deviations.

bimonthly in combination with MTX at dose of 10–20 mg intramuscularly (im)/weekly. In addition to MTX, steroids (maximum daily dose 10 mg of oral prednisone or equivalent), and/or nonsteroidal anti-rheumatic drugs were allowed. After obtaining the informed consent, we collected blood serum samples at baseline and after 2, 6, 14, 22, and 30 weeks of anti-TNF-α treatment (nine patients) or at baseline and after six months of anti-TNF-α treatment (10 patients). The sera were stored at –20°C until assayed. The main clinical and laboratory variables assessed were erythrocyte sedimentation rate, C reactive protein, tender and swollen joint count, patient's assessment of pain, patient's assessment of disease activity, physician's global assessment of disease activity, the health assessment questionnaire and the American College of Rheumatology (ACR) response to therapy (14).

IgG Purification

The IgG purification procedure was performed according to Steinbuch and Audran (15). Briefly, serum samples were treated with 2 volumes of 60 mM sodium acetate buffer (pH 4.0) and 7% caprylic acid. Then, samples were stirred for 30 min at room temperature and centrifuged at 5,000 *g* for 10 min. The supernatants containing predominantly IgG were collected and neutralized by addition of 10% 1 M Tris HCl, pH 8. The protein content was determined by the Bradford assay (16) with some modifications (17,18).

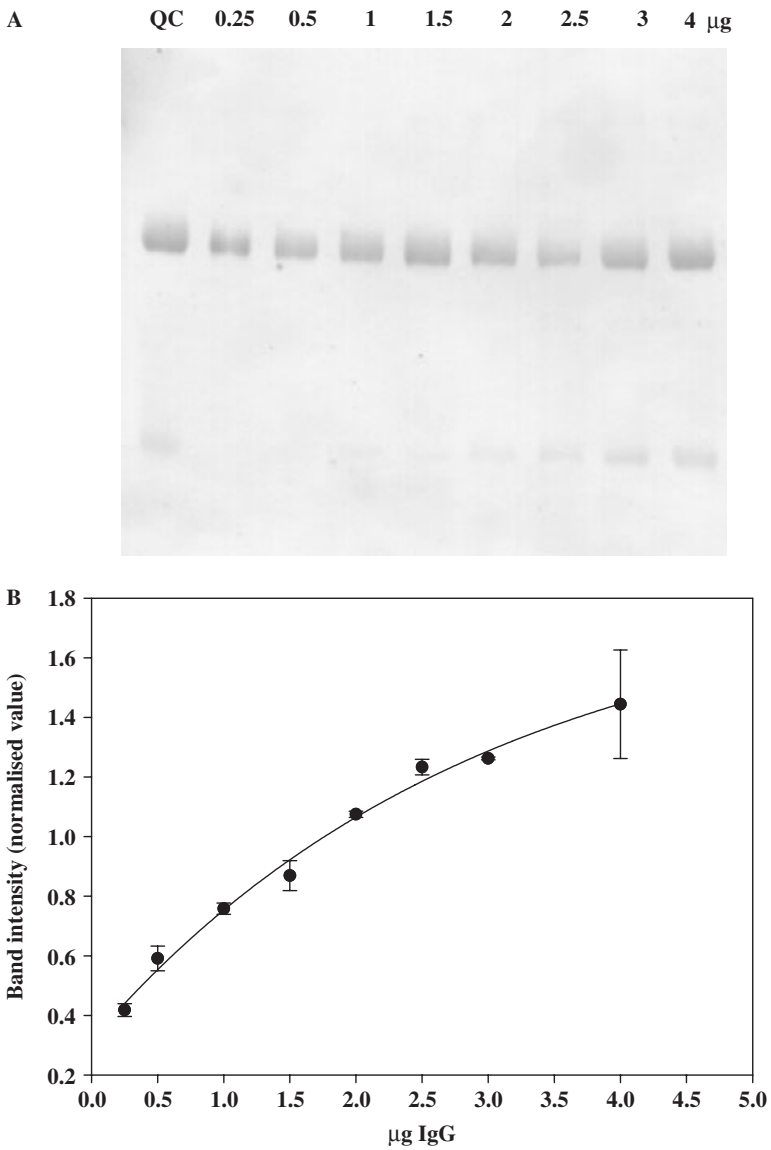

Fig. 3. Analysis of IgG glycosylation: Immunoblotting. **A:** Increasing amounts of standard IgG (0.25–4 μg) were analyzed as described in Materials and Methods. Briefly the proteins were fractionated by SDS-PAGE on a 10% *T* gel, transferred onto PVDF membrane and stained using protein-A/alkaline phosphatase. Quality control (QC) corresponded to 5 μg of standard human IgG. **B:** Exponential regression curve ($r^2 = 0.99$) was obtained following the densitometric analysis by plotting the band intensity vs. μg of IgG. Error bars are standard deviations.

