

# Long term treatment of rheumatoid arthritis with high doses of intravenous immunoglobulins: effects on disease activity and serum cytokines

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## Abstract

**Objective**—To evaluate the effects of long term treatment of rheumatoid arthritis (RA) with high doses of intravenous immunoglobulins (IVIg).

**Methods**—Ten patients with active RA and prior unsuccessful treatment with at least one slow acting antirheumatic drug were treated with 400 mg/kg of IVIg for the first three days and then once a month for 12 months. Clinical evaluation and laboratory analysis were performed every month. Serum levels of tumour necrosis factor  $\alpha$  (TNF $\alpha$ ), soluble interleukin-2 receptor (sIL-2R), IL-1 $\alpha$ , IL-1 $\beta$ , IL-6 and interferon gamma (IFN $\gamma$ ) were measured at baseline and at three monthly intervals for 15 months.

**Results**—Although laboratory parameters were not influenced by the treatment, a late but significant clinical improvement was observed after six months. Serial measurement of cytokines revealed a rapid and persistent decrease in serum TNF $\alpha$  and a late and significant reduction in sIL-2R concentrations.

**Conclusion**—This study suggests that IVIg can ameliorate the symptoms and improve the functional capability of RA patients. This effect is associated with a partial modulation of serum concentrations of inflammatory cytokines and, more interestingly, with a late decrease in sIL-2R which correlated with the late reduction in disease activity.

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Intravenous immunoglobulins (IVIg) have been successfully used as replacement treatment in patients with hypogammaglobulinaemia.<sup>1</sup> More recently, they have also been shown to be effective as immunomodulating therapy in a wide variety of chronic autoimmune diseases including juvenile rheumatoid arthritis (RA).<sup>2-3</sup> However, the role of IVIg therapy in adult RA is unclear, as only a few short term studies have been carried out on small numbers of patients, and most were receiving concomitant treatment with slow acting antirheumatic drugs (SAARDs).<sup>4-6</sup>

The present study evaluated the effects of a one year treatment with high dose IVIg not associated with SAARDs on the clinical and laboratory parameters in adult patients with active RA. In addition, serum concentrations

of interleukins-1 $\alpha$ , -1 $\beta$  and -6, and tumour necrosis factor  $\alpha$  (IL-1 $\alpha$ , IL-1 $\beta$ , IL-6, TNF $\alpha$ ) were measured as indicators of macrophage activity, and soluble interleukin-2 receptor (sIL-2R) and interferon gamma (IFN $\gamma$ ) measured as expressions of T cell activation.

## Patients and methods

### PATIENTS

Ten subjects (seven female) aged 38-72 years (mean 54.5), were enrolled in the study. Six had serological evidence of rheumatoid factor (RF) and four of antinuclear antibodies (ANA). Disease duration ranged from two to 22 years (mean 7.8). All patients fulfilled the American College of Rheumatology diagnostic criteria for RA,<sup>7</sup> had moderately active disease as previously defined (table)<sup>8-9</sup> and were receiving long term treatment with prednisone 2.5-10 mg/day. Unsuccessful treatment with at least one SAARD (hydroxychloroquine, penicillamine or auranofin) was a prior requisite for inclusion in the study. Exclusion criteria were: age less than 18 years or disease onset before 16 years, Steinbroker functional class IV, prior immunosuppressive therapy with methotrexate, azathioprine, cyclophosphamide or cyclosporine A, intra-articular or systemic steroid treatment with more than 15 mg in the 30 days preceding the study, humoral immunodeficiency associated with RA. Written informed consent was obtained from each subject. Patient follow up was also performed during the three months after stopping IVIg therapy.

### IVIg TREATMENT

During the first three days, 400 mg/kg/day of immunoglobulin (Endobulin, Immuno SpA, Pisa, Italy) was infused slowly. Subsequently, 400 mg/kg of IVIg was administered once a month for 12 months. Medication was restricted to a constant dose of prednisone (maximum 10 mg/day) or non-steroidal anti-inflammatory drugs (NSAIDs) (naproxen 500-1000 mg/day or piroxicam 20 mg/day), or both, to obtain pain relief and inflammation reduction for the 30 days preceding the study and during the 15 months of the trial.

