

Evidence for Immunostimulatory Effects of Intramuscular Gold in Patients with Rheumatoid Arthritis: Correlation with Skin Reactions

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ABSTRACT. Objective. Intramuscular gold is a well documented treatment in rheumatoid arthritis (RA), but its mechanism of action is still poorly understood. From an observation that gold sodium thiomalate (GSTM) induces monocyte-derived interleukin 6 (IL-6) and IL-10 production *in vitro*, a hypothesis has been proposed that gold exerts its action mainly as a selective immunostimulator rather than as a general immunosuppressant. In this prospective study we investigated cytokine production in peripheral blood from patients with RA during treatment with GSTM.

Methods. A total of 20 patients with RA were treated with GSTM for at least 3 months. Disease activity was recorded at baseline, 12, 20, and 28 weeks. The ELISPOT method was used to measure spontaneous production of IL-6, IL-10, and interferon- γ (IFN- γ) from peripheral blood mononuclear cells (PBMC) at baseline and 4 and 12 weeks and production after incubation with GSTM *in vitro*, at different concentrations (0, 3, 12.5, 40 μ g/ml) at baseline. IL-6 and IL-10 concentrations in serum were measured with ELISA.

Results. The numbers of IL-10-producing cells were increased after 4 weeks' treatment with GSTM ($p < 0.01$). The numbers of cells spontaneously producing IL-6 were increased after 4 weeks ($p < 0.01$) and 12 weeks ($p < 0.01$). The numbers of IFN- γ -producing cells were increased after 4 weeks ($p < 0.01$). Serum concentrations of IL-10 were increased after 4 weeks ($p < 0.01$). Serum concentrations of IL-6 were not changed at any timepoint. The *in vitro* effect of GSTM on IL-10 production from PBMC at baseline predicted development of skin reactions during GSTM treatment, with lack of skin reactions being associated with high gold induced IL-10 production ($p < 0.05$). There was no correlation between clinical response and cytokine production.

Conclusion. This study indicates an immunostimulatory effect of GSTM treatment in patients with RA. The increase in IL-10 production during GSTM treatment may contribute to the positive effects of gold in RA. (J Rheumatol 2003;30:1748–55)

Key Indexing Terms:

GOLD RHEUMATOID ARTHRITIS
INTERFERON- γ

INTERLEUKIN 10

INTERLEUKIN 6
MONOCYTES

Gold compounds have been used in treatment of rheumatoid arthritis (RA) since 1929¹. The value of intramuscular gold in RA was later confirmed in controlled studies, confirming

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reduction of disease activity²⁻⁵ and also reduction of cartilage destruction^{6,7}. Numerous gold-containing compounds have been used for treatment of RA and the parenterally administered gold sodium thiomalate (GSTM) is most widely used. From treatment studies it is known that the effect of GSTM differs substantially between different individuals and that GSTM, unlike other disease modifying antirheumatic drugs (DMARD), may induce remission lasting several months after the end of therapy⁸⁻¹¹. However, adverse reactions presenting predominantly as dermatitis and stomatitis are more common than for other DMARD¹⁰⁻¹².

The mechanism of action of GSTM in RA is still unclear. *In vitro* studies have shown that GSTM has mostly immunosuppressive properties. Thus GSTM has been shown to reduce the chemotactic activity of monocytes *in vitro*¹³, and the effects of GSTM on T cells include inhibition of proliferation induced by mitogens or interleukin 2 (IL-2)^{14,15}.

However, the relatively frequent presence of dermatitis during GSTM treatment suggests that gold may alter the cytokine balance in RA in favor of more allergic immune reactions. It has been reported that GSTM may induce an upregulation of IL-4 mRNA accompanied by vasculitis in rats, suggesting a shift of the T cell population to the Th2 phenotype with production of antiinflammatory cytokines¹⁶. An immunostimulatory effect on human peripheral blood mononuclear cells (PBMC) was evident when incubated with GSTM *in vitro*¹⁷. Both the beneficial and side effects of gold in RA may be related to divergent effects on cytokine production and the stimulation of antiinflammatory cytokines such as IL-10.

