

Methotrexate therapy of oral corticosteroid-dependent asthmatics reduces serum immunoglobulins: correlation with clinical response to therapy

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Summary

Background Concomitant methotrexate (MTX) therapy of oral corticosteroid (CS)-dependent asthmatics has been shown to spare CS therapy, but the mechanism is unknown. In a previous report, we showed that MTX increases T cell inhibition by CS. In this report we focus on effects of MTX on immunoglobulin concentrations and their possible clinical relevance.

Objective To monitor changes in circulating leucocytes and Ig in a group of these patients during MTX therapy, and to relate these changes to clinical 'response' as defined by oral CS reduction.

Methods Sixteen severe asthmatics dependent on oral prednisolone 15 (7.5–25) mg/day in addition to high dose inhaled CS were treated with MTX 15 mg intramuscularly, weekly for 28 weeks. Prednisolone dosages were maintained constant for 12 weeks then reduced systematically over the next 16 weeks provided that asthma control did not deteriorate. Patients were classified *a priori* as 'responders' or 'non-responders' to MTX (reduction of initial oral prednisolone requirement by $\geq 50\%$ or $< 50\%$, respectively). Patients were followed-up for a further 12 weeks after MTX withdrawal. Serum Ig and differential blood leucocyte counts were measured at baseline, 12, 28 and 40 weeks.

Results MTX therapy allowed significant, but individually variable, reductions in oral prednisolone dosages ($P < 0.00001$) without alteration of lung function or symptoms. This was associated with significant reductions in mean serum concentrations of Ig of all classes, which reversed following MTX withdrawal. Reductions in IgE and IgG were significantly greater in the MTX 'responders' as compared with 'non-responders', and changes in IgE, IgG and IgM correlated with changes in prednisolone requirements. Differential blood leucocyte counts showed no significant variation.

Conclusion MTX therapy reduced oral CS requirements in these severe asthmatics to a degree which correlated with reduced circulating Ig but not lymphopaenia, suggesting a possible cause and effect relationship. These reductions might also contribute to the documented incidence of opportunistic infection in these circumstances.

Keywords asthma, corticosteroid, immunoglobulin, lymphocyte, methotrexate

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Introduction

Methotrexate (MTX) is a folate analogue employed at low dosage to treat a variety of chronic inflammatory diseases. Trials of variable power and robustness of design have addressed the possible benefits of concomitant MTX therapy as a corticosteroid (CS)-sparing agent in oral CS-dependent asthmatics. The consensus, summarized in meta-analyses [1, 2], is that MTX allows reduction of oral CS dosages, without alteration in symptoms or lung function, in a proportion (about 60%) of such patients. Save for the fact that the immunomodulatory actions of MTX probably depend on its accumulation in cells as active polyglutamate complexes [3],

explaining why benefits are both delayed and cumulative, the mechanism by which MTX spares oral CS is unknown.

We recently reported that MTX therapy of oral CS-dependent asthmatics increases the susceptibility of peripheral blood T cells to CS inhibition *ex vivo* [4]. Since T cells influence the amount and class of Ig production by B cells both specifically by cognate, antigen-specific interaction and non-specifically by production of cytokines, this observation raised the possibility that MTX might also alter Ig production in these patients.

The possible role of Ig in regulating asthma severity was little studied until it was highlighted recently by the effects of monoclonal anti-IgE therapy which, like MTX, has been reported to spare CS therapy in asthma [5]. Nevertheless, several previous reports have implicated reduction of serum IgE concentrations as a factor in the mechanism of the CS-sparing and/or disease modifying effects of other immunosuppressive drugs such as gold salts and hydroxychloroquine [6, 7]. In addition to altering the function of cells such as mast

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cells and basophils, in which cross-linking of surface-bound IgE results in degranulation, resulting in release of bronchospastic mediators, reduction of IgE may also affect the functions of cells expressing low-affinity IgE Fc receptors, such as B cells and monocyte/macrophages, which use bound surface IgE to capture and present allergens (and possibly other antigens) to T cells, thus potentiating and nurturing local inflammation. Similarly, recent studies [8, 9] implicate complement fixation in asthma pathogenesis, consistent with a possible role for IgG antibody.

