Anti-Tumor Necrosis Factor-α Therapy Augments Dipeptidyl Peptidase IV Activity and Decreases Autoantibodies to GRP78/BIP and Phosphoglucose Isomerase in Patients with Rheumatoid Arthritis

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ABSTRACT. ***Objective.*** To assess the enzymatic activity and biochemical status of dipeptidyl peptidase IV (DPP IV), an enzyme that participates in the degradation of proinflammatory molecules, in sera from a group of patients with rheumatoid arthritis (RA; n = 15) treated with a human anti-tumor necrosis factor-α (anti-TNF-α) antibody (adalimumab) for 32 weeks. IgG antibody titers against chaperone Bip (GRP78), phosphoglucose isomerase (PGI), lactate dehydrogenase (LDH), fibronectin (FN), and actin were also studied.

Methods. DPP IV activity was measured in sera using Gly-Pro-p-nitroanilide as substrate. The biochemical profile of circulating DPP IV glycoforms was assessed by isoelectric focusing gel electrophoresis. All IgG autoantibody titers and their sialylation levels were determined by ELISA.

Results. Patients showed significant increases in serum DPP IV enzymatic activity from basal values (3.554 ± 1.096) with respect to those obtained at 32 weeks (4.787 ± 0.953; $p < 0.05$). Changes in the biochemical profile of circulating DPP IV from acidic to more neutral isoelectric point glycoforms were also seen during treatment. The elevated titers of anti-GRP78 and anti-PGI IgG observed at the beginning of treatment decreased significantly during therapy, whereas those of anti-LDH, anti-FN, and anti-actin IgG remained unchanged. At the end of treatment, sialylation levels of anti-GRP78 and anti-PGI IgG antibodies increased to nearly normal levels. The DPP IV biochemical changes were accompanied by a significant improvement of the Disease Activity Score (DAS28).

Conclusion. The reduced activity of DPP IV along with increased titers of circulating antibodies to GRP78 and PGI may play a role in the pathogenesis of RA and can be successfully modified by administration of adalimumab. (J Rheumatol 2005;32:2116–24)

Key Indexing Terms:
TUMOR NECROSIS FACTOR-α ADALIMUMAB DIPEPTIDYL PEPTIDASE IV
AUTOIMMUNITY GRP78 PHOSPHOGLUCOSE ISOMERASE RHEUMATOID ARTHRITIS

Rheumatoid arthritis (RA) is a chronic inflammatory disease that often results in significant morbidity and disability. Over the past 20 years a better understanding of the pathogenesis of RA and the success of early biological therapies, including tumor necrosis factor-α (TNF-α) and interleukin 1 (IL-1) antagonists, have led to the development of new approaches to disease treatment[1].

The importance of TNF-α in RA was initially proposed on the basis of analysis of cytokine gene regulation at the synovium, the local site of the disease[2-4]. This finding led to the concept of a TNF-α-dependent cytokine cascade in which TNF-α is a prime factor in coordinating a cytokine response in RA, thereby making the protein a primary target for treatment of this disease[5-7].

Dipeptidyl peptidase IV (DPP IV) is a cell-surface glycoprotein with many distinct functions, expressed on a variety of human tissues, including epithelial cells and leukocyte subsets[8]. A soluble form of DPP IV is also found in human serum[9,10]. Expression of DPP IV is closely associated with T lymphocyte activation and migration across the extracellular matrix[11], and the serum activity shows a correlation with the membrane-bound enzyme found on normal T lymphocytes[12]. The serum enzyme possibly originates from

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T lymphocytes, as it shares exactly the same primary amino acid structure but lacks the amino terminal 39 amino acid sequence required for insertion in the plasma membrane[10].

DPP IV participates in the degradation of many proinflammatory molecules including IL-1, IL-2, and TNF-α, thereby playing a central role in the antiinflammatory response[13]. The expression of DPP IV is increased by IL-12 in phytohemagglutinin-stimulated T lymphocytes, whereas under similar conditions TNF-α downregulates DPP IV expression[14]. Therefore, the interaction between TNF-α and DPP IV plays a multifunctional role in the immune response.

In a previous study we found that the specific activity of serum DPP IV in patients with RA was decreased, although its concentration was similar to that found in healthy controls[15]. The reduction of DPP IV enzymatic activity was caused by hypersialylation as its specific activity was restored after enzymatic desialylation of the protein[15]. In this study, we investigated the biochemical profile of DPP IV in a population of patients with RA treated with adalimumab, a recombinant human IgG1 monoclonal antibody that neutralizes TNF-α, thereby having better efficacy than synthetic disease modifying antirheumatic drugs (DMARD) in the treatment of RA[16].

We assessed the enzymatic activity and biochemical status of DPP IV during adalimumab treatment along with the titers of antibodies to several proteins: the chaperone Bip (GRP78), phosphoglucose isomerase (PGI), fibronectin (FN), lactate dehydrogenase (LDH), and actin, all of which are significantly elevated in patients with RA[17-22]. We noted significant improvement of the Disease Activity Score (DAS28) after 32 weeks of observation, which correlated with positive changes in DPP IV activity and reductions in the titers of anti-GRP78 and anti-PGI IgG antibodies.

