

Methotrexate therapy in asthma increases T cell susceptibility to corticosteroid inhibition

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Abstract

Background Concomitant methotrexate (MTX) therapy of severe, oral corticosteroid-dependent asthmatics has been shown to be corticosteroid sparing, but the mechanism is unknown. We hypothesized that MTX therapy of these patients increases the susceptibility of their T cells to corticosteroid inhibition.

Objective To measure prednisolone inhibition of lectin-induced proliferation of peripheral blood T cells from a group of these patients before, during and following MTX therapy.

Methods Eighteen severe asthmatics (median (range) age 56 (33–68) years, FEV₁ 61 (38–69)% predicted, dependent on oral prednisolone 15 (7.5–25) mg/day in addition to high-dose, inhaled corticosteroids) were treated with MTX 15 mg intramuscularly, weekly for 28 weeks. After 12 weeks of MTX, oral prednisolone dosages were reduced systematically over 16 weeks, provided that asthma control did not deteriorate. Patients were followed for a further 12 weeks after MTX withdrawal. Concentration-dependent, prednisolone inhibition of lectin-induced proliferation of peripheral blood T cells was measured just prior to MTX therapy (week 1) and at weeks 12, 28 and 40, and IC₅₀ concentrations were interpolated.

Results By week 28 of MTX therapy, patients were able to reduce oral prednisolone dosages from (median, SIQR) 15 (10–20.5) to 5.9 (1.4–9.4) mg/day ($P < 0.01$) without alteration of lung function and symptoms, while median IC₅₀ values for prednisolone inhibition of peripheral blood T cell proliferation were reduced from 49 (21–144) to 4 (1–9) nM ($P < 0.0001$). These increased again to 15 (9.4–25.7) mg/day and 36 (18–67) nM, respectively, following MTX withdrawal. A correlation ($P < 0.01$) was observed between percentage reductions in prednisolone dosages *in vivo* and fold changes in prednisolone IC₅₀ *in vitro* between weeks 12 and 28.

Conclusion This effect of MTX may at least partly account for its oral corticosteroid-sparing effect in severe asthma.

Keywords asthma, corticosteroid, methotrexate, prednisolone, therapy, T lymphocyte

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Introduction

Corticosteroids are widely used for the therapy of asthma. Although they have a range of anti-inflammatory effects, one key feature of their activity is thought to be to inhibit T cell activation and the production of asthma-relevant cytokines within the bronchial mucosa *in vivo* [1–6]. Some asthmatic patients are however relatively refractory to corticosteroid therapy, and suffer debilitating symptoms despite treatment with systemic corticosteroids. There is considerable evidence that T cells from these patients are relatively resistant to corticosteroid inhibition, as principally assessed by a shift in the concentration–response curve for corticosteroid inhibition of lectin-induced T cell proliferation and cytokine production [4, 7–9]. Alternative immunomodulators, including methotrexate (MTX), have been advocated as corticosteroid-

sparing therapy for these patients, often by analogy with their immunosuppressive effects in other diseases, but in general the mechanisms by which these drugs exert such an effect *in vivo* are poorly understood.

MTX is a folic acid analogue, which is used at low (non-cytotoxic) dosages for the treatment of a variety of chronic inflammatory diseases including asthma. Clinical trials of variable size and quality performed over the last 10 years have provided disparate conclusions regarding the oral corticosteroid-sparing effect of concomitant MTX therapy in severe asthma. Pooled data from double-blind, long-term prospective studies, summarized in recent meta-analyses [10, 11], do however suggest that MTX allows the reduction of existing oral corticosteroid therapy without deterioration in lung function or symptoms in these patients, although only approximately 60% are able to discontinue oral corticosteroid therapy entirely or substantially. The mechanism of this effect is unknown.