For these reasons, we examined the hypothesis that MTX added to systemic GC therapy in asthmatic patients suppresses Ig, and particularly IgE production to a degree which correlates with the extent of its CS-sparing effect.

Patients, materials and methods

Corticosteroid-dependent asthmatics

These have been described in detail previously [4]. Briefly, patients were adult, severe asthmatics showing at least 20% reversibility of forced expiratory volume in 1 s (FEV₁), and requiring at least 7.5 mg/day oral prednisolone continuously for at least 1 year prior to the study to maintain disease stability, as evidenced by previous failed, supervised attempts to reduce oral CS dosages because of acute exacerbation of symptoms. Starting dosages of oral prednisolone are shown in the 'Results'. In addition, all patients took inhaled CS at high dosage (equivalent to 2000 (1000–4000) µg/day beclometasone) and short-acting β₂-agonist, eight also took an oral xanthine preparation, 12 took an inhaled, long-acting β₂-agonist and eight inhaled ipratropium bromide. Despite these medications, the patients remained symptomatic with impaired lung function (mean FEV₁ 56 (35–67)% predicted) at entry to the study. Nine patients were atopic, as defined by a positive skin prick test to one or more of a range of common aeroallergens. The Ethics Committee of the Hammersmith Hospital approved the study, and all subjects provided informed, written consent.

Of the 22 patients initially recruited, three withdrew without giving a reason, and a fourth was withdrawn because of the development of pancytopenia during MTX therapy in association with commencement of ibuprofen. In two further patients, the analysing laboratory mislaid serum samples for determination of Ig concentrations. The results in this study refer to those 16 patients for whom data are complete.

Methotrexate study protocol (Fig. 1)

This has been described in detail previously [4]. Briefly, after a 'run-in' period of up to 12 weeks in which patients were established on the lowest dosage of prednisolone necessary to maintain disease stability [10], they were treated with MTX 15 mg intramuscularly once weekly for 28 weeks. After the first 12 weeks, prednisolone dosages were reduced systematically if asthma remained stable (defined according to pre-determined diary card and spirometric criteria) or increased in the event of deterioration [10]. Other medication was not changed. After discontinuation of MTX, patients were followed for a further 12-week 'washout' period in which

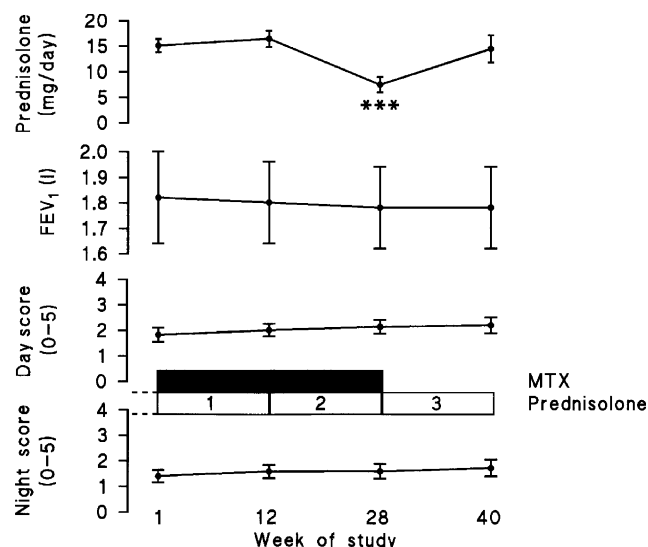


Fig. 1. Changes in oral prednisolone dosages, clinic forced expiratory volume in 1 s (FEV₁) and day and night-time symptom scores of 16 asthmatic patients during the course of the study. Values (mean ± SEM) shown were derived as the means of the measurements in the last week of the run-in or each phase of the study. Horizontal filled bar represents the duration of methotrexate (MTX) therapy. Open horizontal bars represent the phases of oral prednisolone therapy: phase 1, prednisolone dosages maintained constant; phase 2, prednisolone dosages reduced systematically; phase 3, prednisolone dosages readjusted to maintain stable disease following MTX withdrawal. Parametric ANOVA, prednisolone $P = 0.005$; FEV₁ $P = 1.0$; day score $P = 0.79$; night score $P = 0.87$. *** $P < 0.00001$ (Student's paired t -test compared with week 1).