MATERIALS AND METHODS

Patients. We studied 15 patients fulfilling the 1987 revised American Rheumatism Association criteria for classification of RA[23]. All had active disease despite treatment with methotrexate, leflunomide, or sulfasalazine, defined by the presence of ≥ 6 swollen joints, ≥ 9 tender joints, and morning stiffness > 45 min. The dosages of synthetic DMARD must have been stable for at least 8 weeks before enrolling in the study.

All patients received 40 mg adalimumab (Abbott Laboratories, Chicago, IL, USA) subcutaneously every other week during 32 weeks. Patients were allowed to continue the same dose of nonsteroidal antiinflammatory drug, oral glucocorticoid, and DMARD prescribed at the beginning of the study. The study was approved by the institutional review boards at each study site, and all patients gave written informed consent.

Clinical and laboratory assessment. The clinical response and DPP IV activity were evaluated at baseline and at Weeks 8, 16, 24, and 32, before the corresponding adalimumab dose. We evaluated the clinical status by tender joint count and swollen joint count, patient global assessment of disease activity, physician global assessment of disease activity, patient assessment of pain, Health Assessment Questionnaire (HAQ) score, and DAS28 score[23,24]. Laboratory analyses included determinations of erythrocyte sedimentation rate (ESR), blood cell count, and blood chemistry at each visit. All laboratory tests were performed blindly, regardless of response to treatment. Blood samples were taken at 9:00 to 10:00 AM at Weeks 0, 8, 16, 24, and 32 immediately before the corresponding adalimumab dose was administered. Serum samples were stored at –70°C until used. For comparison, we also collected blood samples from 10 patients with osteoarthritis (OA), and 15 healthy controls.

Proteins and peptides. Human FN, rabbit muscle PGI, pig muscle actin, and L-lactate dehydrogenase were purchased from Sigma (St. Louis, MO, USA). Recombinant GRP78 was purchased from Stressgen Biotechnologies (Victoria, BC, Canada).

Enzyme assays. DPP IV activity was measured in 96-well culture plates using Gly-Pro-p-nitroanilide (0.2 mM) as substrate in reaction mixtures (100 μl) containing serum samples (10 μl) and 50 mM Tris-HCl, pH 8.0. The hydrolysis of the substrate was monitored at 405 nm wavelength using an Anthos Labtec kinetic plate reader. Activity was expressed as ΔA_{405} nm/min. All experiments were performed in triplicate unless otherwise specified.

Antibodies. Goat affinity purified $F(ab')_2$ fragments against the Fc region of human IgG and anti-human IgM were purchased from ICN Pharmaceuticals (Aurora, IL, USA). Antibodies to purified DPP IV from human serum were prepared in rabbits as described[15], and purified by affinity chromatography on protein A-Sepharose[25], followed by immunoadsorption to DPP IV coupled to Sepharose 4B.

Analyses of total IgG in serum. Total IgG in serum was assayed by ELISA; 96-well culture plates were coated overnight at 22°C with a solution (200 μl) containing anti-IgG $F(ab')_2$ fragments (5 μg/ml) in 0.1 M Na_2CO_3, 0.01% NaN_3, pH 9.3. After coating, plates were rinsed with 0.1 M NaCl, 0.01 M sodium phosphate, pH 7.4, containing 0.05% Tween-80 (PBS-Tween) to remove unbound proteins. Nonspecific sites were blocked with PBS-Tween containing 2% bovine serum albumin at 25°C for 1 h. For assays, serum aliquots (10 μl, 1:5000 dilution) were added in triplicate in a 200 μl final volume of PBS-Tween and incubated 2 h at 37°C. Plates were then rinsed with PBS-Tween and incubated with 200 μl of a solution containing specific anti-human IgG $F(ab')_2$ fragments conjugated to alkaline phosphatase for 1 h at 37°C, followed by rinsing with PBS-Tween and incubation with 200 μl alkaline phosphatase substrate (1 mg/ml p-nitrophenylphosphate) in 0.1 M glycine, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 10.4. Absorbance was monitored at 405 nm. Bound immunoglobulins were expressed as absorbance measured at 405 nm/min. A calibration curve was constructed with purified human IgG.

IgM rheumatoid factor (RF) measurement. Anti-IgM RF was assayed by ELISA on a 96-well plate coated with highly purified Fc fragments of human IgG, prepared by digestion of human IgG with papain and then purification by chromatography on Sephadex G-75 and protein A-Sepharose. For assays, serum aliquots (200 μl, 1:100 dilution) were added in triplicate in a 200 μl final volume of PBS-Tween and incubated 2 h at 37°C, followed by rinsing in PBS-Tween and incubation with specific anti-human IgM $F(ab')_2$ fragments conjugated to alkaline phosphatase for 1 h at 37°C. Detection of patient-specific IgM RF and their concentrations in serum were assessed as described above.

Analyses of specific autoantibodies. Antibodies against GRP78, PGI, FN, actin, and LDH were assayed by ELISA on 96-well plates coated with each antigen (5 μg/ml) as described. For assays, serum aliquots (200 μl, 1:100 dilution) were added in triplicate in a 200 μl final volume of PBS-Tween and incubated 2 h at 37°C, followed by rinsing in PBS-Tween and incubation with specific anti-human IgG $F(ab')_2$ fragments conjugated to alkaline phosphatase for 1 h at 37°C. Detection of the specific IgG and their concentrations in serum were assessed as described above.

