CONCISE REPORT

Blockade of tumour necrosis factor α significantly alters the serum level of IgG- and IgA-rheumatoid factor in patients with rheumatoid arthritis

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Objective: To determine the effect on the humoral immune system of long term treatment of patients with RA with etanercept.

Methods: 12 consecutive patients with seropositive RA treated with etanercept were studied and followed up for 9 months. Clinical efficacy of treatment was evaluated using the 28 joint count Disease Activity Score (DAS28). Serum samples were collected at baseline and after 9 months and serum immunoglobulin, RF isotypes, and anti-cyclic citrullinated peptide (aCCP), antinuclear, nucleosome, and dsDNA antibodies determined. For comparison 7 patients with seropositive RA treated with adalimumab were studied.

Results: DAS28 decreased significantly after the first month and then was constant for the whole study (5.7 (0.3) *v* 3.8 (0.2), $p \leqslant 0.000$). Serum IgA-RF and IgG-RF increased significantly after 9 months' etanercept treatment (mean (SEM) IgA-RF rose from 19.5 (4.8) to 30.5 (5.9) IU/ml, $p \leqslant 0.01$; IgG-RF from 20.6 (8.1) to 33.8 (11.5) IU/ml, $p \leqslant 0.04$). Serum levels of total immunoglobulin and specific autoantibodies remained unchanged during the study. In patients treated with adalimumab, no significant changes in serum levels of RF isotypes and aCCP antibodies were seen.

Conclusion: Etanercept, although effective in treating the clinical symptoms of RA, seems to have a pivotal effect on RF-producing B cells either directly or indirectly.

Rheumatoid arthritis (RA) is a chronic inflammatory disease characterised by persistent inflammation of synovial tissue. Although the initiating event of RA is still unknown, recent research has demonstrated the importance of the increased production of tumour necrosis factor (TNF) α in the perpetuation of the inflammatory process of this disease. A new class of highly effective antirheumatic drugs has been developed, which target this molecule with soluble receptors—for example, etanercept, or antibodies, like infliximab or adalimumab.[1 2]

In contrast with T cells, the role of B cells in RA is still controversial. Nevertheless, B cells appear to be of importance in the pathogenesis of RA. Thus, in the inflamed synovial tissue of patients with RA, germinal centre-like structures are present, and products of B cells such as the rheumatoid factor (RF) or anti-cyclic citrullinated peptide (aCCP) antibodies are well established indicators of this disease.[3] Several reports have also indicated that a high titre of RF is associated with progressive joint damage, and extra-articular manifestations, especially in patients with raised IgG-RF and IgA-RF isotypes.[4] Yet the sequential measurement of IgG- and IgA-RF is not performed routinely because of conflicting data on the clinical relevance of these isotypes.

Interestingly, the effect of TNF blocking agents on B cells is unclear. It has been shown that during treatment with TNF blockers no change in serum immunoglobulin levels is found.[5] On the other hand, several reports have indicated increased production of autoantibodies specific to systemic lupus erythematosus[6 7] or even the development of B cell lymphomas.[8] Although, in the latter case, it seems that the disease activity and inflammation of RA are associated with the risk for lymphomas.[9]

To study the long term effect of anti-TNF treatment on the production of antibodies we measured the serum levels of immunoglobulin and certain autoantibodies in patients with RA treated with etanercept.

PATIENTS AND METHODS

Patients

Twelve consecutive patients with seropositive RA (table 1 shows detailed demographic data) treated with etanercept were included in the study after informed consent and approval by the local ethic committee had been obtained. Patients fulfilled the American College of Rheumatology (ACR) criteria for the diagnosis of RA and had a 28 joint count Disease Activity Score (DAS28) of ⩾4.0. Patients were followed up for only 9 months because of a shortage of etanercept on the European market at the end of the study period. During the observation period none of the patients had any signs of infection of the respiratory, gastrointestinal, or urinary-genital tract. The clinical efficacy of treatment was evaluated at study entry, after 1, 2, and 3 months, and then every third month using DAS28. Serum samples were collected at baseline and after 9 months. All serum samples were stored at −20℃ until the tests were performed. During the observation period patients were receiving a stable glucocorticoid dose of ⩽10 mg. For comparison seven patients with seropositive RA treated with adalimumab (table 1 shows demographic data) were followed up.

