Anti-TNF antibody treatment improves glucocorticoid induced insulin-like growth factor 1 (IGF1) resistance without influencing myoglobin and IGF1 binding proteins 1 and 3

P Sar zi-Put tin i, F Atzeni, J Schölmerich, M Cutolo, R H Straub

Background: Insulin-like growth factor 1 (IGF1) is an important determinant of muscle mass because it promotes growth and suppresses protein degradation. IGF1 is decreased in rheumatoid arthritis and juvenile idiopathic arthritis because its synthesis is inhibited by inflammation. In parallel, glucocorticoids induce IGF1 resistance and add to muscle degradation.

Objective: To investigate the influence of anti-tumour necrosis factor antibody treatment (anti-TNF) with adalimumab on levels of myoglobin (degradation marker) and IGF1 in patients with rheumatoid arthritis and without prednisolone treatment.

Methods: Subcutaneous adalimumab was given to 32 patients with longstanding rheumatoid arthritis (16 with and 16 without prednisolone) in a longitudinal study. IGF1, IGF1 binding protein 1 (IGFBP-1), IGFBP-3, and myoglobin were measured by enzyme linked immunosorbent assay.

Results: Rheumatoid patients had normal serum myoglobin. Patients on prednisolone had higher myoglobin than patients not receiving prednisolone, indicating increased muscle degradation. On treatment with anti-TNF, myoglobin levels did not change in either patient group. Serum IGF1 was increased in patients with v without prednisolone, indicating IGF1 resistance (mean (SEM): 221 (23) 122 (14) µg/l, p < 0.001). Adalimumab treatment decreased the raised IGF1 levels in patients with prednisolone, so that after 12 weeks of treatment they reached the level of patients without prednisolone. Serum IGFBP-1 and IGFBP-3 did not differ in the two groups, and anti-TNF did not change these concentrations.

Conclusions: Anti-TNF antibody treatment over 12 weeks improved glucocorticoid induced IGF1 resistance without influencing myoglobin and IGF1 binding proteins. Thus, in rheumatoid patients on glucocorticoids with generally decreased muscle mass anti-TNF treatment with adalimumab has favourable effects.

Abbreviations: ACR, American College of Rheumatology; EULAR, European League Against Rheumatism; IGF1, insulin-like growth factor 1; ReAct, Adalimumab Research in Active Rheumatoid Arthritis study; TNF, tumour necrosis factor.

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Many patients with rheumatoid arthritis suffer from decreased muscle function and loss of body cell mass.1–4 The mechanisms involved are unknown at present. High levels of tumour necrosis factor (TNF) and parallel glucocorticoid treatment were thought to be important elements in these alterations in rheumatoid patients.4–5

One key element in maintaining muscle mass is insulin-like growth factor 1 (IGF1), by promoting muscle growth and suppressing muscle degradation.6 IGF1 has additional favourable effects on TNF induced cartilage degradation.7 Furthermore, TNF—a major proinflammatory cytokine in rheumatoid arthritis—inhibits synthesis of IGF1 from liver cells,7,8 and it also inhibits IGF1 mediated anabolic effects on peripheral tissue.9–11 From these data, one would expect an increase of serum IGF1 levels during anti-TNF treatment in patients with rheumatoid arthritis, which has never been investigated.

In addition, parallel glucocorticoid treatment may influence the effects of anti-TNF treatment because glucocorticoids per se can lead to IGF1 resistance.12 IGF1 resistance has been demonstrated during glucocorticoid treatment by an increase in serum IGF1.12–14 A glucocorticoid related increase of IGF1 in the presence of increased muscle degradation is a marker of muscle IGF1 resistance. A similar phenomenon exists with respect to insulin,14 in that glucocorticoids increase insulin resistance. Furthermore, patients with rheumatoid arthritis show insulin resistance, which is improved during anti-TNF treatment.15

As serum levels of IGF1 and its most important binding proteins, IGF1 binding protein 1 (IGFBP-1) and IGFBP-3, have never been studied during anti-TNF treatment in rheumatoid patients, we aimed to shed light on these variables during a 12 week course of adalimumab treatment. We also investigated IGF1 resistance during glucocorticoid treatment.