Isoelectric focusing analysis. Isoelectric focusing was performed in the Mini-protean II electrophoresis chamber (Bio-Rad, Richmond, CA, USA) using IEF Ready gels at pH 5–8 (Bio-Rad). The electrode solutions were 0.1 M NaOH for the cathode and 0.1 M H_3PO_4 for the anode. Human serum DPP IV glycoforms were separated by direct electrophoresis of human serum samples (20 μl) at constant voltage (200 V) for 90 min. The pH gra-

dient was determined using a mixture of 5 standard proteins with known isoelectric point. Following electrophoresis, the gels were immersed in 100 ml of SDS buffer (100 mM Tris-HCl, pH 6.8, containing 2.3 g SDS, 8 ml glycerol, and 0.5 ml ß-mercaptoethanol) for 30 min at room temperature with shaking[26]. After SDS treatment, the proteins were transferred from the gel to nitrocellulose membranes by Western blot method[27]. Detection of proteins was performed by incubating the membranes with 5 μg rabbit anti-human DPP IV IgG in 10 ml of PBS-Tween at room temperature overnight, followed by incubation with a secondary alkaline phosphatase-conjugated secondary antibody at room temperature for 2 h, and developed using the alkaline phosphatase substrate 5-bromo-4-chloroindolyl-3-phosphate nitroblue tetrazolium.

Assay of terminal sialic acid residues of IgG. Specific detection of terminal sialic acid residues of IgG antibodies against GRP78, PGI, LDH, FN, or actin was performed with a glycan differentiation kit (Roche Diagnostics, Indianapolis, IN, USA). Briefly, 200 μl of serum (1:100 dilution) in PBS-Tween were incubated for 1 h at 37°C in 96-well culture plates coated with the antigens as described above. The plates were thoroughly rinsed in PBS-Tween followed by incubation for 90 min at 37°C with 200 μl of a mixture containing equal parts (50 ng each) of digoxigenin-labeled *Sambucus nigra* agglutinin and *Maackia amurensis* agglutinin, which recognize α(2-6) and α(2-3) sialic acid-linked residues, respectively[28,29]. After rinsing with PBS-Tween to remove unbound lectins, the plates were incubated for 1 h at 37°C with a polyclonal sheep anti-digoxigenin Fab fragment conjugated with alkaline phosphatase (7 units/well in 200 μl PBS-Tween). Detection of bound lectin was performed by incubating the plates with the alkaline phosphatase substrate p-nitrophenylphosphate (1 mg/ml) as described above. The total sialic acid concentration of the specific IgG antibodies was calculated from calibration curves constructed with the glycoprotein fetuin as suggested by the kit manufacturer.

Statistics. Overall differences between serum levels from controls and/or baseline DPP IV activity and levels of IgG antibodies to FN, LDH, PGI, GRP78, actin, and RF at 8, 16, 24, and 32 weeks of pharmacological treatment with adalimumab were assessed by means of an analysis of variance test. Post-hoc univariate analysis was perfomed by means of paired t tests. Comparisons with normal controls were nonpaired. Correlation between the mean values of change in the DAS28 score (mean values at 8, 16, 24, and 32 weeks minus mean values at baseline) and similar changes in DPP IV activity, as well as anti-LDH, anti-PGI, anti-actin, anti-GRP78, and anti-fibronectin IgG levels were evaluated by means of Kendall's tau statistics. The sialic acid levels of the IgG antibodies were evaluated by Student's t test using the program Systat® for Windows: Statistics, v. 11 (Systat Inc., Evanston, IL, USA).

RESULTS

Demographic characteristics and clinical evolution of patients with RA. A group of 15 RA patients who received treatment with adalimumab for a period of 32 weeks were studied; clinical and demographic characteristics of the group are shown in Table 1. All presented active disease at the beginning of the study. The DAS28 scores (Figure 1) at 8, 16, 24, and 32 weeks of followup with adalimumab treatment showed a statistically significant improvement compared with baseline values ($p < 0.05$).

RF levels in patients. The mean (± SD) serum IgM RF concentrations were 1.98 (± 0.62) μg/ml for controls, and 10.65 (± 2.84), 8.22 (± 3.15), 4.54 (± 1.97), 2.20 (± 0.79), and 2.06 (± 1.24) μg/ml at baseline and at 8, 16, 24, and 32 weeks, respectively (Figure 2). Levels of IgM RF were higher at baseline and at 8 and 16 weeks compared with controls ($p < 0.000001$, $p < 0.00001$, $p < 0.0005$, respectively). RF concentrations were close to those of controls at Weeks 24 and 32. There was also a statistically significant decline in the concentration of IgM RF from baseline to Week 32 ($p < 0.04$ at Week 8, $p < 0.000001$ at Weeks 16, 24, and 32 compared to baseline).

Table 1. Clinical and demographic details of patients with RA. Data represent means ± SD.

	RA Patients (n = 15)
Sex, F/M	12/3
Mean age, yrs	49.13 ± 9.86
Mean disease duration, mo	96 ± 52.20
Rheumatoid factor-positive patients, n	15
Basal DAS 28	5.82 ± 1.37
Prednisone, n	13
Prednisone dose, mg/day	9.44 ± 5.38
Methotrexate, n	14
Methotrexate dose, mg/week	12.14 ± 3.9
Leflunomide, n	1

DPP IV activity and changes in the profile of circulating enzyme isoforms. Comparison of DPP IV activities in sera of patients (n = 15; Figure 3) shows a gradual increase in enzymatic activity from baseline to 32 weeks. DPP IV activity in patients was statistically lower at baseline and at 8 weeks compared with controls ($p < 0.00003$ and $p < 0.03$, respectively), reaching levels similar to those of controls 16 weeks after initiation of the study. Mean serum DPP IV activity levels increased steadily from baseline to 32 weeks, but differences were statistically significant only at Week 32. A sample of patients with OA (n = 10) shows serum DPP IV activity levels similar to those of the controls.