To study the possible mechanistic role of GSTM on cytokine production we prospectively studied the production of proinflammatory and antiinflammatory cytokines during treatment with GSTM in patients with RA.

MATERIALS AND METHODS

Patients. Twenty patients, 11 women and 9 men, with clinical RA according to the American College of Rheumatology (ACR) criteria¹⁸ were included in this study. Admission criteria were age between 18 and 80 years; a proposal of their attending rheumatologist for treatment with GSTM; and that treatment with other DMARD had been stopped for > 4 weeks prior to inclusion. The exclusion criterion was earlier treatment with GSTM or auranofin. A stable dose of prednisolone was accepted, but not more than 7.5 mg/day.

Medication. The compound used for treatment was 1 ml consisting of 20 mg GSTM and 20 µg phenyl mercury nitrate (Myocrisin®, Aventis, Strasbourg, France), given intramuscularly once a week starting with a dose of 10 mg and thereafter 20 mg/week for 2 weeks followed by 50 mg/week. After a total dose of 1000 mg the dose interval was prolonged. A research nurse who gave the injections also evaluated side effects. If side effects were evident, the dose was lowered or the interval prolonged. No intra-articular steroid injection was allowed 4 weeks prior to inclusion or prior to any visit when disease activity assessment and/or samples for cytokines were taken.

The study was approved by the local ethical committee. All patients gave their informed consent.

Assessments. At inclusion and after 12 weeks treatment with GSTM, disease activity was measured with the Disease Activity Score composite index, using a 28 joint score (DAS28)¹⁹. This includes number of swollen joints, number of tender joints, patient's global assessment of disease activity measured on a 100 mm visual analog scale (VAS), and erythrocyte sedimentation rate (ESR), creating a score ranging from 0 to 10. High disease activity was defined as DAS28 > 5.1 and low activity as DAS28 < 3.2. Good responders were those with improvement of at least 1.2 and an endpoint DAS28 value < 3.2. Moderate responders were patients with either an improvement of at least 1.2 independent of the attending DAS28 value or an improvement of at least 0.6 in combination with an endpoint DAS28 < 5.1²⁰. The primary outcome was good response according to DAS28. If a good response was not reached by the 12 week visit, the patient was followed at time points 20 and 28 weeks until a good response was achieved.

The patients also completed the Swedish version of the Stanford Health Assessment Questionnaire (HAQ)²¹, a self-reporting instrument measuring disability of daily life activities. The created score for the disability index ranges from 0 to 3, where a higher score indicates a higher degree of disability²².

Radiographic examinations. Radiographic examinations of the hands,

wrists, and feet were performed according to the clinical routine of the departments. The investigations were done in the preceding 2 years before inclusion in the study. Patients whose radiographs showed one erosion or more were considered erosive, but not patients with only periarticular osteoporosis.

Blood samples. Blood samples were obtained at baseline and after 4 and 12 weeks' treatment with GSTM and also in some patients after 20 and 28 weeks if a good response was not achieved earlier. Blood analyses included ESR, C-reactive protein (CRP), and serum concentrations of IL-6 and IL-10. PBMC from each patient were analyzed by ELISPOT for unstimulated production of IL-6, IL-10, and interferon-γ (IFN-γ).

Mononuclear cell separation. Peripheral blood was collected into heparinized tubes. PBMC were isolated by density gradient centrifugation (Ficoll, Hypaque) and diluted to 1×10^6 /ml in RPMI-1640 (Flow Laboratories, Irvine, Scotland) supplemented with glutamine, HEPES buffer, penicillin, streptomycin, and 10% of a defined batch of fetal calf serum (FCS) (complete medium). The same batch FCS was used as in the earlier study on GSTM *in vitro*¹⁷. Cell viability was assessed by trypan blue exclusion and always exceeded 95%. Cells were then used without further stimulation for the enumeration of cytokine-producing cells.