prednisolone dosages continued to be adjusted according to disease control. Blood samples were obtained at weeks 1 (baseline, just prior to MTX therapy), 12 (just prior to attempted CS reduction), 28 (just prior to MTX withdrawal) and 40 for differential leucocyte counts and serum Ig concentrations, and more frequently for routine monitoring of full blood count and hepatic function.

Definition of methotrexate 'responders' and 'non-responders'

Since meta-analysis [1, 2] suggests that added MTX permits a mean reduction in oral prednisolone of approximately 50% in oral CS-dependent asthmatics, MTX 'responders' and 'non-responders' were defined *a priori* as those patients able to reduce their baseline dosages of oral prednisolone by ≥ 50% or < 50%, respectively. By coincidence this divided the patients into two groups of eight.

Peripheral blood leucocyte counts and serum immunoglobulin concentrations

Total and differential peripheral blood leucocyte counts were performed using a hospital diagnostic flow cytometer. Concentrations of serum total IgG, IgM and IgA were determined by immunoturbidimetry using an Olympus AU 600 analyser (Olympus, Hamburg, Germany). Serum total IgE concentrations were measured by chemiluminescence using an Access immunoassay analyser (Beckman Coulter, Brea, CA, USA). Reference ranges were as follows: IgE 0–150 IU/mL; IgG 5–16 g/L; IgM 0.5–1.9 g/L; IgA 0.8–2.8 g/L.

Statistics

Analysis was parametric. In Figs 1 and 3, one way parametric ANOVA was followed, where appropriate, by comparison of individual time-points with baseline (day 1) using Student's paired *t*-test. Paired comparisons with baseline were made at three time-points, and statistical significance was therefore taken as $P < 0.02$ (Bonferroni correction). Figs 2 (left) and 4 were analysed by Student's two-sample *t*-test, and the correlations in Figs 2 and 5 by Pearson's linear regression technique.

Results

Concomitant methotrexate therapy allowed oral corticosteroid dosage reduction in severe asthmatics

Taking the patients as a single group, as previously reported [4], concomitant weekly MTX therapy allowed significant reduction in prednisolone dosages (mean (SEM) reduced from 16.4 ± 1.6 mg/day at the end of week 12 to 7.4 ± 1.5 mg/day at the end of week 28 (95% confidence interval of changes -10.6 to -4.8 mg/day, $P < 0.00001$)) without deterioration in disease severity as determined by

clinic FEV₁ measurements, diary-card symptom scores (Fig. 1), short-acting β_2 -agonist usage and morning and evening pre-bronchodilator peak expiratory flow (PEF) (not shown).

There was, however, considerable heterogeneity in the MTX response in individual patients, with eight of the 16 being able to reduce baseline prednisolone dosages by $\geq 50\%$ ('responders'), and eight able to reduce dosages by $< 50\%$ by week 28 (Fig. 2, left panel). One patient actually required increased prednisolone at week 28 despite MTX therapy. There was no correlation between reductions of prednisolone dosage achieved and baseline dosage in individual patients (Fig. 2, right panel). Table 1 shows a comparison of the MTX 'responders' and 'non-responders'. There were no significant differences between the groups in terms of mean age, baseline FEV₁, starting prednisolone dosage and serum concentrations of IgE, IgM and IgA. The mean duration of continuous oral prednisolone therapy was significantly higher, and the mean serum IgG concentration slightly but significantly lower in the 'non-responders' as compared with the 'responders', although no patient had a serum IgG concentration outside the laboratory reference range.