METHODS

Patients, adalimumab treatment, and blood samples

The study involved the administration of the human monoclonal antibody adalimumab (Abbott SpA, Campoverde di Aprilia, Italy) to 32 white patients with rheumatoid arthritis fulfilling the American College of Rheumatology (ACR) criteria for the disease.16 The patients were selected according to the inclusion criteria of the adalimumab research in active rheumatoid arthritis study (ReAct). In all, 16 patients received parallel prednisolone, while the other 16 did not receive parallel prednisolone and.
had not been treated with prednisolone for at least six months. Most patients were also given additional methotrexate (the dose remained stable throughout this study), but no other immunosuppressive drugs. The baseline characteristics of patients are given in table 1.

Patients were assigned to receive single self injections of adalimumab subcutaneously, 40 mg every other week. Efficacy assessments included ACR and EULAR response criteria (carried out by FA and PS-P). A baseline blood sample was taken one to two weeks before the start of adalimumab treatment. Anti-TNF antibodies were infused on weeks 0, 2, 4, 6, 8, 10, and 12. For this study, patients were investigated clinically and blood was drawn between 08:00 and 09:00 in the morning when the patients visited the outpatient clinic on the baseline day, and in weeks 2, 6, and 12. The blood was immediately centrifuged and serum was stored on $-280\,\text{°C}$. The ethics committee of L Sacco University Hospital, Italy, approved the study.

**Laboratory indices**

We used enzyme immunometric assays for the quantitative determination of serum levels of interleukin 6 (IL6) (high sensitivity Quantikine, R&D Systems, Minneapolis, Minnesota, USA), myoglobin (Life Diagnostics Inc, West Chester, Pennsylvania, USA; normal range according to the manufacturer 12 to 90 ng/ml), IGF1 (IDS, Bolden, UK; normal range according to the manufacturer of subjects aged 60 years: 30 to 200 mg/l), IGFBP-1 (Oy Medix Biochemia, Kauninen, Finland), and IGFBP-3 (Biosource Europe, Nivelles, Belgium). Intra-assay and interassay coefficients of variation for all tests were below 10%.

**Statistical analysis**

Medians between different groups were compared by the non-parametric Mann–Whitney test (SPSS/PC, Advanced Statistics, V11.5.1, SPSS Inc, Chicago, Illinois, USA). A decrease or increase in a variable over time (during anti-TNF treatment) was tested using the non-parametric Friedman test (SPSS). An interrelation between two variables was tested by the non-parametric Spearman rank correlation analysis (SPSS). A probability (p) value <0.05 was the significance level.

**RESULTS**

Anti-inflammatory effects of adalimumab treatment

Adalimumab treatment had excellent anti-inflammatory effects in patients with rheumatoid arthritis with or without glucocorticoids, as investigated by the number of swollen

<table>
<thead>
<tr>
<th>Table 1 Characteristics of patients under investigation</th>
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<tbody>
<tr>
<td>Number</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>Sex (F/M)</td>
</tr>
<tr>
<td>Disease duration (years)</td>
</tr>
<tr>
<td>Body mass index (kg/m$^2$)</td>
</tr>
<tr>
<td>Baseline ESR (mm/1st hour)</td>
</tr>
<tr>
<td>Baseline C reactive protein (mg/l)</td>
</tr>
<tr>
<td>Baseline serum IL6 (pg/ml)</td>
</tr>
<tr>
<td>Positive for rheumatoid factor</td>
</tr>
<tr>
<td>Positive for antinuclear antibodies</td>
</tr>
<tr>
<td>Baseline swollen joint score (points)</td>
</tr>
<tr>
<td>Baseline tender joint score (points)</td>
</tr>
<tr>
<td>Baseline DAS28 (points)</td>
</tr>
</tbody>
</table>

*Values are means (SEM) or n (%).

**p < 0.01 vs patients with glucocorticoids. No other variables were different between patients with and without glucocorticoid treatment. No patient received azathioprine, leflunomide, ciclosporine A, or sulfasalazine.

DA28, joint disease activity score; ESR, erythrocyte sedimentation rate; F, female; IL6, interleukin 6; M, male; NSAID, non-steroidal anti-inflammatory drug.
joints, the number of tender joints, patients’ global assessment of pain, and serum levels of IL6 (table 2). It seemed that these effects were more marked in patients who were not receiving glucocorticoids (table 2).

Influence of glucocorticoid treatment on muscle degradation and effects of adalimumab
Baseline serum myoglobin levels were significantly higher in patients on prednisolone than in patients not receiving glucocorticoids (fig 1). This indicates a higher degree of muscle degradation in patients on glucocorticoids. After controlling for age, this difference remained statistically significant at weeks 2 and 12 (but not at baseline or week 6). During the course of anti-TNF treatment, myoglobin levels remained stable in all patients, whether or not they were receiving prednisolone (fig 1). Anti-TNF did not change the myoglobin levels, which may indicate that TNF is not the main player in this glucocorticoid induced phenomenon.