ELISPOT analysis of number of cytokine-producing PBMC. Numbers of cytokine-producing cells were analyzed by ELISPOT as described²³ using plastic ELISA plates coated with primary antibody, 50 µl/well, 15 µg/ml overnight (16–20 h) at 4°C. PBMC were added (2.5×10^4 /well for IL-10, 2000/well for IL-6, 1×10^5 /well for IFN-γ) in complete medium and incubated overnight (16–20 h). Wells were washed and biotinylated secondary antibodies were added at 1 µg/ml overnight. Avidin-alkaline phosphatase (Dako, Glostrup, Denmark) was added at a dilution of 1:250 and allowed to bind for 2 h, and BCIP 710-3 (Sigma, St. Louis, MO, USA) was added for 5 h after washing to develop the spots. The number of cytokine-producing cells was counted using an inverted microscope. Antibodies used were 1-D1K (MabTech, Stockholm, Sweden) and biotinylated 7-B6-1 (MabTech) for IFN-γ; 19F1 (Pharmingen, San Diego, CA, USA) and biotinylated 12G8 (American Type Culture Collection, Manassas, VA, USA) for IL-10; and IL-6-I (MabTech) and biotinylated 39C3 (Pharmingen) for IL-6.

Determination of serum IL-10 and IL-6. IL-10 and IL-6 concentrations were analyzed using an ultra-sensitive ELISA (R&D Systems, St. Paul, MN, USA).

Incubation of PBMC with GSTM. Before treatment, PBMC from each patient were incubated with GSTM at 37°C for 16–20 h at concentrations of 0, 3, 12.5, or 40 µg/ml and analyzed for production of IL-10 with ELISPOT according to our protocol¹⁷. Concentrations in this range have been used in previous *in vitro* studies and proven nontoxic²⁴.

Statistical analysis. Nonparametric methods were used throughout the report. Differences between groups were analyzed with the Mann-Whitney U test, and analyses for matched pairs were performed with Wilcoxon's signed rank test. For analyses of relations between cytokine production and clinical response with several variables, the Kruskal-Wallis test was performed. A finding of $p < 0.05$ was considered significant.

RESULTS

Clinical characteristics, response, and skin reactions. The mean age for patients was 58 years (range 29–78), with a mean disease duration of 9 years (range 0–41); 15/20 were RF-positive and 14/20 had erosive disease. The mean number of previous DMARD was 1.25 (range 0–5). At the start, the mean DAS28 was 5.84 (range 4.66–7.35), HAQ score 1.34 (0.38–2.75), ESR 40 mm Hg (15–90), and CRP 31 mg/l (10–87). Two patients were treated with prednisolone in a stable dose of 5–7.5 mg/day.

The total dose of GSTM differed because of side effects. At 3 months the total dose ranged from 75 to 800 mg. One patient reacted immediately with a severe dermatitis after one injection of GSTM and was withdrawn from treatment. A total of 10 patients developed skin reactions. Seven of these withdrew from GSTM treatment as a result of their skin reactions (time points are displayed in Table 1). The remaining 3 patients with mild skin reactions continued with a lower dose of GSTM, without aggravated dermatitis.

DAS28 values and response according to the European League Against Rheumatism (EULAR) response criteria are shown in Table 1.

Skin reactions observed in patients did not correlate with EULAR individual responses.

GSTM treatment and ex vivo IL-10, IL-6, and IFN- γ production. After 4 weeks of treatment with GSTM, an increase in the number of cells spontaneously producing IL-10 was recorded (Table 2, Figure 1a). IL-10 production after 12 weeks was not changed. The numbers of IL-6-producing cells were significantly increased after 4 and 12 weeks (Table 2, Figure 1b). The numbers of IFN- γ -producing cells were significantly increased after 4 weeks (Table 2, Figure 1c).

The numbers of peripheral blood monocytes after 4 and 12 weeks' treatment remained unchanged, and there were no correlations between monocyte concentrations and the number of cytokine-producing cells (data not shown).