When MTX was withdrawn at the end of week 28, a sharp increase in prednisolone dosages was necessary to maintain

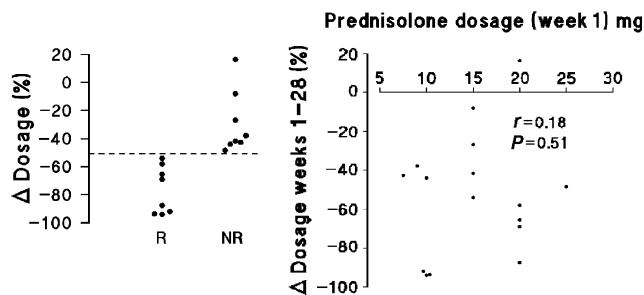


Fig. 2. Left panel: percentage reductions in baseline prednisolone dosages in the methotrexate 'responders' (R) and 'non-responders' (NR) between weeks 1 and 28. Right panel: relationship between percentage prednisolone dosage reductions achieved between weeks 1 and 28 and baseline prednisolone dosages. Pearson's linear regression.

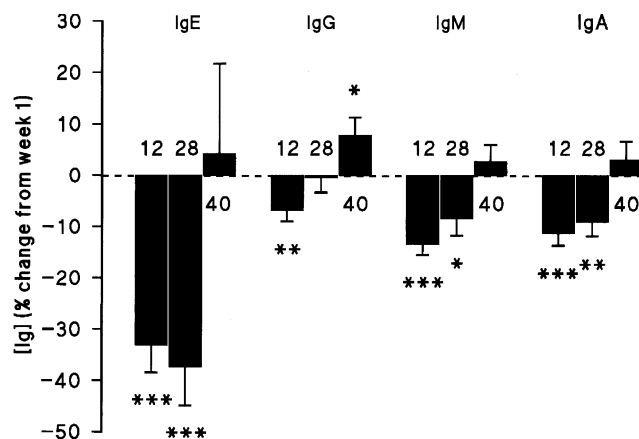


Fig. 3. Percentage changes in serum IgE, IgG, IgM and IgA concentrations in the 16 patients at weeks 12, 28 and 40 of the study as compared with week 1. Parametric ANOVA, IgE $P = 0.02$; IgG $P = 0.003$; IgM $P = 0.001$; IgA $P = 0.002$. * $P < 0.02$; ** $P < 0.01$; *** $P < 0.001$ compared with week 1 (Student's paired *t*-test).

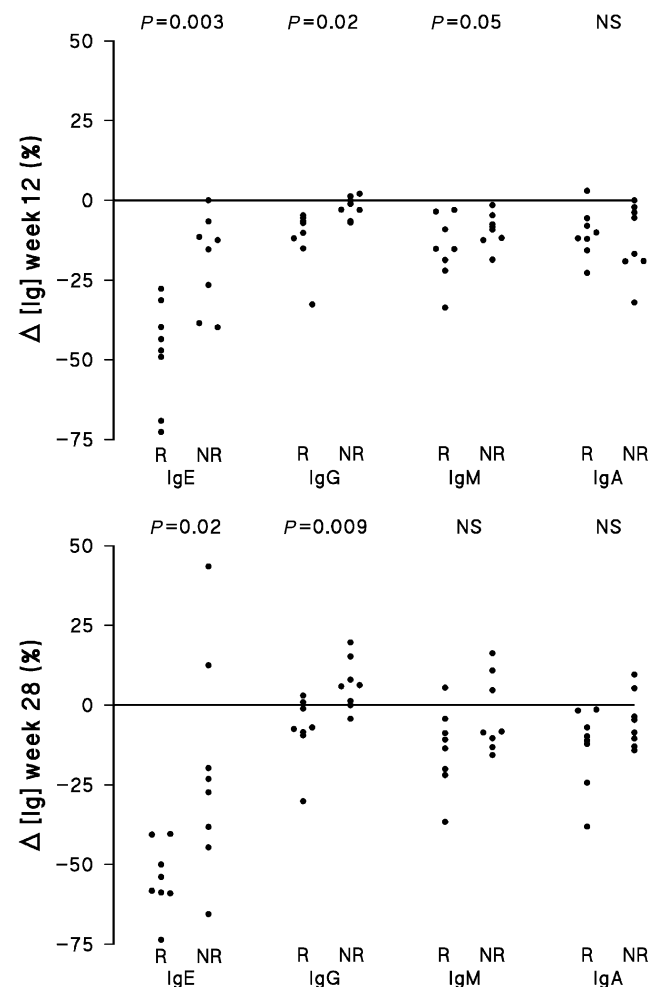


Fig. 4. Percentage changes in serum concentrations of Ig between weeks 1 and 12 (top) and weeks 1 and 28 (bottom) in the 'responders' (R) and 'non-responders' (NR) to methotrexate. Student's two-sample *t*-test.