Analysis of IgG Glycosylation

Lectin blotting and immunoblotting

All analyses were performed in duplicate. The linear concentration range corresponded to 0.25–4 μg/μL of standard IgG. Then, aliquots of purified IgG (10 μL) were denatured at 100°C for 2 min in Tris buffer (62.5 mM, pH 6.8), containing 10% SDS, 5 mM DTT and bromophenol blue, and were fractionated by SDS-polyacrlamide gel electrophoresis (PAGE) on a 10% *T* gel according to Laemmli (1970) (19). The proteins were transferred on PVDF membrane, using semidry blotting system (20), and stained using *Griffonia (bandeiraea) simplicifolia* lectin II (BS II) with a modification of the method previously described (21,22). Briefly, the blot was:

- blocked overnight at 4°C in Tris buffered saline (TBS) (50 mM Tris, pH 7.5, containing 150 mM NaCl) containing 0.1% Tween 20 and 0.1% I-block;
- incubated with the BS II lectin (0.01 μg/μl) in TBS buffer for 1 hr at room temperature;
- washed three times for 5 min in TBS buffer;
- incubated with 0.1% streptavidin-alkaline phosphatase in TBS buffer for 1 hr at room temperature;

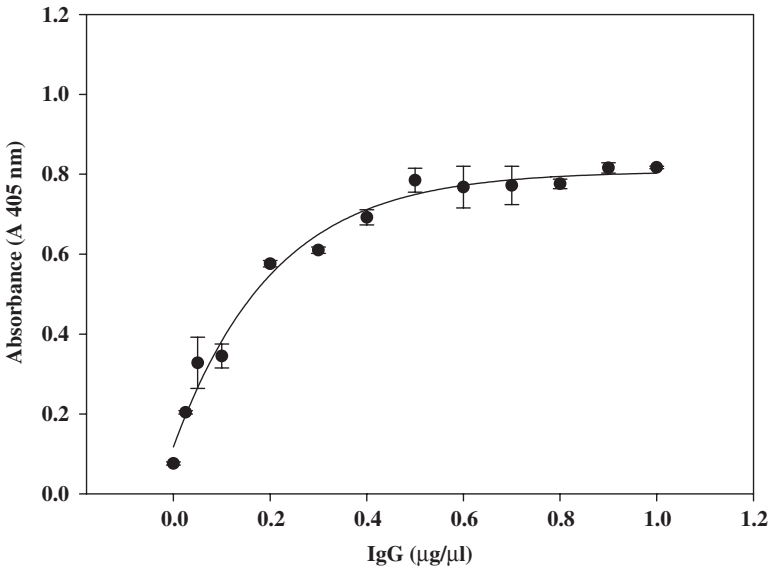

Fig. 4. Analysis of IgG glycosylation: ELLA. Exponential regression curve obtained by plotting the absorbance values at 405 nm vs. increasing amounts of standard IgG concentrations (0.025—1 μg/μL). Error bars are standard deviations.