GSTM treatment and circulating IL-10 and IL-6 concentrations. There was a significant increase of IL-10 in serum after 4 weeks of treatment (Table 3). The IL-10 serum concentrations after 12 weeks were not changed. No effect

on IL-6 serum concentrations was recorded at any of these time points (Table 3).

Correlations between cytokine concentrations and clinical variables. There was no correlation between changes in IL-10 or IL-6 production and clinical response measured as individual EULAR responses, DAS28 values, CRP, or HAQ score. Further, there was no correlation between cytokine production or cytokine concentrations and age, sex, disease duration, positive rheumatoid factor, or presence of erosive disease.

Incubation of PBMC with GSTM increases production of IL-10. When PBMC from each patient were incubated with GSTM at different concentrations, before intramuscular treatment with the same compound was started, there was a dose dependent increase in the number of IL-10-producing cells (Figure 2).

Correlation between in vitro cytokine production and skin reactions. The ratio between IL-10 production achieved following incubation of PMBC with the highest concentration of GSTM (40 μ g/ml) and IL-10 production in unstimulated cultures was calculated for each patient. Patients with skin reactions (n = 10) had a significantly lower ratio than patients without any kind of skin reaction (n = 10; p < 0.05) (Figure 3). The ratio between IL-10 production after incubation with 12.5 μ g GSTM and unstimulated production displayed a tendency in the same direction, but did not reach statistical significance (data not shown). The increase in IL-10 production after 4 weeks of GSTM treatment did not correlate with the presence of skin reactions, however (data not shown).

Table 1. Disease activity and side effects during GSTM treatment.

| Age | Sex | RF | DAS28 Score | | | | EULAR Individual Response | Time at Appearance of Skin Reaction |
|-----|-----|-----|-------------|----------|----------|----------|---------------------------|-------------------------------------|
| | | | 0 Weeks | 12 Weeks | 20 Weeks | 28 Weeks | | |
| 29 | F | Neg | 5.49 | 2.49 | | | Good | — |
| 38 | F | Pos | 4.66 | 2.36 | | | Good | — |
| 64 | F | Pos | 5.47 | 2.89 | | | Good | w8 |
| 50 | M | Neg | 5.61 | 3.53 | 2.06 | | Good | — |
| 66 | F | Pos | 5.1 | 3.9 | 2 | | Good | w15 |
| 69 | M | Pos | 5.2 | 3.61 | 3.91 | 3.14 | Good | — |
| 62 | M | Pos | 6.42 | 4.67 | 4.22 | 4.12 | Moderate | — |
| 60 | F | Pos | 6.42 | 4.39 | 4.58 | 4.46 | Moderate | — |
| 73 | F | Pos | 5.31 | 4.6 | 4.3 | 3.83 | Moderate | — |
| 57 | M | Pos | 5.26 | 4.18 | 5.39 | | Moderate | — |
| 49 | F | Pos | 5.81 | 5.48 | 5.32 | 3.41 | Moderate | — |
| 63 | F | Neg | 6.81 | 6.69 | 6.62 | | None | — |
| 60 | F | Pos | 6.05 | | | | Withdrawn | w1 |
| 78 | M | Pos | 5.86 | | | | Withdrawn | w8 |
| 52 | M | Pos | 7.26 | | | | Withdrawn | w8 |
| 64 | M | Pos | 7.35 | 4.99 | | | Moderate | w11 |
| 53 | F | Neg | 5.52 | 5.59 | | | None | w13 |
| 43 | M | Neg | 6.61 | 5.37 | | | Moderate | w14 |
| 55 | M | Pos | 5.52 | 4.15 | 5.68 | | Moderate | w20 |
| 54 | F | Pos | 4.97 | 4.41 | 4.77 | 5.93 | None | w28 |

w: week.