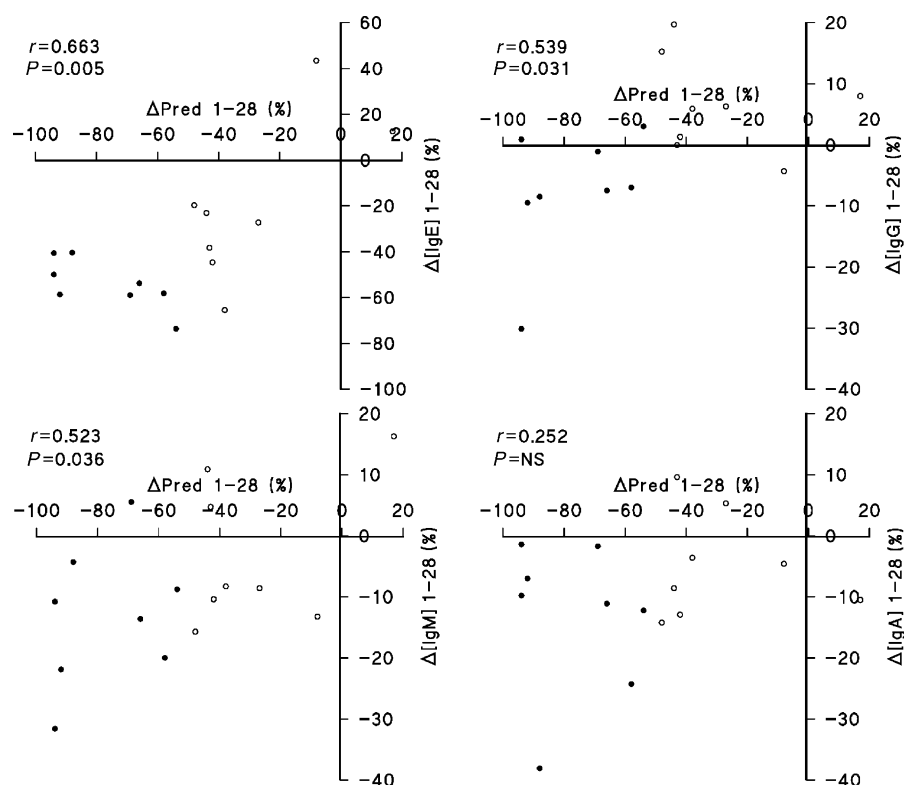


Fig. 5. Correlations between percentage changes in concentrations of Ig and percentage changes in prednisolone dosages between weeks 1 and 28. Closed and open circles represent 'responders' and 'non-responders' to methotrexate, respectively. Pearson's linear regression.

Table 1. Clinical and demographic features of 'responders' and 'non-responders' to MTX therapy

Feature	Responders (n = 8)	Non-responders (n = 8)	P-value*
Age (years)	53.0 ± 4.0	53.0 ± 2.4	NS
Time on continuous oral prednisolone (years)	6.1 ± 2.1	18.1 ± 3.1	0.008
Baseline FEV ₁ (%)	63.6 ± 6.2	47.7 ± 9.2	NS
Initial prednisolone dosage (mg/day)	15.6 ± 1.8	14.6 ± 2.1	NS
Baseline [IgE] (IU/mL)	303 ± 126	318 ± 207	NS
Baseline [IgG] (g/L)	9.1 ± 0.5	6.6 ± 0.5	0.005
Baseline [IgM] (g/L)	1.25 ± 0.33	1.01 ± 0.09	NS
Baseline [IgA] (g/L)	1.93 ± 0.28	1.78 ± 0.28	NS

*Student's two-sample *t*-test. Values represent mean ± SEM. NS, not significant; MTX, methotrexate; FEV₁ forced expiratory volume in 1 s.

asthma stability (Fig. 1). By the end of week 40, mean daily prednisolone requirements had risen again to 14.4 ± 2.7 mg/day, not significantly different from baseline ($P > 0.05$).