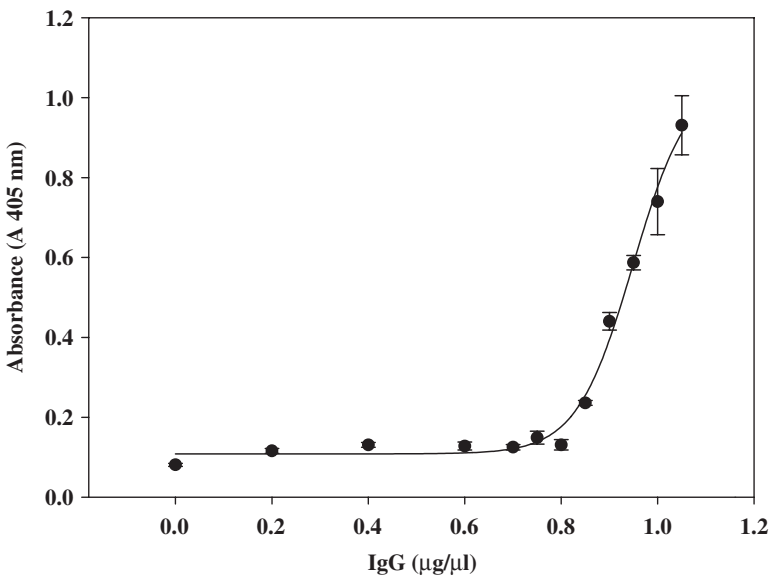

Fig. 5. Analysis of IgG glycosylation: ELISA. Sigmoidal regression curve obtained by plotting absorbance values at 405 nm vs. increasing amounts of standard IgG concentrations (0.025–1.05 μg/μL). Error bars are standard deviations.

- washed three times for 5 min in TBS buffer;
- stained with 0.5% NBT and 0.37% BCIP in TBS buffer containing 10% $MgSO_4$ for 5 min.

The quantity of IgG was determined following staining by protein-A/alkaline phosphatase. Briefly, the blot was:

- blocked overnight at 4°C in TBS buffer containing 0.1 % Tween 20 and 0.1% I-block;
- incubated with 0.1% protein-A/alkaline phosphatase in TBS buffer for 1 hr at room temperature;
- washed three times for 5 min in TBS buffer;
- stained as described above for 20 min.

Densitometric analysis was performed as previously described (23), using the software Quantiscan 2.1 for Windows (Biosoft, Cambridge, UK; www.biosoft.com).

In all analyses a quality control (QC), consisting of standard IgG (0.25 μg/μL) was run to determine the

TABLE 1. Analysis of IgG glycosylation by lectin blotting/immunoblotting (M1) and ELLA/ELISA (M2) of serum samples of nine patients (P) with rheumatoid arthritis (RA) at baseline and after 2, 6, 14, 22 and 30 weeks of anti-TNF-α treatment

P	T	M1	M2
1	0	1.15	0.55
	2	0.73	0.96
	6	0.77	0.39
	14	–	–
	22	0.89	0.48
	30	–	–
2	0	–	–
	2	0.74	0.25
	6	0.82	0.55
	14	–	–
	22	–	–
	30	–	–
3	0	1.02	0.74
	2	1	0.73
	6	0.92	0.62
	14	0.91	0.45
	22	0.72	0.45
	30	0.64	0.43
4	0	–	–
	2	0.51	0.26
	6	0.33	0.23
	14	0.54	0.32
	22	–	–
	30	–	–
5	0	1.26	0.38
	2	1.76	0.54
	6	0.5	0.35
	14	0.31	0.28
	22	0.53	0.35
	30	–	–
6	0	1.12	0.19
	2	–	–
	6	–	–
	14	1.63	0.44
	22	–	–
	30	–	–
7	0	–	–
	2	1.35	0.18
	6	1.28	0.27
	14	1.4	0.23
	22	1.5	0.37
	30	2.73	1.4
8	0	–	–
	2	0.99	0.4
	6	1.12	0.42
	14	–	–
	22	–	–
	30	–	–
9	0	0.95	0.49
	2	1.19	0.8
	6	0.71	0.24
	14	0.74	0.28
	22	–	–
	30	–	–

TABLE 2. Analysis of IgG glycosylation by lectin blotting/immunoblotting (M1) and ELLA/ELISA (M2) of serum samples of 10 patients (P) with rheumatoid arthritis (RA) at baseline and after 6 months of anti-TNF-α treatment

P	T	M1	M2
1	0	1.81	0.45
	24	1.83	0.71
2	0	1.53	0.98
	24	1.37	0.81
3	0	2.1	0.83
	24	1.91	0.56
4	0	1.6	0.74
	24	1.48	0.47
5	0	1.52	1.25
	24	1.61	1.69
6	0	1.23	0.93
	24	1.27	1
7	0	1	0.45
	24	0.73	0.4
8	0	0.68	0.95
	24	0.78	1
9	0	1.041	0.87
	24	0.98	0.7
10	0	0.72	0.75
	24	0.75	1

intra- and interassay coefficients of variation (CV) following the densitometric analysis of each band.