Table 2. Effect of GSTM treatment on spontaneous production of IL-10, IL-6, and IFN- γ . Values are mean \pm SD. One patient discontinued treatment before 4 weeks. Data on IL-10 production in one patient at baseline, in 2 after 4 weeks, and in 3 after 12 weeks were excluded due to method failures. Data on IL-6 production in 2 patients were excluded due to the change of antibody during the study. However, this did not affect the statistics. IL-6 data in one patient at baseline, in one after 4 weeks, and in 3 after 12 weeks were excluded due to method failures. Data on IFN- γ production in one patient after 4 weeks and in 3 after 12 weeks were excluded due to method failures.

| | 0 Weeks | 4 Weeks | 12 Weeks |
|-------------------------------------|--------------------------|--|---|
| IL-10, spots/100,000 cells | 37 \pm 42 (n = 19) | 79 \pm 68 (n = 17) p < 0.01 ^{†*} | 53 \pm 31 (n = 16) p = NS [†] |
| IL-6, spots/100,000 cells | 2248 \pm 1750 (n = 17) | 4260 \pm 2274 (n = 16) p < 0.01 ^{†*} | 5302 \pm 2883 (n = 14) p < 0.01 [†] |
| IFN- γ , spots/300,000 cells | 2 \pm 6 (n = 20) | 10 \pm 21 (n = 18) p < 0.01 ^{†*} | 7 \pm 25 (n = 16) p = NS [†] |

[†] p value versus 0 Weeks. * 4 Weeks versus 12 Weeks: nonsignificant.

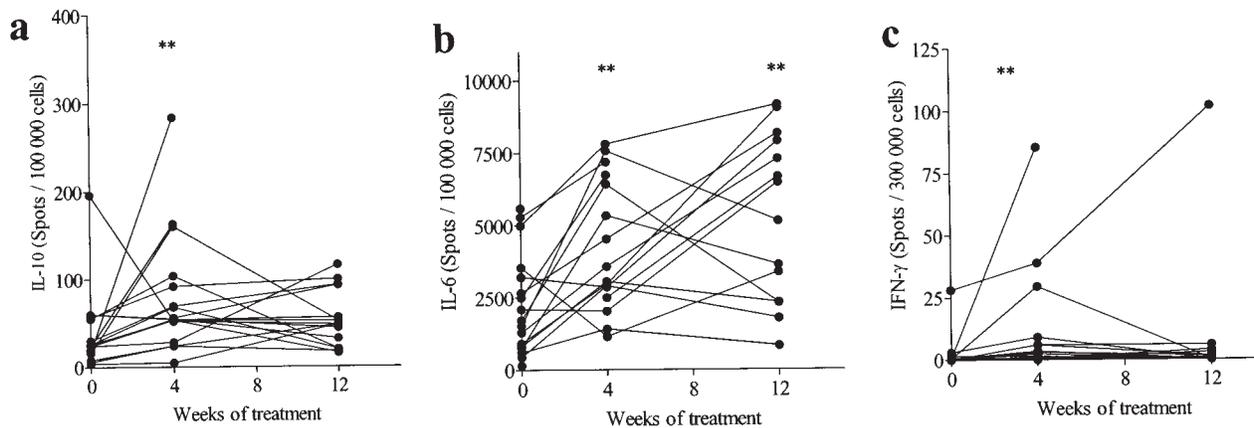


Figure 1. Spontaneous production of IL-10 (a), IL-6 (b), and IFN- γ (c) after treatment with GSTM. **p < 0.01 compared to baseline cytokine production. See also legend to Table 2.

Table 3. Effect of GSTM treatment on serum concentrations of IL-6 and IL-10. Values are mean \pm SD. One patient discontinued treatment before 4 weeks. Data on IL-10 concentrations in 2 patients after 4 weeks and in one after 12 weeks were excluded due to method failures. IL-6 data from one patient were excluded after 4 weeks and from one after 12 weeks due to method failures.

| | 0 Weeks | 4 Weeks | 12 Weeks |
|--------------|---------------------------|---|---|
| IL-10, pg/ml | 7.0 \pm 7.4 (n = 20) | 8.0 \pm 4.4 (n = 17) p < 0.01 [†] | 5.9 \pm 3.8 (n = 18) p = NS [†] |
| IL-6, pg/ml | 117.6 \pm 90.6 (n = 20) | 145.3 \pm 130.2 (n = 18) p = NS [†] | 78.4 \pm 53.2 (n = 18) p = NS [†] |

[†] p value versus 0 Week.