Laboratory examinations

Serum immunoglobulin levels (IgG (normal range 7.0–16.0 g/l), IgM (normal range 0.4–2.3 g/l), and IgA (normal range 0.7–4.0 g/l)), β_2-microglobulin (normal range <2.5 mg/l; DADA-Behring, Marburg, Germany), and IgM-RF (normal range ⩽14 U/ml) were determined by nephelometry. For detection of RF isotypes (Autostat II IgA-RF (normal range <20 U/ml), Autostat II IgG-RF (normal range <30 U/ml); Hycor Biomedical, Kassel, Germany), IgG-aCCP antibodies (normal range ⩽25 U/ml, Immunoscan RA; Euro

Abbreviations: aCCP, anti-cyclic citrullinated peptide; ACR, American College of Rheumatology; DAS28, 28 joint count Disease Activity Score; RA, rheumatoid arthritis; RF, rheumatoid factor; TNF, tumour necrosis factor

Table 1 Demographic features of patients with RA at baseline

Characteristics	Etanercept (n = 12)	Adalimumab (n = 7)
Sex		
Men, No (%)	4 (33)	1 (14)
Women, No (%)	8 (67)	6 (86)
Age (years), mean (SD)	60 (7)	53 (12)
Disease duration (years), mean (SD)	13 (6)	10 (7)
DMARDs before TNFα blocker (n), mean (SD)	5 (2)	5 (3)
Previous DMARDs (n)		
Methotrexate	12	7
Hydroxychloroquine	5	4
Sulfasalazine	5	6
Ciclosporin A	5	2
Gold (intramuscular)	4	1
Gold (oral)	4	2
Leflunomide	6	5
Azathioprine	1	0

Diagnostica, Malmö, Sweden), dsDNA antibodies (normal range <55 U/ml; Pharmacia Diagnostic, Vienna, Austria), and antinucleosome antibody (normal range ⩽25 U/ml; Nucleosome-IgG ELISA; D-tek, Mons, Belgium) commercially available enzyme linked immunosorbent assay (ELISA) kits were used according to the manufacturer's instructions. Antinuclear antibodies were detected using indirect immunofluorescence on rat liver sections.

Statistical analysis

The Wilcoxon rank test was used for statistical analysis. The correlation coefficient (r_s) was calculated using the Spearman rank correlation (StatView for Windows; SAS Institute Inc. copyright © 1992–1998; version 5.0.1). The threshold for significance was $p<0.05$.

RESULTS

All patients with RA showed a rapid, significant, and sustained clinical response to etanercept treatment throughout the observation period (data not shown). After 9 months of treatment the ACR20, ACR50, and ACR70 responses were 92% (11/12), 58% (7/12), and 25% (3/12), respectively. Furthermore, there was a significant decrease in the DAS28 after the first month, which was constant for the whole observation period. At baseline the mean (SEM) DAS was 5.7 (0.3) and after 9 months 3.8 (0.2) ($p \leqslant 0.000$). Surprisingly, a significant increase in IgG-RF and IgA-RF serum levels after 9 months of etanercept treatment was seen (figs 1A and 2). Thus, IgG-RF rose from 20.6 (8.1) to 33.8 (11.5) U/ml ($p \leqslant 0.04$) and IgA-RF from 19.5 (4.8) to 30.5 (5.9) U/ml ($p \leqslant 0.01$).