In view of the probable central role of corticosteroid-mediated T cell inhibition in the therapy of asthma, and the

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corticosteroid-sparing effect of MTX therapy in patients with severe disease, we hypothesized that concomitant MTX therapy of oral corticosteroid-dependent asthmatics increases susceptibility of their T cells to corticosteroid inhibition. We measured the susceptibility of peripheral blood T cells from a group of these patients to corticosteroid inhibition *ex vivo* during a period of oral corticosteroid-sparing MTX therapy, and subsequently following MTX withdrawal. A secondary aim was to see if it was possible to predict favourable responses to MTX by the measurement of MTX-induced modulation of thymidine uptake by T cells from these patients *in vitro*.

Patients, materials and methods

Corticosteroid-dependent asthmatics

We recruited, from the outpatient clinics of the Hammersmith Hospital and other regional respiratory clinics, patients with specialist-diagnosed asthma, showing at least 20% reversibility of the FEV₁ in response to inhaled β_2 -agonist (salbutamol 200 μ g). Patients were required to be 18–70 years old and to have been taking oral prednisolone therapy continuously at a daily dosage of 7.5 mg/day or more, for at least a year prior to the study, in addition to high-dosage inhaled corticosteroids. All patients were also required to have shown failed previous supervised attempts to reduce oral corticosteroid therapy, resulting in acute exacerbation of symptoms. Patients at risk of pregnancy, patients with any chronic renal, hepatic or haematological disease, any chronic infection, any history of malignancy within the previous 5 years, or any significant lung disease other than asthma were excluded. The Ethics Committee of the Hammersmith Hospital approved the study, and all subjects provided informed, written consent to participate.

Of the 22 patients initially recruited for this study, three withdrew during MTX therapy without giving a reason, as they were ethically entitled to do, while a fourth was withdrawn because of the development of pancytopenia during MTX therapy, possibly in association with commencement of ibuprofen. The data in this study refer to those 18 patients who completed it. The median (range) age of the patients was 56 (33–68) years. They had been taking oral prednisolone at a dosage of 15 (7.5–25) mg/day for a total of 9.5 (1–33) years. Ten of the 18 patients were atopic, as defined by one or more positive skin prick tests to a range of common aeroallergens. All the patients were taking inhaled corticosteroids at high dosage (2000 (1000–4000) μ g/day). In addition, all patients were taking a short-acting β_2 -agonist, 10 using a nebulizer, eight were taking an oral xanthine preparation, 12 were taking an inhaled, long-acting β_2 -agonist and nine were taking nebulized ipratropium bromide. Three were smokers. Despite these medications, the patients remained symptomatic with impaired lung function (FEV₁ 61 (38–69)% of the predicted value) at entry.

MTX study protocol

Throughout the study all patients kept a daily diary card, documenting morning and evening pre-bronchodilator PEF,

severity of day and night symptoms on a scale from 0 (no symptoms) to 5 (very severe symptoms) and use of medications. The study physician reviewed patients every 4 weeks. At each visit, post-bronchodilator FEV₁ was measured in the clinic and routine blood samples were taken for blood counts and safety monitoring.

Patients were observed over a 'run-in' period of up to 12 weeks, during which they were maintained on the lowest dosage of oral prednisolone required to prevent exacerbations and deterioration in lung function. Once patients were free of exacerbations for at least 4 weeks, as defined by objective criteria [12], they were treated with MTX 15 mg intramuscularly once weekly for a period of 12 weeks, while all existing asthma medication was maintained unaltered, with the exception of inhaled β_2 -agonists which were allowed 'as required'. This initial 12-week phase was employed because previous studies had suggested that any oral corticosteroid-sparing effect of MTX does not become apparent until at least 12 weeks following commencement of therapy [12].

Over the following 16 weeks, MTX therapy was continued and oral prednisolone dosages were reduced by the trial physician. In order to eliminate bias, weaning of systemic prednisolone therapy was subject to strict adherence to previously determined diary card criteria (no deterioration of lung function or asthma symptoms, with no other alteration in asthma therapy) as previously described [12]. MTX was then discontinued and the patients followed up for a further 12 weeks. During this 'wash-out' period, the trial physician once again adjusted prednisolone dosages to maintain disease control. Samples of heparinized peripheral venous blood (100 mL) were obtained from the patients at weeks 1 (just prior to commencement of MTX therapy), 12 (just prior to commencement of attempted reduction of oral prednisolone), 28 (just prior to MTX withdrawal) and 40 of the study.