DISCUSSION

In this prospective study we observed that gold may act as an immunostimulator *in vivo*. There was an increase in the number of IL-10, IL-6, and IFN- γ -producing cells and circulating IL-10 concentrations, especially early in GSTM treatment. Moreover, there was a negative correlation between individual ability to respond to GSTM *in vitro* with increase

in IL-10 production at baseline and recorded skin reactions during the study.

The antirheumatic effect of gold usually comes into action not sooner than after 12 weeks treatment^{6,10}. In this study we chose to focus on the early phase of gold treatment, i.e., after 4 and 12 weeks, with the hope that this approach would provide an opportunity to detect the initial

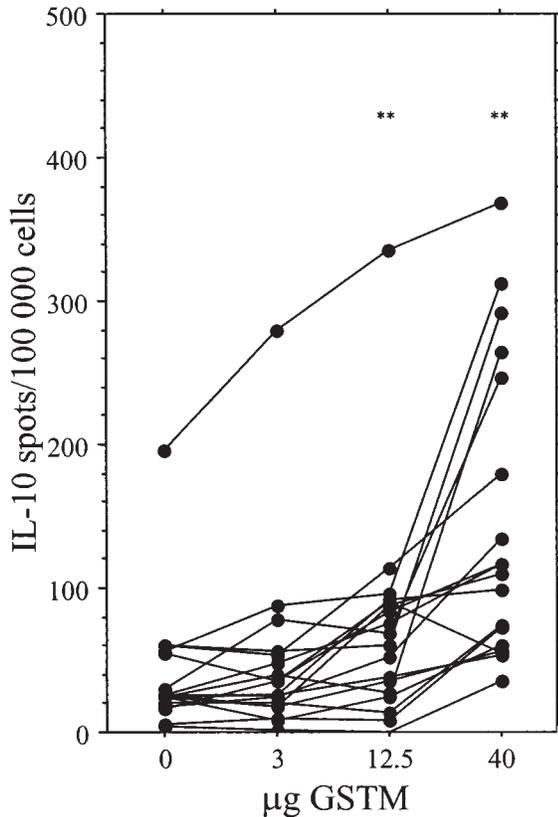


Figure 2. Effects of incubation with different concentrations of GSTM on spontaneous IL-10 production *in vitro* from PBMC of all patients at baseline. ** $p < 0.01$.

mechanistic effects of gold in the immune system of patients with RA.

For detection of cytokine production we used the ELISPOT technique, in which cytokines are bound to the detection surface directly after secretion from the producing cells. This technique mirrors the actual cytokine production and shows a notable sensitivity, advantageous for cytokines that are found in relatively minute amounts in peripheral blood, such as IFN- γ and IL-10. Previous reports have described ELISPOT to be 10 to 200 times more sensitive than ELISA measurements²⁵. Previous serial measurements in our laboratory confirmed that IL-10 production *ex vivo* remains stable with time. For reasons of comparison we also analyzed serum concentrations of IL-6 and IL-10. It is important to consider that ELISPOT and ELISA do not necessarily have to display the same results, since ELISPOT indicates only the cytokine production, whereas ELISA measures the result of both production and consumption of cytokines. Serum analyses of IFN- γ was not feasible due to low detection levels.