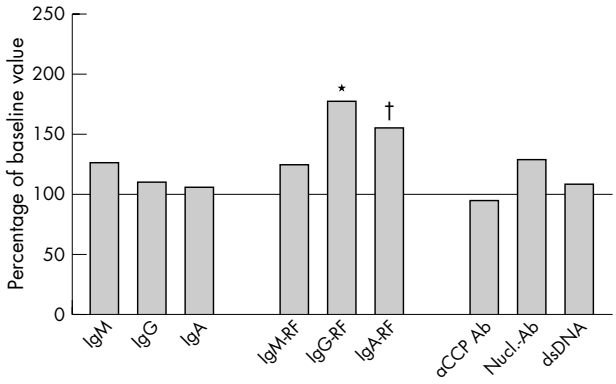

Figure 2 Serum levels of total IgM, IgG, IgA, RF isotypes and specific autoantibodies as a percentage of the baseline value after 9 months of etanercept treatment. *$p \leqslant 0.04$; †$p \leqslant 0.01$ by Wilcoxon's test.

No significant change in the serum level of total IgM, IgG, and IgA after 9 months of treatment with etanercept was found (fig 2). Thus, the mean (SEM) IgM level was 1.2 (0.2) g/l *v* 1.5 (0.2)g/l (baseline *v* month 9, respectively), the mean IgG level was 10.8 (0.9) g/l *v* 12.0 (1.1) g/l, and the mean IgA level was 2.5 (0.2) g/l *v* 2.7 (0.1) g/l. In addition, the mean IgM-RF level was not significantly changed after 9 months of anti-TNF-treatment (247 (96) *v* 312 (129) U/ml; figs 1A and 2). Similarly, the serum level of aCCP antibodies appeared to be uninfluenced by the treatment (176 (17) *v* 168 (11) U/ml; fig 1A and 2). None of the patients developed

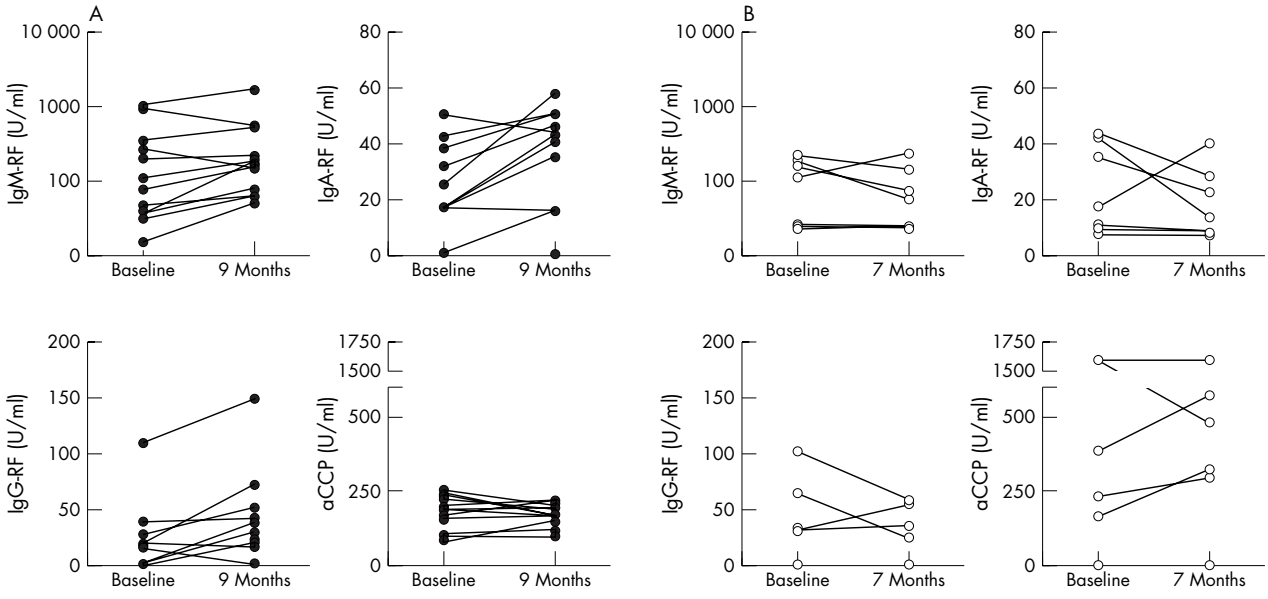

Figure 1 Individual serum levels of IgM-RF, IgG-RF, IgA-RF, and aCCP before and after 9 months of treatment with etanercept (A) and before and after 7 months of treatment with adalimumab (B). IgM-RF values are shown on a logarithmic scale.