### CLINICAL AND LABORATORY ASSESSMENT

Clinical evaluation, performed in each patient every month, is described in the table.

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Clinical and laboratory features of the 10 RA patients before IVIg treatment\*

Patient	Joint score	Morning stiffness (min)	Patient pain assessment§	Physician's global assessment§	Grip strength (mm Hg)	ESR (mm/1st h)	CRP (mg/dl)
1	7	240	45	50	240	76	5.75
2	28	180	75	83	200	44	2.46
3	16	60	95	75	100	84	5.24
4	23	360	50	50	140	38	5.53
5	10	150	47	50	170	45	4.13
6	18	180	80	72	95	46	2.7
7	21	120	63	86	110	53	3.16
8	22	150	70	77	100	42	4.23
9	30	240	60	40	130	20	6.5
10	13	180	50	60	110	55	4.26

\*Active disease was defined by the presence of six or more tender joints, and two of the following conditions: nine or more swollen joints, morning stiffness lasting more than 45 minutes or Westergren erythrocyte sedimentation rate (ESR) > 28 mm/1st h.

†Obtained by summing the score of the single tender joints (1 = mild, 2 = intermediate, and 3 = severe pain).

§Patient's pain and physician's overall assessments were evaluated using a visual analogue pain scale (0 mm = absence of pain/disease activity; 100 mm = the maximum pain/disease activity).

Laboratory analysis included evaluation of erythrocyte sedimentation rate (ESR), C reactive protein (CRP), full blood count, RF (latex agglutination assay), IgG, IgA, IgM (nephelometry) and ANA (indirect immunofluorescence on HEp-2 cells). Hand, feet and any other involved joints were radiographed in each patient at the start and end of the trial and scored according to Sharp's criteria.<sup>10</sup> All films were evaluated blind by the same observer.

LYMPHOKINE DETERMINATION

Serum samples were obtained from each patient every three months, and from 40 healthy volunteers to evaluate the normal range. Commercially available enzyme linked immunosorbent assay kits were used for assaying serum IL-1 $\alpha$  (Immunotech SA, Marseille, France), IL-1 $\beta$  (Immunotech SA),

IL-6 (Genzyme, Cambridge, MA), IFN $\gamma$  (Byk Gulden Italia, Milan, Italy), sIL-2R (T Cell Diagnostics, Cambridge MA) and TNF $\alpha$  (Genzyme).

STATISTICAL ANALYSIS

Variations in clinical and laboratory parameters were evaluated by analysis of variance for repeated measures, testing both parametric and non-parametric methods. Contrast analysis was used to evaluate, within subjects, the effect of treatment over time. Values of  $p \leq 0.05$  were considered significant.

Results

Only eight patients completed the trial as two (Nos 7 and 8) suffered minor side effects (mild hypotension, lumbar pain, nausea, and hyperemesis) immediately after starting the IVIg infusion. Although the adverse effects reversed spontaneously after IVIg was stopped, the patients refused to continue therapy. The other eight patients did not suffer from any adverse effects or illness, related either directly to IVIg or to immunosuppression during the course of the study.

Figure 1 shows the mean changes in some clinical parameters and ESR values in the eight patients who completed the trial. Although an increase in both ESR (fig 1) and CRP (data not shown) was documented in all patients after the first month of IVIg infusion, a gradual subjective and objective improvement of clinically assessed disease activity was observed from the second month of treatment. However, a statistically significant improvement was