Influence of glucocorticoid treatment on serum IGF1 and the effects of adalimumab
Patients on prednisolone had markedly higher serum IGF1 levels than those not receiving prednisolone (fig 2A), even though the former were somewhat older and a lower serum IGF1 would be expected (table 1). This was particularly evident at baseline, where serum IGF1 values exceeded the normal age related range given by the manufacturer (30 to 200 µg/l) in more than half the patients on prednisolone. In the presence of increased muscle degradation (myoglobin release), this phenomenon is called IGF1 resistance.

During the course of anti-TNF treatment, serum IGF1 values did not change markedly in patients not receiving prednisolone (fig 2B). However, in patients who were receiving prednisolone the raised IGF1 values decreased on average by more than 50 µg/l (fig 2B). The decrease was statistically significant (Friedman test, fig 2B). After 12 weeks of adalimumab treatment, serum IGF1 values were not different between the two groups (fig 2B), which indicates normalisation of IGF1 resistance.

Influence of glucocorticoid treatment on IGF1 binding proteins and effects of adalimumab
At baseline, concentrations of the two major binding proteins of IGF-1, IGFBP-1 and IGFBP-3, did not differ in patients receiving or not receiving glucocorticoid treatment (data not shown). The values remained stable throughout the course of treatment (fig 2B). The Friedman p values show whether values changed during the treatment (that is, there was a significant decrease in glucocorticoid treated patients).

Table 2  Response indices during 12 weeks of adalimumab treatment

<table>
<thead>
<tr>
<th></th>
<th>Number of swollen joints</th>
<th>Number of tender joints</th>
<th>Pain, patient’s global assessment</th>
<th>Serum IL6 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td>8.8 (0.7) [9.1 (0.6)]</td>
<td>10.5 (0.8) [9.5 (0.5)]</td>
<td>54.8 (4.5) [54.8 (3.7)]</td>
<td>21.6 (7.4) [33.9 (12.2)]</td>
</tr>
<tr>
<td>Week 2</td>
<td>7.3 (0.9) [7.0 (0.7)]</td>
<td>9.6 (0.7) [8.1 (0.5)]</td>
<td>40.8 (3.5) [40.7 (5.3)]</td>
<td>4.1 (1.8) [7.0 (1.8)]</td>
</tr>
<tr>
<td>Week 6</td>
<td>4.3 (0.9) [4.3 (0.6)]</td>
<td>7.5 (0.80) [6.5 (0.7)]</td>
<td>32.3 (3.8) [38.5 (3.8)]</td>
<td>8.7 (5.3) [5.5 (2.1)]</td>
</tr>
<tr>
<td>Week 12</td>
<td>2.8 (0.5) [3.4 (0.6)]</td>
<td>6.1 (0.5) [5.4 (0.7)]</td>
<td>23.3 (3.7) [25.2 (4.7)]</td>
<td>2.9 (0.9) [13.8 (8.5)]</td>
</tr>
</tbody>
</table>

Data are mean (SEM). Data on patients receiving glucocorticoid treatment are given in square brackets.
*p<0.003, indicating a decrease as assessed by the Friedman test.
IL6, interleukin 6.
adalimumab treatment (data not shown). Adalimumab treatment did not affect the serum levels of these binding proteins (data not shown).

Influence of IL6 and drug treatment on IGF1 and its binding proteins

There was no correlation between serum levels of IGF1 and IL6 in patients receiving or not receiving prednisolone (data not shown), neither was there a correlation between C reactive protein or erythrocyte sedimentation rate and serum IGF1 (data not shown).

In patients on prednisolone, serum IL6 correlated positively with serum IGFBP-1 in week 2 ($R_{\text{Rank}} = 0.500$, $p = 0.048$), in week 6 ($R_{\text{Rank}} = 0.598$, $p = 0.015$), and in week 12 ($R_{\text{Rank}} = 0.797$, $p < 0.001$). The positive correlation increased during adalimumab treatment, becoming evident at much lower levels of IL6 (table 2). However, no such correlation was present in patients not receiving prednisolone (data not shown). IL6 did not correlate with IGFBP-3 in either patient group. Furthermore, no correlation was found between IGF1, IGFBP-1, or IGFBP-3 and the intake of non-steroidal anti-inflammatory drugs or chloroquine/hydroxychloroquine (data not shown).