Electrophoresis analysis of serum DPP IV glycoforms from 2 RA patients, at baseline and 32 weeks of therapy, shows changes in their profiles from more acidic isoforms at the beginning of therapy to glycoforms resembling those of

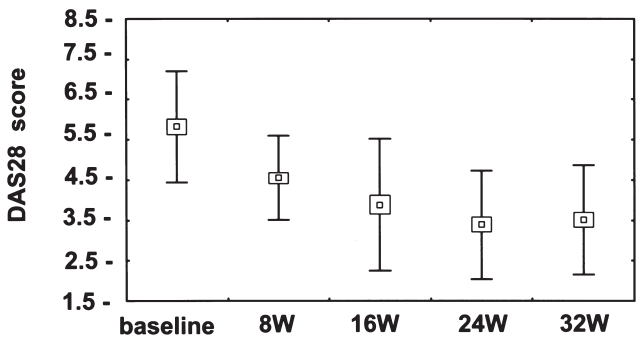

Figure 1. Comparison of DAS28 scores of patients with RA (n = 15) who received adalimumab treatment. Mean DAS28 value at baseline (5.82 ± 1.37) is compared with mean values at 8 weeks (4.53 ± 1.17), 16 weeks (3.72 ± 1.54), 24 weeks (3.43 ± 1.34), and 32 weeks (3.51 ± 1.40) of therapy. Comparison by pairs shows statistically significant differences between these values ($p < 0.05$).

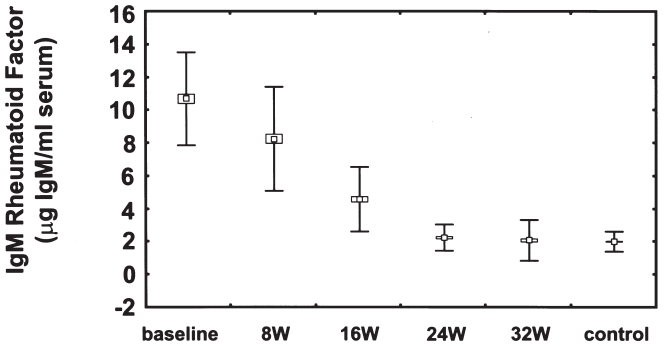

Figure 2. Rheumatoid factor levels in RA patients who received adalimumab therapy. Means of serum IgM RF levels were 10.65 (± 2.84), 8.22 (± 3.15), 4.54 (± 1.97), 2.20 (± 0.79), and 2.06 (± 1.24) μg IgM/ml serum at baseline, 8, 16, 24, and 32 weeks, respectively, and 1.98 (± 0.62) for controls.

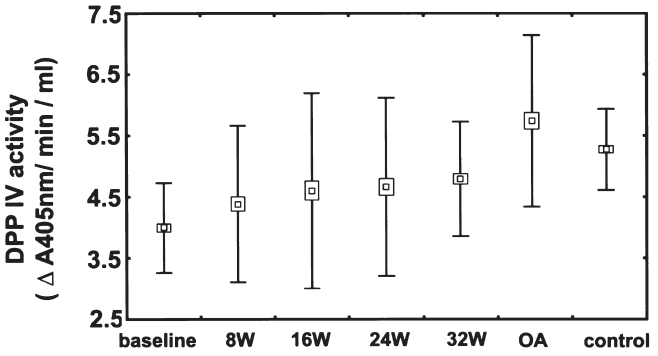

Figure 3. Comparison of DPP IV activities in sera of RA patients (n = 15) who received adalimumab treament. Enzymatic activities were determined directly in serum (20 μl) at baseline and at 8, 16, 24, and 32 weeks of therapy. Activity levels were compared with normal serum activity in healthy controls (n = 15). Difference between mean activity at baseline (3.554 ± 1.096) and at 32 weeks (4.787 ± 0.953) is statistically significant ($p < 0.05$).

a healthy control at the conclusion of treatment (Figures 4A, 4B, lanes 1 and 2, respectively). These serum samples were selected from patients showing DPP IV activity levels above the mean DPP IV activity after 32 weeks of adalimumab therapy. They were compared with 2 OA patients and 2 individual healthy controls (Figures 4A, 4B, lanes 3 and 4, respectively). These results suggest that therapy with adalimumab promotes a reversal in the glycosylation pattern of DPP IV, which is also reflected in an increase of its enzymatic activity. Similarly, we analyzed 2 serum samples (Figures 4C, 4D, lanes 1 and 2, respectively) from the population of RA patients showing DPP IV activity below the mean normal activity after 32 weeks of adalimumab therapy. These samples were compared with 2 OA patients and 2 healthy individuals (Figures 4C, 4D, lanes 3 and 4, respectively). The glycosylation patterns of DPP IV in these patients do not appear substantially changed by therapy with adalimumab, although the intensity of the more acidic DPP IV glycoform protein bands is stronger in the baseline serum