Enzyme-linked lectin assay (ELLA)

The ELLA was performed as previously described (24,25), using the BS II lectin. In all analyses, the standard curves were obtained by analyzing increasing amounts (0.025–1.05 μg/μL) of standard IgG. Then, aliquots of all samples (50 μL) containing purified IgG with 1% 2-mercaptoethanol in phosphate buffered saline (PBS) (10 mM sodium phosphate, 150 mM sodium chloride pH 7.4) were analyzed in duplicate on 96-well microplates. Briefly, the samples were incubated at 70°C for 1 hr and later nonspecific binding sites were saturated with 1% BSA in PBS pH 8 for 1 hr. The plate was incubated with 0.05 % BS II lectin in PBS for 1 hr and streptavidin-AP in PBS. Finally, the staining was performed in the presence of a commercially available phosphatase substrate and the absorbance of the wells was measured at 405 nm with an automatic microplate reader (Model 3550 from Bio-Rad).

Enzyme linked immunosorbent assay (ELISA)

A modification of the ELISA method previously described (24,25), using protein-A/alkaline phosphatase was performed. In all analyses, the standard curve was obtained with increasing amounts (0.025–1.05 μg/μL) of standard IgG. Then, aliquots of all samples (50 μL)

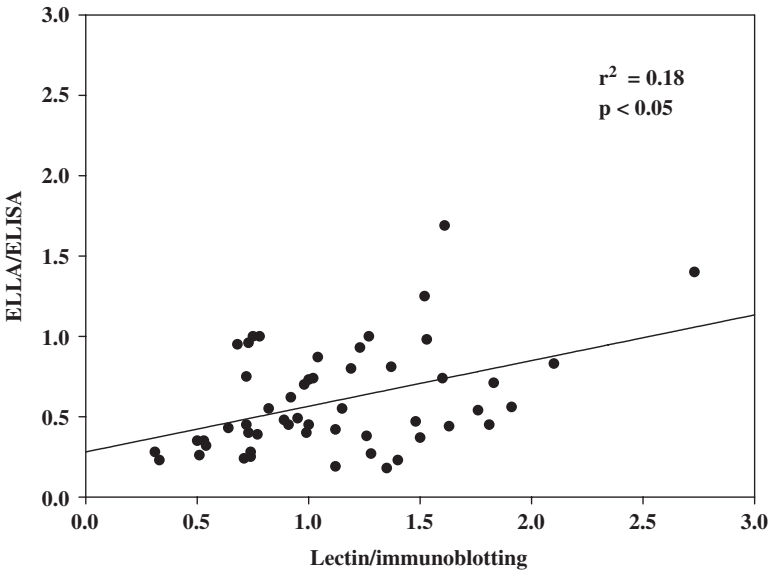

Fig. 6. Analysis of IgG glycosylation: Correlation between the lectin blotting/immunoblotting and the ELLA/ELISA approaches. When the glycosylation data of 53 serum samples of 19 patients with RA were measured by the two different approaches, and plotted against each other, a linear regression curve ($r^2 = 0.18$, $P < 0.05$) was obtained.

containing purified IgG with 1% 2-mercaptoethanol in PBS buffer, were analyzed in duplicate on 96-well microplates. Briefly, the samples were incubated at 70°C for 1 hr and later nonspecific binding sites were saturated with 1% BSA in PBS pH 8 for 1 hr. The plate was incubated with 0.01% protein-A/alkaline phosphatase in PBS for 1 hr and the colorimetric reaction was developed as described above.

Calculation of IgG glycosylation

IgG glycosylation was calculated based on the ratio G0 (quantified by lectin blotting or ELLA)/total IgG (quantified by immunoblotting or ELISA).

Statistical Analysis

The calibration curves were obtained using SigmaPlot 8.0 for Windows (SPSS, Chicago, IL; www.spss.com). To assess whether the changes of the IgG glycosylation correlated with the clinical and laboratory parameters included in the ACR criteria for response to therapy, the Wilcoxon paired test was used. CV were calculated dividing the standard deviations by the means, in the same (intra-) or different (inter-) assay.

RESULTS

IgG purification Using Caprylic Acid

The purity of IgG was estimated to be higher than 90% (Fig. 1).