DISCUSSION

The hypothalamus-pituitary-liver-muscle (HPLM) axis is a delicate reflex loop stabilising muscle mass (fig 3): the liver, upon stimulation with growth hormone, produces IGF1, IGF1 stimulates muscle growth, and IGF1 itself inhibits its own secretion from the liver.25 IGF1 induced inhibition of liver IGF1 is probably dependent on consumption of liver IGF1 within the muscle (binding to receptors and local degradation). Both TNF and exogenous glucocorticoids inhibit muscle growth by stimulating protein degradation pathways in the muscle and by interfering with IGF1 signalling (fig 3).

Glucocorticoids exert an additional inhibiting effect on muscle by inducing the muscle growth inhibiting factor myostatin.21 One study in burn induced muscle degradation showed that exogenous and endogenous glucocorticoids induce myostatin.22 Furthermore, it was shown that glucocorticoids inhibit the production of muscle IGF1, leading to a decrease in negative feedback regulation of liver IGF1 (fig 3).23 The decrease in negative feedback would increase serum levels of IGF1 (fig 3).

In addition, TNF inhibits growth hormone induced IGF1 production from the liver.24, 25 We have not tested serum levels of growth hormone, a decrease in which might cause low serum levels of IGF1 (as observed). Such a decrease in growth hormone would be an unwanted effect of high TNF levels, which could add to the negative sequelae of rheumatoid arthritis, such as atherosclerosis and raised serum lipids. However, as growth hormone kinetics are not notably changed in rheumatoid patients in general,26 the effect is likely to be dependent on a mechanism other than growth hormone secretion and action in the liver. In addition, because exogenous glucocorticoids inhibit pituitary growth hormone release,27 one would have expected low levels of growth hormone stimulated IGF1 in patients with prednisolone treatment. However, exactly the opposite was observed, refuting a marked influence of growth hormone on the observed phenomena.

In patients with rheumatoid arthritis, TNF plays a dominant proinflammatory role, and affected individuals are often treated with exogenous glucocorticoids (as in the ReAct study). In such patients, therefore, two important muscle growth inhibiting factors are present which may have a major influence on muscle homeostasis (fig 3). This is adds to the disability suffered by these patients and contributes to rheumatoid cachexia.28 Our study shows that neutralisation of TNF by anti-TNF therapy decreases serum IGF1 in patients receiving glucocorticoids (in the presence of somewhat raised myoglobin levels). It is possible that our patients who were on prednisolone were more ill than those not receiving prednisolone, which may have resulted in increased myoglobin levels. However, this was not supported by the baseline characteristics given in table 1. It is noteworthy that myoglobin did not change during anti-TNF treatment, but this may depend on other proinflammatory factors and longstanding effects on muscle degradation. Removal of the important proinflammatory agent TNF will probably attenuate muscle wasting by increasing IGF1 effects (after removal of IGF1 resistance). We did not test muscle strength during the treatment but it is thought that muscle function improves during anti-TNF therapy, as indicated by improved scores on the Health Assessment Questionnaire.29 Positive effects of TNF neutralising strategies on growth rates have been described in children with juvenile idiopathic arthritis.29 In addition, positive effects of improved IGF1 signalling on TNF induced cartilage degradation are also expected.76 8

Our two patients groups—with and without glucocorticoid treatment—were different in age, which may have influenced
our results. Patients on prednisolone were on average 10 years older than those not receiving glucocorticoids. As IGFI levels decrease with aging, one would have expected a lower serum IGFI in the older patients (who were also more likely to be on prednisolone). However, we observed exactly the opposite, with increased IGFI levels in these patients, which is additional evidence of IGFI resistance.

Furthermore, the observed effects of anti-TNF are most probably independent of IGFI binding proteins because serum IGFBP-1 and IGFBP-2 did not change during treatment. In this study, we observed a positive correlation between serum IL6 and serum IGFBP-1, which confirms a positive influence of IL6 on the synthesis of these binding proteins.12

Conclusions
Our study suggests an attenuation of IGFI resistance in steroid treated patients with rheumatoid arthritis who are also receiving an anti-TNF therapy. This may be an important new favorable effect of anti-TNF treatment in such patients. Future studies in larger samples over a longer period of time should address whether or not anti-TNF therapy increases both muscle mass (as measured, for example, by magnetic resonance imaging) and muscle strength on clinical testing.

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REFERENCES