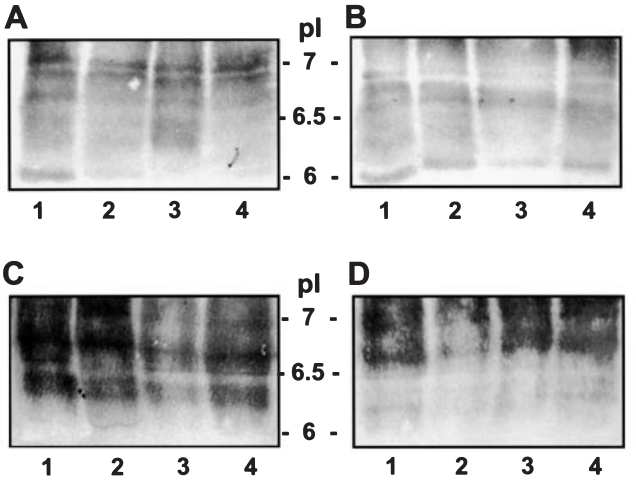

Figure 4. Isoelectric focusing (IEF) electrophoretic analysis of circulating DPP IV glycoforms in patients who received adalimumab treatment. The serum DPP IV glycoform profiles at baseline and 32 weeks of therapy of 4 patients with RA were compared with those of 4 with OA and 4 controls. Lanes 1 and 2 represent isoelectric point profiles of serum DPP IV glycoforms of 4 RA patients at baseline and 32 weeks, respectively; lanes 3 and 4 are isoelectric point profiles of 4 OA patients and 4 controls, respectively. Proteins in serum samples (20 μl) were separated by electrophoresis on pH 5–8 IEF gels, transferred to nitrocellulose membranes, and processed.

samples when compared with the samples at 32 weeks of treatment, OA or healthy controls.

Anti-GRP78 and anti-PGI antibodies. Analyses of anti-GPR78 IgG levels in patients (Figure 5A) shows a gradual decrease of their mean titers from 3.48 (± 1.10) μg IgG/mg total IgG at baseline to 2.86 (± 1.21), 2.14 (± 0.84), 1.86 (± 1.05), and 1.41 (± 1.14) μg IgG/mg total IgG at 8, 16, 24, and 32 weeks, respectively. Analyses of serum anti-GRP78 IgG show levels higher than controls at baseline and at 8, 16, and 24 weeks that are statistically significant ($p < 0.000001$, $p < 0.000007$, $p < 0.002$, and $p < 0.009$, respectively). At 32 weeks their anti-GRP78 IgG levels were not significantly different from those of controls. There was also a steady decline of anti-GRP78 IgG between baseline and 32 weeks. This reduction is statistically significant at Weeks 16 ($p < 0.01$), 24 ($p < 0.009$), and 32 ($p < 0.006$) compared with baseline levels. Similarly, the mean titers of anti-PGI IgG antibodies (Figure 5B) decreased from 35.98 μg IgG/mg total IgG at baseline to 30.64, 14.03, 10.28, and 2.34 μg IgG/mg total IgG at 8, 16, 24, and 32 weeks, respectively. These reductions were also statistically significant at Weeks 16 ($p < 0.009$), 24 ($p < 0.005$), and 32 ($p < 0.002$) compared with baseline levels. The titers of anti-PGI IgG antibodies were not statistically different from those of controls only at Week 32.

Anti-actin, anti-FN, and anti-LDH antibodies. Analyses of the anti-actin IgG antibodies in patients' sera (Figure 6A) reveal no statistically significant changes in mean titers from 3.03 μg IgG/mg total IgG at baseline to 4.09, 2.72,

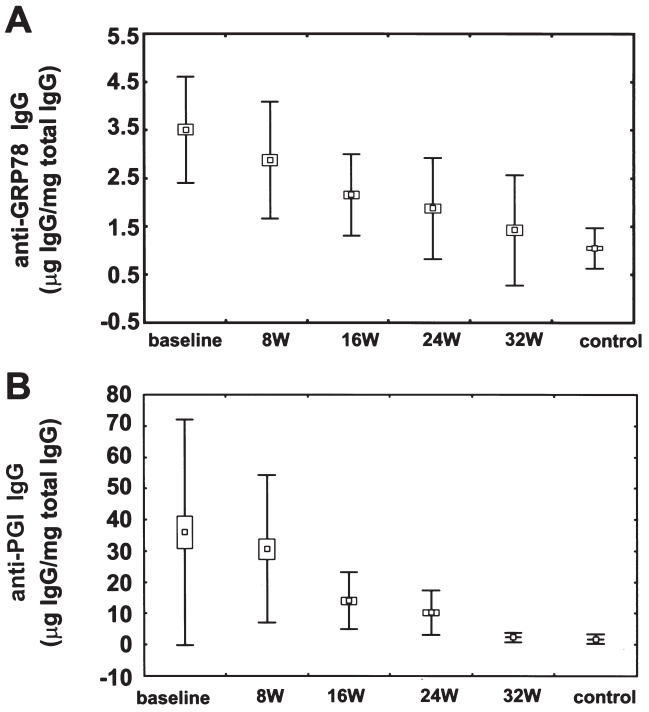

Figure 5. Analysis of serum titers of anti-GRP78 and anti-PGI antibodies in RA patients (n = 15) who received adalimumab treatment at baseline and 8, 16, 24, and 32 weeks compared with controls. (A) Anti-GRP78 antibodies. (B) Anti-PGI antibodies.