Cell isolation and culture

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized venous blood using the Ficoll gradients as previously described [2, 6–9], washed twice and resuspended in RPMI-1640 medium supplemented with 2 mM L-glutamine, 5% v/v human AB serum and antibiotics (all Sigma, Poole, UK), subsequently referred to as 'complete medium'.

A stock solution of prednisolone (Sigma) was prepared by dissolving the drug in 100% ethanol at 10^{-2} M, filtering sterile (0.2 μ m pore size) and storing at -20°C in aliquots. MTX for parenteral administration (Wyeth, Maidenhead, UK) was dissolved directly in complete medium at a concentration of 10^{-2} M. Serial 10-fold dilutions of these stocks were made in complete medium prior to each experiment. The same stock solutions were used for all the experiments. The PBMC were cultured (72 h, 37°C , 5% CO₂ atmosphere) in 96-well round-bottomed plates at 2×10^6 /mL in 200 μ L total volumes, in triplicate, in complete medium supplemented with 5 μ g/mL phytohaemagglutinin (PHA-P, Sigma) in the absence and presence of both prednisolone and MTX, at concentrations of 10^{-6} – 10^{-9} M. In experiments where cells were cultured with prednisolone, positive control wells containing PHA and diluent vehicle at a concentration corresponding to that of the highest final concentration in the prednisolone-containing wells, and negative control wells (complete medium with

vehicle only) were also employed. In experiments where cells were cultured with MTX, positive control wells contained PHA in complete medium, while negative control wells contained complete medium only. Tritiated thymidine ($0.5 \mu\text{Ci}$ in $10 \mu\text{L}$, Amersham, Little Chalfont, UK) was added to the cells for the final 6 h of culture and label incorporation was assessed by harvesting and liquid scintillation spectroscopy. After subtraction of background counts (negative control wells), the results were expressed as the geometric means of triplicate counts per minute. An investigator (C. J. C.) unaware of the provenance of the blood samples performed the cell culture experiments and collected the relevant data.

Statistics

Data were analysed using non-parametric statistics (Friedman's method for repeated measures, Dunn's post-test comparison and Spearman's rank correlation) and are generally presented as the median and semi-interquartile range (SIQR). For clarity, however, the data in Figs 2 and 5 are expressed as the mean and standard error of the mean (SEM). Prednisolone and MTX concentration–response curves for individuals and the asthmatics as a group (Figs 2 and 5) were constructed using a third-order, non-linear equation-fitting programme (Fig.P Software Corp., Durham, NC, USA).

Results

Concomitant MTX therapy allowed oral corticosteroid dosage reduction in severe asthmatics without deterioration in disease severity

Concomitant weekly MTX therapy of the patients allowed progressive, clinically and statistically significant reductions in oral prednisolone dosages between weeks 12 and 28 (Fig. 1a). The median (SIQR) daily dosage of prednisolone was reduced from 15 (10–20.5) at the end of week 12 to 5.9 (1.4–9.4) mg/day at the end of week 28 (95% confidence interval of changes -12.1 to -4.4 mg/day; $P < 0.01$) (Fig. 1b). As in previous studies, the degree by which individual patients were able to reduce oral prednisolone varied considerably. Despite this, asthma control, as measured by clinic FEV₁ measurements, diary card day and night symptom scores and short-acting β_2 -agonist usage (median (SIQR) FEV₁ (l) 1.63 (1.31–2.18), 1.80 (1.31–2.01), 1.81 (1.40–2.31), 1.85 (1.22–2.10), $P = 0.95$; day symptom scores (0–5) 2.00 (0.85–2.95), 2.15 (1.14–2.73), 2.60 (1.14–3.02), 2.02 (1.19–3.15), $P = 0.25$; night symptom scores (0–5) 1.10 (0.45–2.05), 1.30 (0.91–2.08), 1.29 (0.49–2.45), 1.48 (0.20–3.00), $P = 0.88$; short-acting β_2 -agonist usage (puffs/day) 5.2 (3.5–6.4), 4.9 (3.4–6.5), 4.7 (3.2–6.8), 5.0 (3.6–6.1), $P = 0.67$ on days 1, 12, 28 and 40, respectively; Friedman analysis of variance across all four time-points) did not alter significantly during the course of the study.