The cumulative gold dosage varied among the patients as the doses were individually reduced because of side effects. These reductions may not necessarily affect the results of

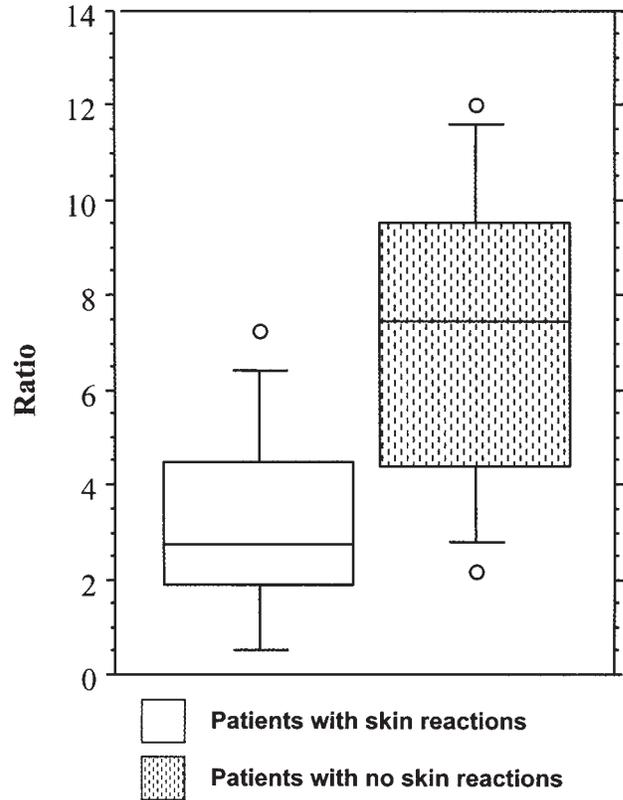


Figure 3. Ratio between pretreatment IL-10 production from PBMC incubated with GSTM 40 $\mu\text{g/ml}$ and from unstimulated PBMC in patients with skin reactions ($n = 10$) and patients with no skin reactions ($n = 10$) after GSTM treatment. Error bars indicate standard deviation. $p < 0.05$.

treatment, however, since low doses of GSTM have also been reported to be efficacious using a flexible dose schedule²⁶. The serum concentration of gold during maintenance therapy is between 0.75 and 1.25 $\mu\text{g/ml}$ ²⁷, and it has been shown that serum gold concentrations have little relation to the clinical effect of gold therapy²⁸. As GSTM is retained into the tissue, and inflamed tissue in particular²⁹, it is likely that the GSTM concentration during therapy is higher in the synovial membrane than in serum. The frequency of dermatitis and the need to discontinue the therapy in one-third of the patients is in accord with others' reports³⁰. We could not confirm observations⁹ that patients with dermatitis respond more advantageously to gold than those without.

Studies of cytokine production during gold treatment are sparse, but Lacki, *et al* reported from a cross-sectional study an increase of circulating IL-10 concentrations, whereas IL-6 levels were decreased in GSTM treated patients compared to controls³¹. In a prospective study, Madhok, *et al* described downregulation of IL-6 concentrations in serum of GSTM treated patients that correlated with disease activity³², but the same group did not detect any effect of GSTM treatment on spontaneous or lipopolysaccharide

(LPS) induced IL-6 production from blood monocytes³³. Immunostimulatory effects by GSTM *in vitro* have been reported^{17,34}, but as far as we know there are no reports on stimulatory effects on cytokine production *in vivo* during GSTM treatment.

IL-10 is an immunoregulatory cytokine produced by T cells^{35,36} and monocytes/macrophages³⁷, and it has inhibitory effects on several proinflammatory cytokines such as IL-1, IL-2, IL-4, IL-5, IL-6, IL-8, tumor necrosis factor- α , IFN- γ , and granulocyte monocyte-colony stimulating factor (GM-CSF)^{35,37-39}. In RA, IL-10 is increased in serum and synovial fluid⁴⁰.

The increase of IL-10-producing cells in peripheral blood during GSTM treatment recorded in this study is in accord with our earlier *in vitro* results¹⁷, and together with the observed increase of circulating concentrations of IL-10 supports the hypothesis that gold may act in RA not only by direct suppression of inflammatory cells such as monocytes, but through stimulation of antiinflammatory cytokine production. This conclusion is supported by the observation that the increase in IL-10 was already apparent after 4 weeks of treatment, suggesting that the early stimulation of IL-10 production may be the cause and not the result of the antiinflammatory effect of gold in RA.