2.74, and 3.77 μg IgG/mg total IgG at 8, 16, 24, and 32 weeks, respectively. No statistically significant differences between baseline levels and those at 8, 16, 24, and 32 weeks were found for anti-LDH IgG (Figure 6B). The mean titers of anti-FN IgG antibodies increased, however, from 20.53 μg IgG/mg total IgG at baseline to 31.52, 32.45, 35.10, and 53.07 μg IgG/total IgG at 8, 16, 24, and 32 weeks (Figure 6C). These increases were statistically significant at Weeks 8 ($p < 0.001$), 16 ($p < 0.002$), 24 ($p < 0.00009$), and 32 ($p < 0.000001$) compared with baseline titers. At Week 32, anti-fibronectin IgG levels were also higher than those of controls ($p < 0.000001$). No statistically significant differences between baseline levels and those at 8, 16, 24, and 32 weeks were found for anti-LDH IgG (Figure 6B).

The mean of the changes in DAS28 scores between baseline and those at 8, 16, 24, and 32 weeks correlated significantly with the mean values of the changes between baseline and the anti-PGI and anti-GRP78 IgG titers at 8, 16, 24, and 32 weeks, respectively.

Sialic acid levels of anti-GRP78 and anti-PGI IgG antibodies. In patients with RA, circulating IgG lacking terminal sialic acid in their Fc regions are secreted with exposed terminal galactosyl or mannosyl carbohydrate residues[30]. Nonreducing terminal mannoses in the IgG Fc region are ligands for the serum mannose binding protein (MBP), which, associated to complement-type proteases like C1r and C1s, leads to complement activation through the classical pathway[31]. This is the case for asialylated anti-FN IgG, which bind MBP and have a greater complement-activating activity than normal IgG, as we previously reported for RA patients[21]. For these reasons, we assessed the effect of adalimumab on sialylation levels of anti-GRP78 (Figure 7A) and anti-PGI antibodies (Figure 7B). The mean sialic acid concentrations of anti-GRP78 antibodies were 0.5578 ± 0.1638 and 1.914 ± 0.2996 mole sialic acid/mole IgG at baseline and 32 weeks, respectively ($p < 0.0045$). The mean sialic acid concentrations of anti-PGI antibodies were 0.9254 ± 0.2233 and 1.726 ± 0.2991 mole sialic acid/mole IgG at baseline and 32 weeks, respectively ($p < 0.005$). These results suggest that the decrease in titers of anti-GRP78 and anti-PGI IgG is also accompanied by an increase in their sialylation levels.

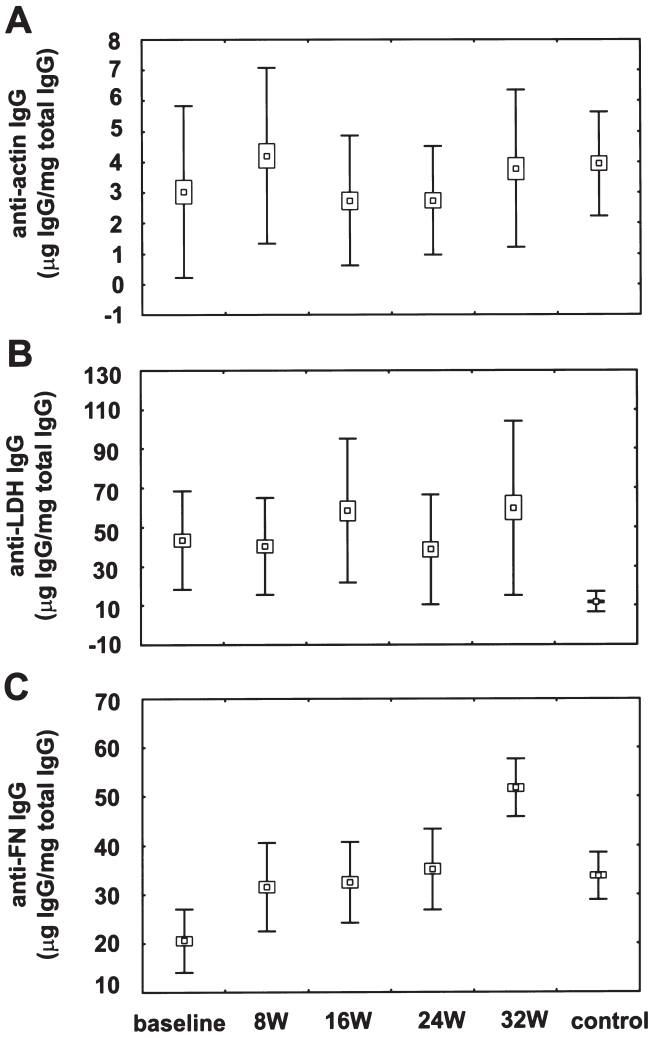

Figure 6. Analysis of serum titers of anti-actin, anti-LDH, and anti-FN antibodies in RA patients (n = 15) who received adalimumab treatment at baseline and 8, 16, 24, and 32 weeks of therapy compared with controls. (A) Anti-actin antibodies. (B) Anti-LDH antibodies. (C) Anti-FN antibodies.