When MTX was withdrawn at the end of week 28, a sharp increase in oral prednisolone dosages was necessary to maintain asthma stability (Fig. 1). By the end of week 40, median (SIQR) daily prednisolone requirements had risen

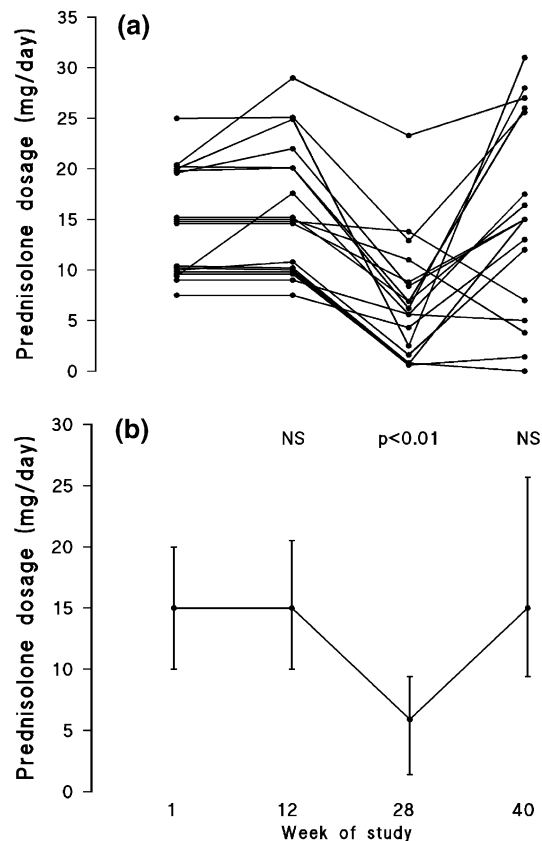


Fig. 1. Absolute individual (a) and median (SIQR) (b) prednisolone dosages (mg/day) taken by the patients at weeks 1, 12, 28 and 40 of the study. Friedman's analysis of variance across all four time-points $P < 0.0001$. Individual P -values by Dunn's post-test (comparing with week 1).

again to 15 (9.4–25.7) mg/day, not significantly different from the patients' starting dosages.

Median peripheral blood total lymphocyte counts fell slightly but statistically significantly during the course of MTX therapy, but normalized following MTX withdrawal (median (SIQR) counts ($\times 10^9$ cells/L) 1.60 (1.19–2.36) at week 1, 1.17 (0.85–1.67) at week 12 ($P < 0.05$), 1.29 (1.06–1.62) at week 28 ($P = 0.056$) and 1.63 (1.04–2.67) at week 40; Friedman's test $P < 0.05$).

Concomitant MTX therapy increased peripheral blood T cell sensitivity to prednisolone inhibition

Measurements of *in vitro* inhibition of tritiated thymidine uptake by prednisolone at each of the concentrations tested (10^{-6} , 10^{-7} , 10^{-8} , 10^{-9} M) at each time-point in the study (1, 12, 28, 40 weeks) were used to construct concentration–response curves, using an objective, 'best-fit' procedure for prednisolone inhibition of thymidine uptake at each time-point for each patient, allowing IC₅₀ values (the concentration of prednisolone causing reduction of thymidine uptake to 50% of that observed in the positive control culture) to be interpolated. A summary of the concentration–response curves in all the patients is presented in Fig. 2. For each concentration of prednisolone there was significant variation in the inhibition of thymidine uptake across the four time-