Concomitant methotrexate therapy was associated with reduced serum immunoglobulin concentrations

At the beginning of the study, serum concentrations (mean, SEM) of IgG, IgM and IgA were, respectively, 7.84 ± 0.49, 1.13 ± 0.17 and 1.85 ± 0.19 g/L. Mean (SEM) concentration of IgE was 310 ± 117 IU/mL.

Percentage changes in serum IgE, IgG, IgM and IgA concentrations at weeks 12, 28 and 40 as compared with week

1 of the study (baseline) are shown below and in Fig. 3. During the first 12 weeks of the study, when MTX was introduced but prednisolone dosages not altered, there was a significant reduction in mean serum concentrations of all four Ig classes: (IgE – 33.2%; 95% CI – 44.4%, – 22.0%, $P < 0.00001$; IgG – 6.9%; 95% CI – 11.3%, – 2.5%, $P = 0.004$; IgM – 13.4%, 95% CI – 17.9%, – 8.9%, $P < 0.00001$; IgA – 11.3%, 95% CI – 16.3%, – 6.4%, $P = 0.0002$).

At the end of the subsequent 16 weeks of MTX therapy, despite reduction of prednisolone dosages, the mean serum concentration of IgE was further reduced (– 37.3%, 95% CI – 53.3%, – 21.2%, $P = 0.0002$; Fig. 3). The mean serum concentrations of IgM and IgA remained significantly depressed as compared with week 1 (IgM – 8.4%, 95% CI – 15.4%, – 1.4%, $P = 0.02$; IgA – 9.1%, 95% CI – 15.0%, – 3.1%, $P = 0.005$; Fig. 3) while that of IgG was not significantly different from week 1 (– 0.5%, 95% CI – 6.6%, 5.6%, $P > 0.05$; Fig. 3).

As with changes in prednisolone dosages, there was considerable heterogeneity of individual responses: reductions in serum IgE, IgG and IgM concentrations between weeks 1 and 12, and reductions in serum IgE and IgG, but not IgA and IgM concentrations between weeks 1 and 28 were significantly greater in the MTX 'responders' as compared with the 'non-responders' (Fig. 4). Furthermore, percentage reductions in serum IgE, IgG and IgM between weeks 1 and 28 correlated with percentage reductions in prednisolone dosage in all 16 patients considered as a single group (Fig. 5). On the other hand, percentage reductions in IgE and IgG concentrations in individual patients did not correlate with baseline concentrations (data not shown).

At week 40, 12 weeks after MTX withdrawal, mean serum concentrations of IgE, IgM and IgA showed a rebound increase to ranges not statistically different from those at the start of the study, while the mean serum IgG concentration actually rebounded to be higher than baseline (7.8%, 95% CI 0.5%, 15.2%, $P = 0.04$; Fig. 3). These increases following withdrawal of MTX were seen despite a sharp increase in prednisolone requirements.

Concomitant methotrexate therapy did not alter circulating leucocyte numbers

Throughout the study, mean total and differential peripheral blood leucocyte counts remained within normal laboratory ranges and showed no significant variation, with the exception of one patient who developed pancytopenia related to introduction of ibuprofen, who was withdrawn early from the study and not included in this analysis.

Discussion

For the first time we have shown that concomitant MTX therapy of CS-dependent asthmatics reduces serum immunoglobulin (IgE, IgM, IgA and IgG) concentrations. This effect was apparent before dosages of prednisolone were changed, persisted despite prednisolone dose reduction and reversed on MTX withdrawal. There was no associated lymphopenia. These data extend and complement our previous study on these patients [4] showing that MTX therapy increases susceptibility of their peripheral blood T cells to CS inhibition.