Analysis of IgG Glycosylation

Lectin blotting and immunoblotting

Figure 2A shows the quantitative analysis of increasing amounts (0.25–4 μg) of standard IgG using lectin blotting with BS II lectin. The exponential regression curve was obtained by plotting the band intensity vs. the amount of IgG (Fig. 2B).

Similarly, Fig. 3 Ashows the quantitative analysis of increasing amounts (0.25–4 μg) of standard IgG by immunoblotting, using protein-A/alkaline phosphatase. An exponential regression curve was obtained by plotting the band intensity vs. μg of IgG (Fig. 3B).

The inter- and intraassay CV were 1.3% and 9.0% for lectin blotting and 4.6% and 8.3% for immunoblotting, respectively.

ELLA

Figure 4 shows the quantitative analysis of increasing amounts (0.025–1 μg/μL) of standard IgG, using BS II lectin. The exponential regression curve was obtained by plotting the absorbance values at 405 nm vs. the IgG concentration. The inter- and intraassay CV were 6.2% and 7.7%, respectively.

ELISA

Figure 5 shows the quantitative analysis of increasing amounts (0.2–1.05 μg/μL) of standard IgG, using protein-A/alkaline phosphatase. A sigmoidal regression curve was obtained by plotting the absorbance at

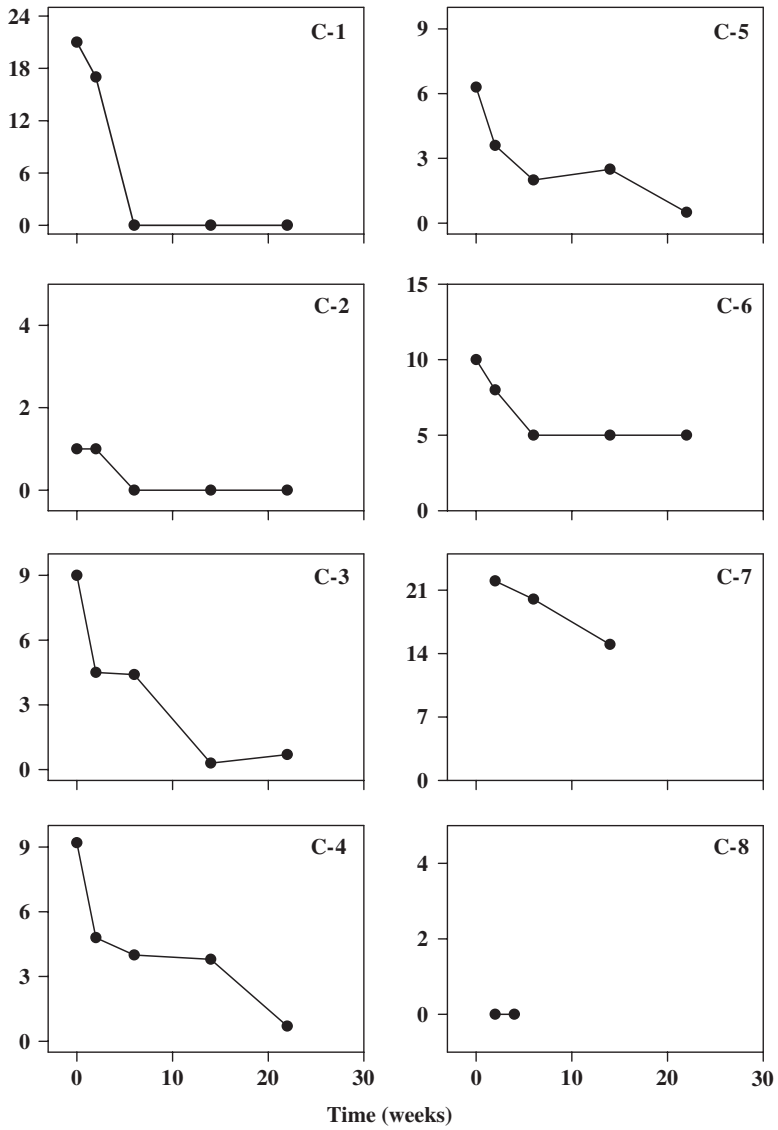

Fig. 7. Changes of the ACR criteria in a representative patient with active RA who responded to infliximab and methotrexate. C-1, tender joints count; C-2, swollen joints count; C-3, patient's assessment of pain; C-4, patient's assessment of disease activity; C-5, physician's global assessment of disease activity; C-6, health assessment questionnaire; C-7, erythrocyte sedimentation rate; and C-8, C reactive protein.