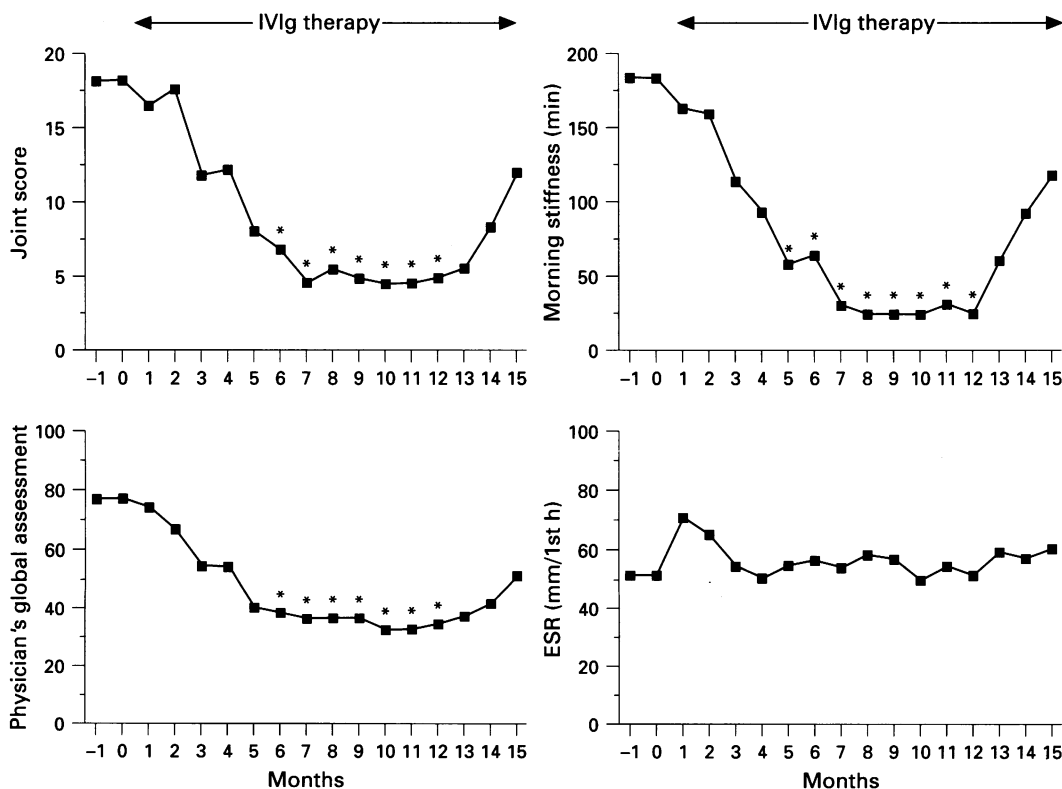


Figure 1 Average of joint score, morning stiffness, physician's global assessment values and ESR in eight RA patients treated with IVIg. \* $p < 0.03$  for joint score and morning stiffness and  $p < 0.05$  for physician global assessment, compared with baseline.

obtained only after six months of therapy, and was maintained throughout the treatment phase, in joint score ( $p < 0.03$ ), duration of morning stiffness ( $p < 0.03$ ) and physician's global assessment ( $p < 0.05$ ). Improvements in patient pain assessment and grip strength did not reach statistical significance (data not shown). In contrast ESR, CRP concentrations and other laboratory variables studied (haemoglobin, RF, ANA, IgG, IgA, IgM or complement components C3 and C4) were not affected by treatment (data not shown). According to previously described criteria for assessing individual response to treatment,<sup>8</sup> six out of the eight patients (75%) could be considered responders.

As shown in figure 1, disease relapse was clinically evident in most patients about one month after stopping IVIg.

There were no significant radiological changes, as assessed by the Sharp's score, after 12 months of therapy (data not shown).

Large concentrations of soluble TNF $\alpha$  were detected in the serum of five of the eight patients (mean 166 (SD 58) pg/ml) but, interestingly, could not be detected in any patient after three months of therapy or during the treatment phase ( $p < 0.001$ ) (fig 2). The abnormally high levels of sIL-2R (924 (326) IU/ml), detected in six of the eight patients, decreased significantly from the ninth month of treatment (486 (65) IU/ml;  $p < 0.05$ ). Interestingly, pathological levels of both TNF $\alpha$  and sIL-2R were revealed after IVIg was stopped. IL-1 $\alpha$  and IFN $\gamma$  serum concentrations in all patients were within the normal range both at baseline and during the

study (data not shown). Although IL-1 $\beta$  concentrations were higher in all and IL-6 concentrations greater in five patients with respect to normal values, the changes in these two cytokines were not significant during the trial (fig 2).

