Anti-Tumor Necrosis Factor-α Therapy Augments Dipeptidyl Peptidase IV Activity and Decreases Autoantibodies to GRP78/BIP and Phosphoglucone 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 patho-
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. 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. 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. 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. The reduction of DPP IV enzymatic activity was caused by hyperglyalation as its specific activity was restored after enzymatic desialylation of the protein. 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.

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), phosphoglucone isomerase (PGI), fibronectin (FN), lactate dehydrogenase (LDH), and actin, all of which are significantly elevated in patients with RA. 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-PIG IgG antibodies.

MATERIALS AND METHODS

Patients. We studied 15 patients fulfilling the 1987 revised American Rheumatism Association criteria for classification of RA. 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 anti-inflammatory 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 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 ΔA405 nm/min. All experiments were performed in triplicate unless otherwise specified.

Antibodies. Goat affinity purified F(ab’), 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, and purified by affinity chromatography on protein A-Sepharose, 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’), fragments (5 µg/ml) in 0.1 M Na2CO3, 0.01% NaNO3, 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’), 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 MgCl2, 1 mM ZnCl2, 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’), 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’), 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-protein 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 H3PO4 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-
dent 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 8-mercaptoethanol) for 30 min at room temperature with shaking. After SDS treatment, the proteins were transferred from the gel to nitrocellulose membranes by Western blot method. 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. 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 performed 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).

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
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.

Figure 4.
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/mg 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. 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. 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. 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 < 0.0045). 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 < 0.0045). The mean sialic acid concentrations of anti-GRP78 and anti-PGI IgG is also accompanied by an increase in their sialylation levels.

Sialic acid levels of anti-actin, anti-FN, and anti-LDH IgG
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 RA1-7,32. Although IL-1, IL-6, and IL-8 also play a role in the pathogenesis of RA4, TNF-α is considered pivotal, since it is found at biologically significant

![Figure 7](image_url).

![Figure 8](image_url).

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.

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\textsuperscript{33}. By contrast, it is not present in either synovial fluid or tissue from patients with OA\textsuperscript{33}. In RA synovia, TNF-\(\alpha\) levels correlate with both the extent of inflammation and bone erosion\textsuperscript{34}. In our study, the efficacy of adalimumab, a recombinant fully human IgG1 monoclonal antibody that neutralizes TNF-\(\alpha\), 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\textsuperscript{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-\(\alpha\) therapy has been observed by others\textsuperscript{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 autoantibodies 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-\(\alpha\)-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\textsuperscript{21,22}, as well as their sialylation levels, which were not significantly altered during adalimumab therapy. Together, these results provide evidence that TNF-\(\alpha\) 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\textsuperscript{38}. Changes in glycosylation also affect DPP IV\textsuperscript{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\textsuperscript{15}. Our data suggest that TNF-\(\alpha\) 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\textsuperscript{8}. In this context, a recent study in bovine synoviocytes showed that TNF-\(\alpha\) stimulates enzymes potentially important to the inflammatory process like core 2 \(\beta 6\)-GLcNAc-transferase, \(\beta 4\)-Gal-transferase, and \(\alpha 6\)-sialyltransferase\textsuperscript{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\textsuperscript{41}, and it has been suggested to play a protective role in RA against ongoing inflammation both in humans and in a mouse model\textsuperscript{41}. Our data, however, suggest that sialylation of circulating GRP78 IgG autoantibodies was negatively affected by TNF-\(\alpha\), 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-\(\alpha\) simultaneously stimulated an increase in O-glycan core 1 synthesis, but a decrease in core 2 synthesis\textsuperscript{42}.

The autoimmune response to PGI in RA has been assessed in humans\textsuperscript{43,44} and a mouse model\textsuperscript{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\textsuperscript{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\textsuperscript{47}. Although this scenario is hypothetical and the efficacy of PGI as an autoantigen in humans has been challenged\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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\textsuperscript{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. 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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