405 nm vs. the IgG concentration. The inter- and intraassay CV were 5.1% and 14.1%, respectively.

Relationship between lectin/immunoblotting and ELLA/ELISA

A good correlation ($r^2 = 0.18$, $P < 0.05$) was obtained between the two different approaches (Tables 1 and 2) used to measure the IgG glycosylation (Fig. 6).

Changes of IgG Glycosylation in RA Patients

Figures 7 and 8 show the changes of each clinical and laboratory parameter and the modification in glycosylation of IgG in a patient with active RA partially responsive to infliximab/MTX (ACR response to therapy = 20%) at baseline and after 2, 6, 14, 22 and 30 weeks, in comparison with the IgG glycosylation of 11 normal subjects (N).

Figures 9 and 10 show the changes of the clinical and laboratory parameters included in the ACR criteria for clinical response and of glycosylation of IgG in five patients (ACR 20% and 50%) with active RA treated with intravenous infliximab/MTX at baseline and after 6 months, in comparison with the IgG glycosylation of 11 normal subjects (N).

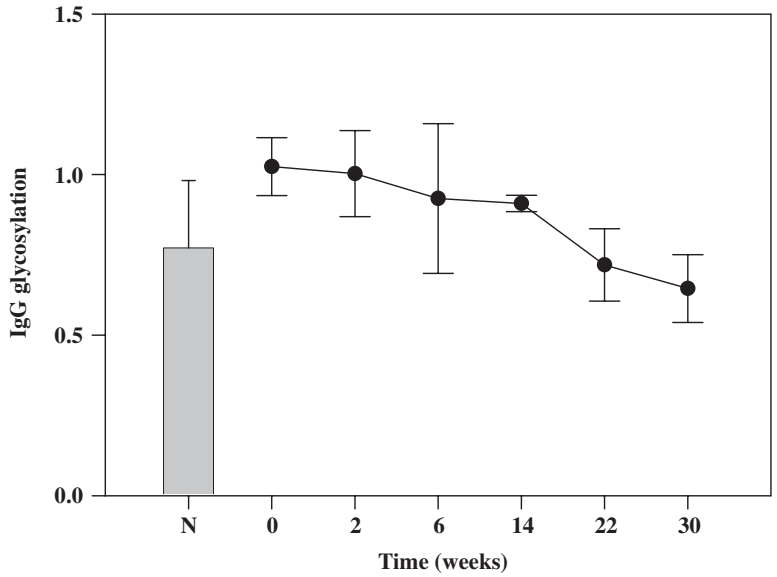

Fig. 8. Changes in IgG glycosylation in a representative patient with active RA who responded to infliximab and methotrexate. IgG glycosylation measured by lectin blotting/immunoblotting of the same patient of Fig. 7 with active RA who responded to infliximab and methotrexate (ACR response to therapy = 20%). N, IgG glycosylation of 11 normal subjects measured by lectin blotting/immunoblotting. Error bars are standard deviations.