## Discussion

The results of the present investigation support a positive effect of IVIg in the treatment of adult RA. However, in contrast with previous studies which reported that IVIg administration exerted a rapid influence on clinical and laboratory inflammation indexes,<sup>3,4</sup> we found clinical improvement only after six months of therapy. In addition, despite the fact that the clinical benefit was maintained throughout the trial, it was not associated with improvement in values of laboratory variables which, on the contrary, underwent a transitory worsening at the beginning of the treatment, perhaps because of IVIg overload.

As it is well known that RA is a heterogeneous disorder in which clinical course, prognosis, and response to therapy can differ between subjects,<sup>11</sup> these conflicting results may be explained, at least in part, by differences in selection of patients, different levels of disease activity, and different doses of IVIg. Furthermore, the fact that IVIg preparations, although similar in Ig composition, are not identical but vary in purity, antibody activity, and content of other immunomodulatory proteins,<sup>12</sup> may account for the discrepancies between our results and those from other trials.

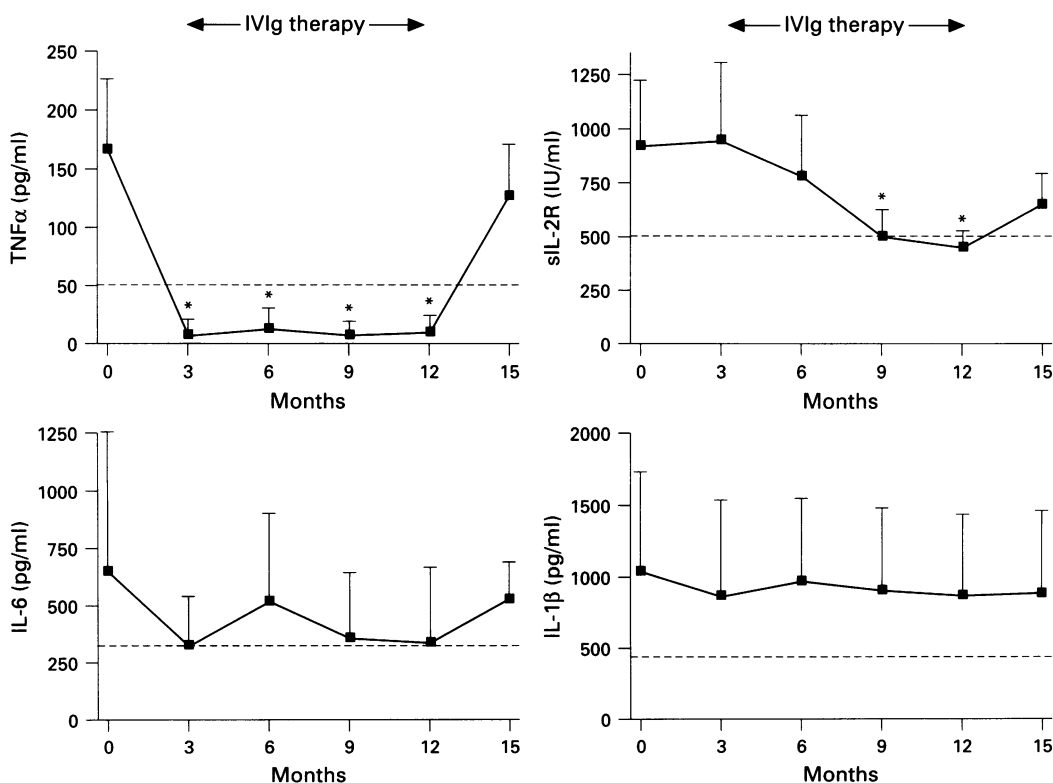


Figure 2 Average of TNF $\alpha$ , sIL-2R, IL-1 $\beta$  and IL-6 serum concentrations in eight RA patients treated with IVIg. Broken horizontal line indicates upper limit of range of normal values. \* $p < 0.001$  for TNF $\alpha$  and  $p < 0.05$  for sIL-2R compared with baseline.