Sialic acid levels of anti-actin, anti-FN, and anti-LDH IgG

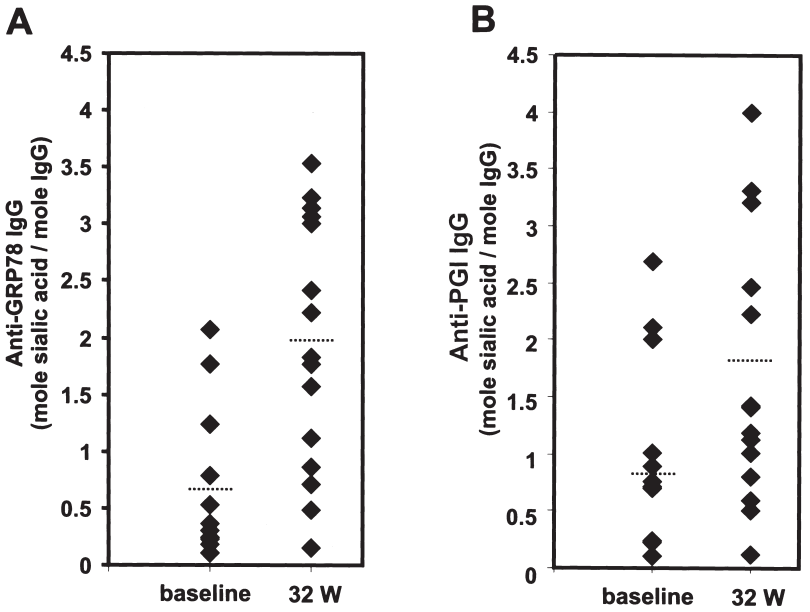

Figure 7. Analysis at baseline and at 32 weeks of sialic acid levels of anti-GRP78 and anti-PGI IgG antibodies in RA patients (n = 15) who received adalimumab treatment. (A) Anti-GRP78 IgG antibodies. (B) Anti-PGI IgG antibodies.

antibodies. There were no significant changes between the mean sialic acid concentrations of anti-actin IgG antibodies (Figure 8A). Their values were 1.522 ± 0.3569 and 1.200 ± 0.2232 mole sialic acid/mole IgG at baseline and at 32 weeks, respectively. Similarly, no significant changes were observed between the mean concentrations of sialic acid of anti-FN (Figure 8B) IgG antibodies (1.426 ± 0.2102 and 1.339 ± 0.2075 moles of sialic acid/mole IgG) or anti-LDH (Figure 8C) IgG antibodies (2.206 ± 0.3095 and 1.975 ± 0.2687 moles sialic acid/mole IgG) at baseline and 32 weeks, respectively.

DISCUSSION

Targeting TNF-α with therapeutic antibodies has been the objective of numerous studies in animal models of arthritis and in patients with RA[1-7,32]. Although IL-1, IL-6, and IL-8 also play a role in the pathogenesis of RA[4], TNF-α is considered pivotal, since it is found at biologically significant

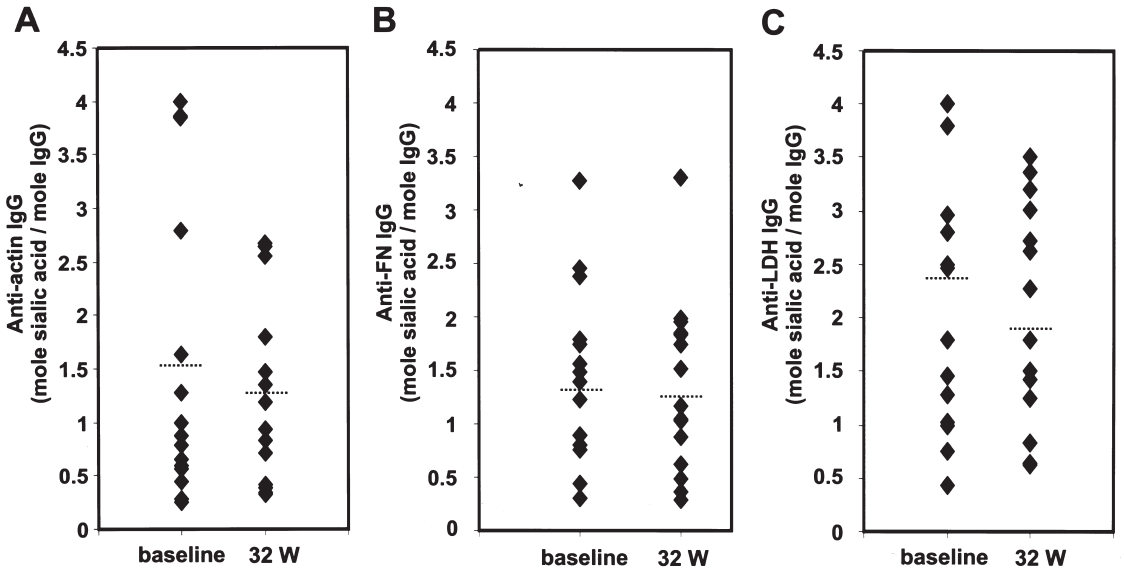

Figure 8. Analysis at baseline and at 32 weeks of sialic acid levels of anti-actin, anti-FN, and anti-LDH IgG antibodies in RA patients (n = 15) who received adalimumab treatment. (A) Anti-actin IgG antibodies. (B) Anti-FN IgG antibodies. (C) Anti-LDH IgG antibodies.

concentrations in RA synovial tissue and fluid[33]. By contrast, it is not present in either synovial fluid or tissue from patients with OA[33]. In RA synovia, TNF-α levels correlate with both the extent of inflammation and bone erosion[34]. In our study, the efficacy of adalimumab, a recombinant fully human IgG1 monoclonal antibody that neutralizes TNF-α, was evaluated in a group of 15 RA patients who received adalimumab therapy. We assessed DPP IV enzymatic activity and profiles of circulating DPP IV glycoforms in sera from these patients. We also evaluated their IgM RF, anti-GRP78, anti-PGI, anti-actin, anti-FN, and anti-LDH IgG autoantibody titers along with their sialylation levels.