Reductions in circulating concentrations of IgE, IgG and IgM (in the latter case after 12, but not 28 weeks of MTX therapy) were significantly greater in those asthmatics classified as 'responders' to MTX, and correlations were observed between percentage reductions in serum IgE, IgG and IgM concentrations and reductions in required prednisolone dosages. In contrast, reductions in IgE, IgG and IgM did not correlate with baseline concentrations of these Ig in individual patients, arguing against a non-specific effect of MTX. Taken together, these observations strongly suggest that this action of MTX may be of relevance to its CS-sparing effect in asthma.

The fact that MTX responder status did not correlate with baseline prednisolone dosage (Fig. 2) also suggests that reductions in prednisolone dosages achieved in individual patients represent a true effect of added MTX therapy, rather than a reduction of dose in excess of clinical need (when larger reductions might have been expected in patients taking higher baseline dosages). In addition to baseline prednisolone dosages, 'responders' were not distinguishable by age or baseline lung function, although they had been taking oral CS continuously for significantly less time than the 'non-responders', and had a slightly, but significantly higher mean serum IgG concentration at baseline (Table 1). The significance of these differences, if any, is unclear.

The present study emphasizes that systemic CS therapy alone does not reduce serum Ig concentrations, as previously noted [11]. In fact, CS actually increases IgE production by B cells both *in vitro* [12] and *in vivo* [13]. Consequently, MTX,

either alone or in combination with CS, is likely responsible for this alteration in Ig metabolism. This effect does not, however, reflect MTX toxicity, which has been reported to reduce serum Ig concentrations in the context of lymphopenia [14, 15]. Since MTX enhances T cell CS sensitivity [4], it is tempting to speculate that reduced Ig concentrations might reflect reduced T cell activation of B cells to form Ig secreting plasma cells, although this effect could also reflect other direct actions of MTX/CS on T cells, B cells or other targets which regulate Ig production and/or consumption. Enhanced T cell CS sensitivity may also result in reduced production of asthma-relevant cytokines, providing another possible mechanism for the CS-sparing effect of MTX. MTX alone can alter T and B cell function by inhibiting the enzyme amidophosphoribosyl transferase, on which lymphocytes depend for blastogenesis, and by increasing extracellular concentrations of adenosine, which act on cellular adenosine receptors [16–18]. MTX does not alter prednisolone pharmacokinetics [19]. Studies in rheumatoid arthritis suggest that MTX can reduce production of rheumatoid factor by lymphocytes cultured *ex vivo* [20], consistent with a possible direct action on B cells.

At first sight, the present study can be criticized for its lack of a formal placebo control group. This omission was deliberate, since our hypothesis concerned the effects of MTX on circulating Ig concentrations, rather than its CS-sparing effect compared with placebo. The latter has already been adequately demonstrated (and our findings in this regard conform well with the conclusions from meta-analysis). Our patients served as their own controls when measuring changes at the various time-points, and all samples were analysed objectively. Unmanageably large numbers of these scarce patients would have been needed for a placebo-controlled study. The demonstrated probability of the changes in Ig concentrations ($P < 0.001$) during MTX therapy being because of chance, coupled with our observations that both oral CS requirements to maintain asthma stability, as well as circulating Ig concentrations showed very marked changes following MTX withdrawal, provide compelling evidence for a causal relationship between the two phenomena.

In summary, we demonstrate that concomitant MTX therapy of oral CS-dependent asthmatics is associated with reduction in circulating concentrations of Ig of all classes, but particularly IgE, to a degree which correlates with the extent of its CS-sparing effect. We hypothesize that these changes result from a combined effect of MTX and CS. At our present state of knowledge, we can only speculate as to whether this is relevant to the CS-sparing effect of MTX in asthma, and to the occasional reports of severe opportunistic infections in CS-dependent asthmatics treated with MTX [21, 22], or simply epiphenomenal. The findings invite more prolonged, prospective studies of Ig metabolism in CS-dependent asthmatics treated with MTX, as well as further study of the combined effects of MTX and GC on T and B cell function *in vivo*.

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