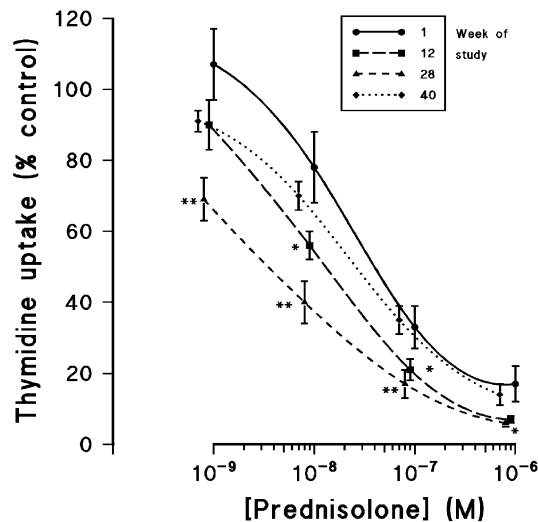


Fig. 2. Concentration–response curves for prednisolone inhibition of tritiated thymidine uptake at each sampling time-point (1, 12, 28 and 40 weeks). For clarity, data are expressed as mean $\pm$ SEM, and means and error bars for each sampling time-point are shown slightly offset on the abscissa against the corresponding concentration of prednisolone. Friedman's analysis of variance across all four time-points $P = 0.027$ (10^{-6} M), $P < 0.0001$ (10^{-7} M), $P < 0.0001$ (10^{-8} M), $P = 0.002$ (10^{-9} M). * $P < 0.05$, ** $P < 0.01$ (Dunn's post-test comparing with week 1).

points, with increasing inhibition from weeks 1 to 28 and a rebound decrease at week 40 following MTX withdrawal. There was, on the other hand, no significant variation in the median thymidine uptake in the positive control wells across all four time-points (Friedman's analysis of variance; data not shown).

Concomitantly with MTX therapy, IC_{50} values for prednisolone inhibition of PHA-induced peripheral blood T cell proliferation were reduced progressively (Figs 3a and b) from (median, SIQR) 49 (21–144) nM at the commencement of the study to 16 (5–45) nM ($P = 0.014$) at the end of week 12 (during which period oral prednisolone dosages were not adjusted), and to 4 (1–9) nM ($P < 0.0001$) at the end of week 28 (when oral prednisolone dosages were reduced) (95% confidence interval of changes from week 1 to week 28 – 20 to –107 nM). When MTX was withdrawn at the end of week 28, IC_{50} values showed a rebound increase to 36 (18–67) nM, a range not significantly different from the measurements at the commencement of the study (Figs 3a and b).

Figure 4 shows a comparison, in individual patients, of fold changes in peripheral blood T cell prednisolone IC_{50} values between weeks 12 and 28 and percentage changes in oral prednisolone dosages over the same period. Those patients showing a significant clinical response to MTX therapy in terms of reduction of oral prednisolone dosages tended to be those showing the largest fold reduction in prednisolone IC_{50} ($P < 0.01$).

Effects of MTX on uptake of tritiated thymidine by PHA-stimulated peripheral blood T cells

In vitro, MTX increased the uptake of tritiated thymidine by PHA-stimulated peripheral blood T cells from the severe asthmatics in a concentration-dependent fashion (Fig. 5). This effect varied significantly across the four time-points of