After 12 weeks there was a tendency to increased IL-10 production, but this did not reach statistical significance, possibly indicating that there were substantial individual variations in cytokine production.

PBMC from patients who later developed dermatitis had a lower increase in IL-10 production in response to gold *in vitro* than patients with no skin reactions. However, the increase in IL-10 production after 4 weeks of GSTM treatment did not correlate with the presence of side effects (data not shown). Thus it seems that the grade of gold-induced IL-10 production *in vitro* may be of importance for the immune-modulating effects of gold, leading to skin reactions. Although these results require confirmation in larger populations, they support findings that increased IL-10 production is associated with protection for dermatitis in animal models⁴¹ and in cross-sectional studies of nickel-allergic and nonallergic subjects⁴².

IFN- γ is a proinflammatory cytokine⁴³ produced in RA synovium⁴⁴. In our previous study, there was no influence of GSTM on IFN- γ *in vitro*¹⁷. The stimulation of IFN- γ production during the first 4 weeks of GSTM treatment corresponds to reports that IFN- γ increases in the early phase of DMARD treatment^{45,46}. One explanation may be that gold inhibits the traffic of inflammatory cells to the joints through downregulation of adhesion molecules⁴⁷.

IL-6 is thought to have an important role in RA, suggested by reports that IL-6 activity in RA serum correlates with serum concentrations of CRP, α 1-antitrypsin, fibrinogen, and haptoglobin⁴⁸. *In vitro* studies of GSTM's effects on IL-6 production have yielded contradictory

results. Koda and collaborators reported decreased IL-1 β -dependent production of IL-6 after incubation *in vitro* with GSTM⁴⁹, whereas we detected a stimulating effect of GSTM on spontaneous IL-6 production *in vitro*¹⁷. Moreover, there have been reports of antirheumatic drugs interfering with bioassays for IL-6⁵⁰, but no such reports have included ELISPOT measurements. *In vivo*, one prospective study³² showed decreased concentrations of IL-6 in serum after 24 weeks of treatment with gold, whereas one year later the same group could not detect any significant effect on spontaneous or LPS induced IL-6 production from RA PBMC during gold treatment³³. Another prospective study detected a decrease of IL-6 serum concentrations during treatment with 2 DMARD during 12 months, but made no distinction whether this decrease was induced by gold (n = 9) or methotrexate (n = 11)⁵¹.

In this study, there were no differences in IL-6 serum concentrations before and after 4 and 12 weeks of GSTM treatment. The difference between these results and earlier studies on IL-6 serum concentrations during GSTM treatment may be explained by different time points for cytokine detection. Interestingly, we were able to detect an increase in spontaneous IL-6 production after 4 weeks, when the action of gold on joint inflammation was not noted clinically. This augmented IL-6 production was also sustained after 12 weeks' treatment. The discrepancy between serum concentrations and spontaneous production of IL-6 in this context is difficult to explain. One possibility might be that GSTM treatment through stimulation of monocytes may induce increased consumption of cytokines such as IL-6, affecting serum concentrations but not spontaneous production of the cytokine. Although IL-6 exerts many proinflammatory effects⁴⁸, several studies have reported that IL-6 may also have antiinflammatory properties. Thus, Shingu, *et al* among others have described IL-6 stimulated production of tissue inhibitor of metalloproteinases (TIMP)-1, suggesting a protective effect on cartilage degradation⁵²⁻⁵⁴.

We describe a prospective study of GSTM treated patients with RA, showing that gold has immunostimulatory properties *in vivo* preceding the clinical effect of the drug. The initial response to the same gold compound *in vitro* correlated negatively with the presence of subsequent skin reactions. These data indicate an immune-modulating effect of GSTM and may provide a hypothesis for the mechanism of action of gold in RA.

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