The reduction of the mean DAS28 score during adalimumab treatment shows that this is a DAS28 responder group of patients with RA. Our results show a gradual increase from low enzymatic activity and highly sialylated DPP IV glycoforms[15] to nearly normal levels of enzymatic activity and glycoform profiles in the serum of these patients. These measures were also compared with a population of 10 patients with OA, who showed normal DPP IV activity as well as normal serum DPP IV glycoform profiles. Analyses of IgM RF in sera of the RA patients showed a steady decline in the IgM RF titers from baseline to Week 32 of therapy, suggesting that the improvement in their clinical symptoms reflected in DAS28 scores was accompanied by a reduction in circulating IgM RF. A similar response to anti-TNF-α therapy has been observed by others[35-37], showing that a decrease in serum titers of RF is a significant feature of clinical improvement in RA.

Similarly, titers of anti-GRP78 and anti-PGI IgG antibodies returned nearly to levels found in healthy controls. Further, the sialylation levels of these autoantibodies were significantly increased after adalimumab therapy. We hypothesize these results indicate a TNF-α-specific effect on the IgG titers against these antigens in comparison with titers of other IgG autoantibodies, like anti-actin and anti-LDH, also elevated in RA[21,22], as well as their sialylation levels, which were not significantly altered during adalimumab therapy. Together, these results provide evidence that TNF-α is a key factor in both control of glycosylation and secretion of serum DPP IV, as well as the production and sialylation of IgG autoantibodies to GRP78 and PGI.

A decrease of DPP IV activity due to hypersialylation has been observed in peripheral blood T cells from patients with acquired immune deficiency syndrome[38]. Changes in glycosylation also affect DPP IV[39]. However, in RA, hypersialylation does not influence secretion of DPP IV, as its mean concentration in RA serum samples was not significantly different from that of healthy controls[15]. Our data suggest that TNF-α is involved in the process leading to DPP IV hypersialylation, a biological modification that affects DPP IV activity, thereby influencing the multiple roles that this enzyme plays in the immune response[8]. In this context, a recent study in bovine synoviocytes showed that TNF-α stimulates enzymes potentially important to the inflammatory process like core 2 ß6-GLcNAc-transferase, ß4-Gal-transferase, and α6-sialyltransferase[40], suggesting this mechanism as a possible explanation for the hypersialylation observed in DPP IV in RA.

The autoimmune response to GRP78 has been recently evaluated[41], and it has been suggested to play a protective role in RA against ongoing inflammation both in humans and in a mouse model[41]. Our data, however, suggest that sialylation of circulating GRP78 IgG autoantibodies was negatively affected by TNF-α, a biological effect opposite to that observed with DPP IV, suggesting that this cytokine affects sialylation in a protein-specific manner. For example, in aortic endothelial cells TNF-α simultaneously stimulated an increase in O-glycan core 1 synthesis, but a decrease in core 2 synthesis[42].

The autoimmune response to PGI in RA has been assessed in humans[43,44] and a mouse model[45-47]. In arthritic mice, deposits containing immune complexes of PGI and anti-PGI IgG accumulated on the lining of the normal articular capsule, most visibly along the cartilage surface, localized with C3 complement[47]. Similar deposits were also found in human arthritic joints, suggesting that PGI-anti-PGI complexes on articular surfaces initiate an inflammatory cascade via the alternative complement pathway[47]. Although this scenario is hypothetical and the efficacy of PGI as an autoantigen in humans has been challenged[48,49], abnormal changes in the physiology of the inflamed RA synovium certainly suggest a pathogenic role for PGI in this disease. For instance, the intraarticular hypoxia in the inflamed rheumatoid joint may upregulate synthesis of PGI[50], which, under the acidic conditions of this environment, may experience a conformational change leading to both an increased cell surface association and its deposition on fibronectin fibrils[51]. Thus, conditions exist that may serve to induce and perpetuate the autoimmune response to PGI. Further, the reduction of RA symptoms induced by adalimumab in our patients was accompanied by a reduction of anti-PGI IgG autoantibody titers and an increase in their sialylation. Hypothetically, these changes may inhibit the complement activating capacity of the PGI-anti-PGI immune complexes[31,45], thereby supporting a pathogenic role for PGI in human RA.

We observed no significant changes in anti-actin and anti-LDH IgG antibody titers or their sialic acid levels in these patients. However, the immune response to FN appears to increase after adalimumab therapy. The mean titer of anti-FN IgG antibodies increased significantly during the treatment; however, its sialic acid levels did not change significantly. Although these autoantibodies are potentially pathogenic[21], their existence did not influence the improvement in symptoms observed in these patients during therapy, as indicated by a reduction in the mean DAS28 score at the end of the treatment.

Our results support studies showing that targeting TNF-α with adalimumab induces improvements in the symptoms of RA[52]. We observed that these improvements correlate with biochemical changes in the structure of DPP IV and changes in the titers and structure of anti-GRP78 and anti-PGI IgG antibodies.

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