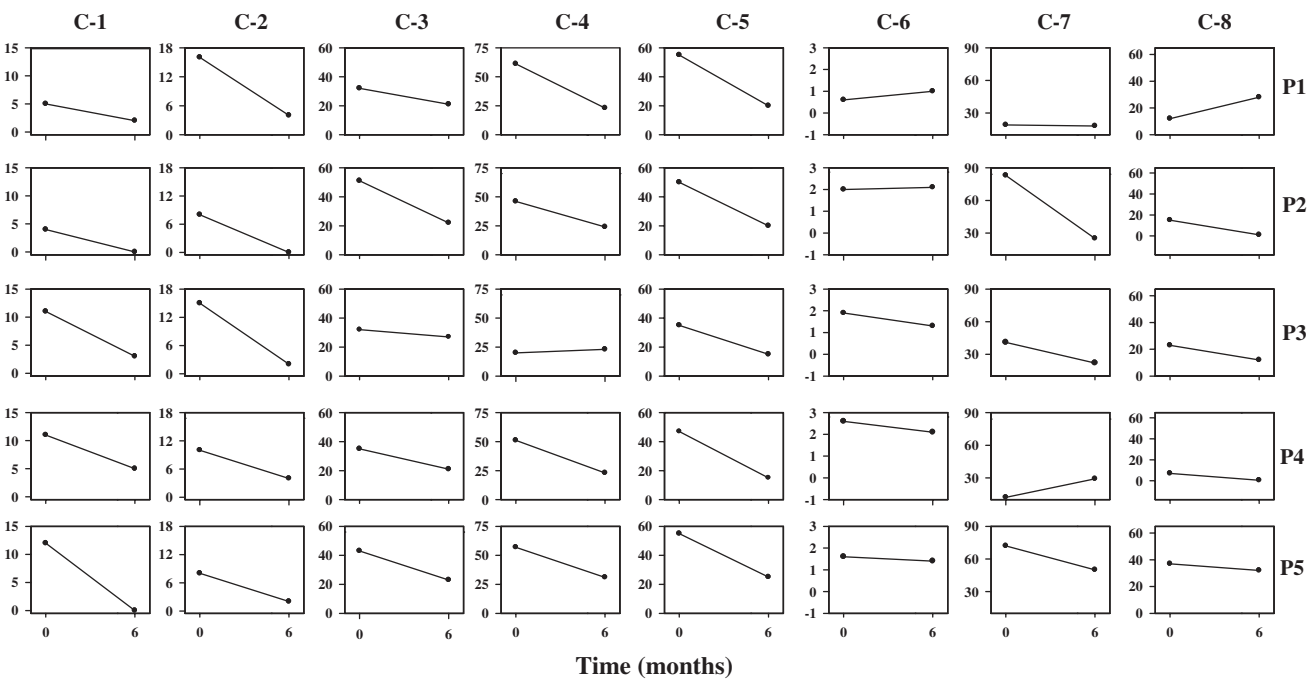

Fig. 9. Changes of the ACR criteria in representative patients with active RA who responded to infliximab and methotrexate. C-1, number of tender joints; C-2, number of swollen joints; C-3, patient's assessment of pain; C-4, patient's global assessment of disease activity; C-5, physician's global assessment of disease activity; C-6, health assessment questionnaire; C-7, erythrocyte sedimentation rate; C-8, C reactive protein; P1, ACR response to therapy = 20%; P2, ACR response to therapy = 50%; P3, ACR response to therapy = 20%; P4. ACR response to therapy = 50%; P5, ACR response to therapy = 20%.

DISCUSSION

In the present work, we describe for the first time, to our best knowledge, the effect of infliximab, a new biological drug for RA, on the glycosylation of IgG.

The analyses were performed by two different experimental approaches (lectin/immunoblotting and ELLA/ELISA) following the purification of IgG from single serum samples. IgG purification was performed by using caprylic acid according to Steinbuch and Audran (15). Most plasma proteins were precipitated and the purity of IgG, assessed by SDS-PAGE using Coomassie blue staining, was estimated to be higher than 90%, in agreement with other authors (26). It is surprising that this method, which is simpler, faster and cheaper than conventional chromatographic techniques (anion-exchange, protein G or protein A affinity chromatography, etc.), was not used by many other authors in the last 30 years (27).

IgG glycosylation was performed using the BS II lectin and protein-A/alkaline phosphatase: this lectin is capable of distinguishing G0 from normal glycosylated IgG, being specific for terminal N-acetylglucosamine (GlcNAc) residues on G0 (Fig. 2A) (22). Protein-A/alkaline phosphatase is specific for the Fc region of IgG (28), thus staining only the IgG subunits (Fig. 3A). Two different experimental approaches were used:

1. Lectin-blotting and immunoblotting. These methods are usually considered qualitative, but can be made quantitative if internal QCs are analyzed in each blot (23) and proper standard curves are set up (Fig. 2B). In particular, the QC were used to normalize the intensity of the bands of samples analyzed in different blots and to calculate the inter- and intraassay CV (1.3% and 9.0% for lectin blotting, 4.6% and 8.3% for immunoblotting, respectively). The sensitivity of both methods was of about 0.25 μg of standard IgG (Fig. 2B). A similar experimental approach was used by Gornik et al. (21) who, however, did not normalize the changes of lectin affinity by the amount of the IgG analyzed.
2. ELLA and ELISA were performed by modifying methods previously described (24,25). Proper standard curves were set up to select the optimal concentration of samples for analyses (Figs. 4 and 5). The precision of the assays was evaluated by calculating the inter- and intraassay CV (6.2% and 7.7% for ELLA; 5.1% and 14.1% for ELISA, respectively). The sensitivity of the methods was 0.025 μg/μL (Figs. 4 and 5).