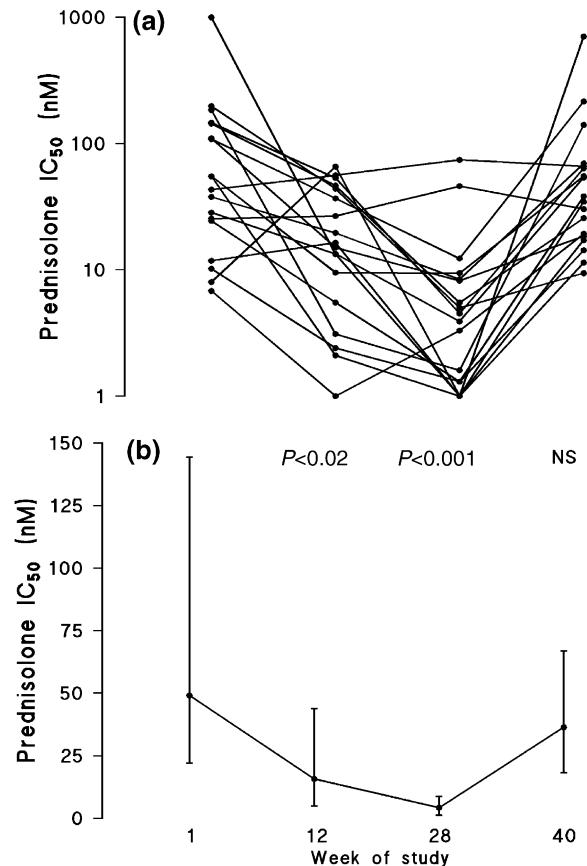


Fig. 3. Absolute individual (a) and median (SIQR) (b) IC_{50} concentrations (nM) for prednisolone inhibition of PHA-stimulated peripheral blood T cell proliferation at weeks 1, 12, 28 and 40 of the study. Friedman's analysis of variance across all four time-points $P < 0.0001$. Individual P -values by Dunn's post-test (comparing with week 1).

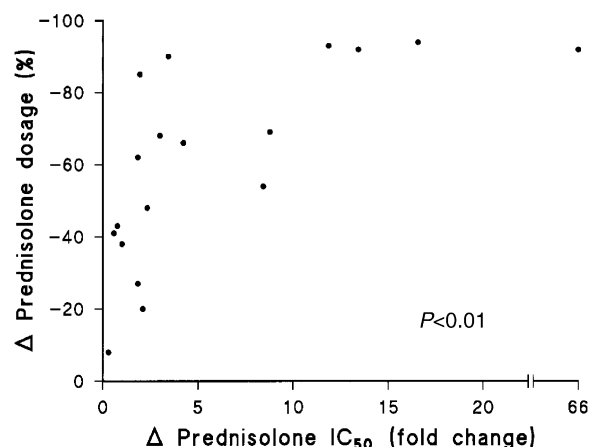


Fig. 4. Correlation of reduction in oral prednisolone dosages (expressed as percentages of the dosages at week 12) and fold changes in prednisolone IC_{50} in all 18 patients between weeks 12 and 28 of the study. $P < 0.01$ (Spearman's rank correlation).

the study at the higher concentrations of MTX (10^{-6} , 10^{-7} , 10^{-8} M) but not the lowest concentration (10^{-9} M), owing in large part to the reduced enhancement of thymidine uptake at week 40, following MTX withdrawal. Enhancement of thymidine uptake by MTX in T cells from individual patients,

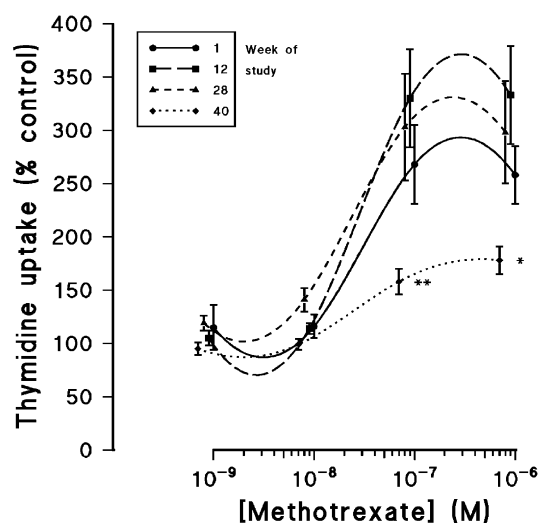


Fig. 5. Concentration–response curves for methotrexate enhancement of tritiated thymidine uptake at each sampling time-point (1, 12, 28 and 40 weeks). For clarity, data are expressed as mean $\pm$ SEM, and means and error bars for each sampling time-point are shown slightly offset on the abscissa against the corresponding concentration of prednisolone. Friedman's analysis of variance across all four time-points $P = 0.004$ (10^{-6} M), $P = 0.001$ (10^{-7} M), $P = 0.009$ (10^{-8} M), $P = 0.07$ (10^{-9} M). * $P < 0.05$, ** $P < 0.01$ (Dunn's post-test comparing with week 1).

analysed at weeks 1, 12 and 28 of MTX therapy, did not correlate with their clinical response to MTX as assessed by both absolute and percentage reductions in oral prednisolone dosages achieved between weeks 12 and 28 of MTX therapy (data not shown).