pathological antibodies against nucleosomes (7.5 (1.2) *v* 9.8 (1.3) U/ml), dsDNA (6.8 (1.4) *v* 7.2 (2.2) U/ml) or antinuclear antibodies (data not shown). No significant correlation between IgG-RF and IgA-RF before or after 9-months of etanercept treatment was seen ($r_s = 0.08$ and 0.27, respectively).

Furthermore, the surrogate marker for B cell proliferation, β_2-microglobulin, remained unaltered during the observation period (1.8 (0.1) *v* 1.8 (0.1) mg/l).

As with etanercept, adalimumab treatment significantly decreased the DAS28 in patients with RA (the mean (SE) value at baseline was 5.1 (0.7) and after 7 months 3.3 (1.6); $p \leqslant 0.01$) but had no significant effect on the serum levels of IgG-RF (32.5 (14.7) *v* 24.7 (9.8)), IgA-RF (23.1 (6.1) *v* 17.9 (4.7)), IgM-RF (104 (31) *v* 80 (30)), and aCCP-antibodies (796 (287) *v* 697 (243)) (fig 1B). The baseline serum levels of the IgG-RF, IgA-RF, IgM-RF, and aCCP antibodies were not significantly different between the two treatment groups ($p = 0.55$, 0.83, 0.43, and 0.12, respectively).

DISCUSSION

Cytokine blockers have revolutionised the treatment of RA and are routinely used to treat patients not adequately responding to traditional disease modifying antirheumatic drugs. Blocking of TNF interrupts the disease process by blocking the activation of T cells, macrophages, and fibroblasts.

In this study we demonstrate that treatment with the soluble TNF receptor etanercept induced a significant increase in serum IgG-RF and IgA-RF that was not seen in patients treated with adalimumab. This suggests that etanercept has a pivotal effect on certain B cells because the total serum immunoglobulin isotype levels were unaltered.

Whether the raised RF isotypes are due to an increase in the number of post-switch cells or an activation of RF-producing memory B cells is as yet unknown. The stable serum levels of β_2-microglobulin indicate activation rather than proliferation of post-switch RF-producing B cells. The activation of post-switch B cells might be similar to that postulated for RF-producing memory cells.[10] Thus, IgG-RF-producing B cells could keep themselves alive by generating sufficient antibodies that form stimulating immune complexes. Our observations are in accordance with this hypothesis because only down stream RF antibodies and no other autoantibodies were raised (fig 2). Another possibility for the increase in IgG- and IgA-RF might be switching of IgM-RF-producing B cells. Two observations would argue against this possibility. Firstly, in contrast with all other TNF blockers, etanercept also inhibits lymphotoxin, which is essential for the formation of germinal centres where isotype switching occurs.[11] Secondly, IgM-RF and IgM total serum levels remained unaltered.

A final explanation might be that etanercept could counteract inhibiting signals on autoreactive plasma cells, leading to an increase in IgG- and IgA-RF serum levels.

In line with recent reports,[12] we were unable to detect antibodies specific to systemic lupus erythematosus in etanercept treated patients. Whether these autoantibodies are not produced or were missed because our test system detects only IgG isotypes has to be clarified by further studies.

In summary, this study shows for the first time that patients treated with etanercept have a significant increase in IgG- and IgA-RF antibodies. The clinical relevance of this observation is as yet unknown and further studies are necessary to estimate the impact on the course of the disease. It might be possible that these RF-producing B cells can produce proinflammatory cytokines that to some degree would counteract the TNF blockade. The observation that the combination of etanercept and methotrexate, a drug that also targets B cells,[13] is better than etanercept alone[14] supports this hypothesis.

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