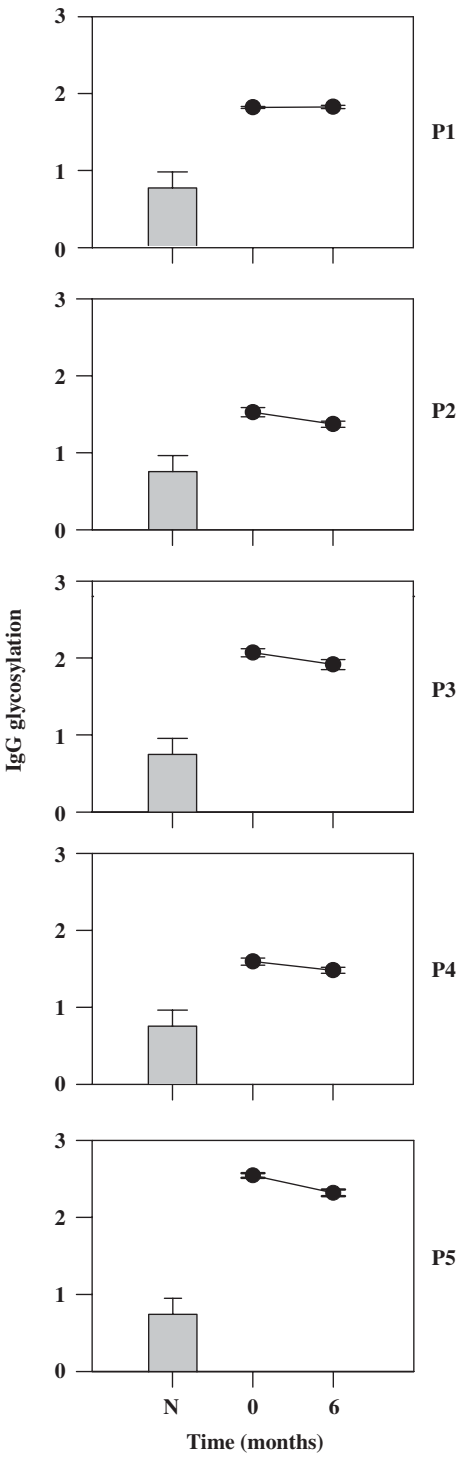

Fig. 10. Changes of IgG glycosylation in representative patients with active RA who responded to infliximab and methotrexate. IgG glycosylation measured by lectin blotting/immunoblotting of five representative patients (P1, ACR response to therapy = 20%; P2, ACR response to therapy = 50%; P3, ACR response to therapy = 20%; P4, ACR response to therapy = 50%; P5, ACR response to therapy = 20%) treated with infliximab/methotrexate. N, IgG glycosylation of 11 normal subjects measured by lectin blotting/immunoblotting. Error bars are standard deviations.

A good linear correlation between the two different experimental approaches described above (Tables 1 and 2) indicates that these can be considered equivalent (Fig. 6).

A decrease of G0 was observed in patients who clinically improved according to the ACR criteria (20% and 50% ACR response to therapy) following the infliximab/MTX treatment. In Figs. 7,8,9,10 representative cases are reported.

It is well known that abnormal glycosylation of IgG can occur in RA (3,4), causing the production of RFs directed against IgG molecules containing less terminal galactose on their oligosaccharide moieties (29). Indeed, the changes of galactosylation of IgG are known to correlate with the RF activity (30). Infliximab, a chimeric antibody with anti-TNF-α activity, is a well established therapeutic option for RA. In patients showing clinical improvement after the pharmacological treatment with this drug, a decrease in the serum titers of RF and anti-cytrullinated cyclic peptides antibodies was observed (31). The mechanism responsible for these phenomena is not known and, in addition to its actions on selected inflammatory cytokines, a direct effect of the drug on IgG glycosylation and citrullination could be involved.

In conclusion, our data indicate that infliximab can reduce the concentration of G0 in patients with active RA.

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