In the majority of patients in whom IVIg has been of benefit, it is uncertain whether its immunomodulatory effects were the results of alterations in T or B cell function, or modulation of antigen-presenting cell activity.<sup>2</sup> Moreover IVIg may, in certain clinical situations, directly reduce the inflammatory response without exerting any immunomodulatory effect.<sup>2</sup>

Accumulating evidence indicates that a number of cytokines are crucial in the pathogenesis of RA synovitis and joint damage.<sup>9</sup> Cytokines such as IL-1 $\alpha$  and -1 $\beta$ , TNF $\alpha$  and IL-6, expressed as a result of macrophage activation, have been demonstrated in rheumatoid synovium, and increased concentrations of these monokines have been found in the synovial fluid and serum of RA subjects;<sup>11</sup> increased concentrations of sIL-2R, considered a marker of T cell activation, have also been detected in this disorder.<sup>9</sup> Despite the fact that serum concentrations of IL-1 $\alpha$  and IFN $\gamma$  in our patients were within normal values, all had abnormally high serum concentrations of IL-1 $\beta$  and six had increased concentrations of sIL-2R, TNF $\alpha$ , or IL-6, or both. None displayed simultaneously normal values of these immune active proteins, which have been associated with disease severity in RA patients.<sup>9, 11</sup>

In the present study, IVIg administration had a partial influence on the synthesis of macrophage derived cytokines, as there was a dramatic and rapid decrease in TNF $\alpha$ , while IL-6 and IL-1 serum concentrations remained unaffected. The monocyte-macrophage system is one of the potential targets of IVIg therapy.<sup>2</sup> IVIg may decrease or block reticuloendothelial system function by occupying, downregulating or decreasing the affinity of the Fc receptors, thereby interfering with the binding of immune complexes.<sup>2</sup> Our findings agree with the recent demonstration of a downregulation of TNF $\alpha$  secretion following IVIg treatment in experimental models of T cell mediated autoimmune disease.<sup>13</sup> Moreover, a partial inhibition of monokine synthesis by SAARD treatment has recently been described in RA patients: Danis and colleagues noted that clinically successful treatment with sulphasalazine was associated with reduction in serum concentrations of IL-1 and TNF $\alpha$ , but not IL-6.<sup>14</sup> The different in vitro effects of sulphasalazine on monokine production observed by these investigators may mean that high concentrations of this drug are needed to inhibit IL-6 synthesis than those required to block IL-1 and TNF $\alpha$  production. In consequence, although the different effects on these cytokines are not completely clear, the possibility cannot be excluded that the IVIg doses we adopted were too small to inhibit IL-1 and IL-6 synthesis completely.

A significant reduction in sIL-2R serum concentrations was reached late in the course of treatment, when a significant clinical improvement was also obtained. This suggests that changes in sIL-2R serum values reflect the effect of IVIg on disease activity. However, although T lymphocytes play a key role in the

development of RA synovitis,<sup>11</sup> we do not have enough data to support the hypothesis that T cells have been the main target of IVIg immunomodulation in determining long lasting remission in our RA patients.

In conclusion, our data indicate that IVIg can ameliorate symptoms and improve functional capability of RA patients. Although it had an effect on some immunological parameters, complete remission was not obtained. The lack of effect on aspecific inflammation indexes, which is consistent with the effect of other new drugs used in the treatment of RA,<sup>15</sup> may have implications in the evolution of joint disease. However, the improvement in quality of life induced by IVIg may predict a long term gain in morbidity. Two patients withdrew from the trial early because of minor side effects, but tolerance was otherwise optimal throughout the 12 months of treatment. Although the present trial was uncontrolled and the results may therefore be too limited to permit definitive conclusions, these observations suggest that, despite its high cost, IVIg may be a valid, tolerable, alternative combination drug for maintenance therapy in RA.

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