Discussion

The principal finding in this study is that concomitant MTX therapy of chronic, severe, oral corticosteroid-dependent asthmatics increases the susceptibility of their peripheral blood T cells to corticosteroid inhibition *ex vivo*, as assessed by inhibition of lectin-induced proliferation. In view of the correlation between the clinical effect of MTX in reducing oral prednisolone requirements in these patients and the *ex vivo* effect of MTX in shifting prednisolone IC_{50} concentrations, we hypothesize that this effect of MTX is at least partly responsible for its corticosteroid-sparing effect *in vivo*. As far as we are aware, this is a previously undocumented effect of MTX in asthma, although it should be noted that in a previous preliminary study [13] it was observed that nanomolar, but not higher, concentrations of dexamethasone inhibited lectin-induced proliferation of PBMC from eight atopic asthmatics to a slightly, but significantly, lesser degree as compared to that measured in a group of non-asthmatic controls, a difference that disappeared following 8 weeks of therapy of the asthmatics with MTX.

Our data are also in line with previous controlled studies [10–12] showing that concomitant MTX therapy of these patients allows reduction of oral corticosteroid dosages without deterioration in lung function and symptoms. This effect was demonstrable despite our precautions to ensure that all patients were on the minimal dosages of oral

corticosteroid necessary to maintain their disease stable. Once again, as in previous studies, the range of clinical benefit in terms of sparing of oral corticosteroid dosages was variable.

It could be argued that inhibition of lectin-induced T cell proliferation is not necessarily the best functional correlate of the anti-asthma activity of corticosteroids *in vivo*, and that other outcome measures, for example, inhibition of asthma-relevant cytokine production, might be more appropriate. While this may be so, both we [7, 8] and others [9, 14] have clearly shown that the susceptibility of peripheral blood T cells of asthmatics to corticosteroid inhibition of lectin-induced proliferation correlates well with their clinical responses to corticosteroid therapy *in vivo*. This further underlines our contention that the observed effect of MTX *in vitro* is relevant to its corticosteroid-sparing effect *in vivo*.

We can only speculate as to the mechanism whereby MTX might alter T cell corticosteroid sensitivity, since while the molecular effects of corticosteroids in inhibiting certain T cell functions such as cytokine production have been extensively investigated [15], the precise mechanism whereby they ultimately inhibit T cellular proliferation is unknown. The mechanism of the immunomodulatory effect of MTX on T cells is also controversial. A definitive study addressing this question [16] showed that low-dose MTX inhibits human T cell proliferation through inhibition of the enzyme amidophosphoribosyl transferase, the enzyme catalysing the first committed step of *de novo* purine synthesis, on which T cells are dependent for blastogenesis. Interestingly, neither of the two folate-dependent steps on this synthetic pathway is the target for MTX, which is in accord with clinical experience in rheumatoid arthritis, where folic acid supplements are often given to counter unwanted effects of MTX without diminution of its therapeutic effect [17]. This effect of MTX almost certainly depends on its sustained intracellular accumulation as polyglutamates [18], which may explain why it is both delayed and cumulative. Since it has been suggested that exposure of T cells to IL-2 and IL-4 *in vivo* may reduce their glucocorticoid sensitivity, possibly at least partly by reducing the binding affinities of their glucocorticoid receptors [19], we are examining the possible effects of MTX on T cell production of these and other cytokines both *in vitro* and *ex vivo*.

This study can obviously be criticized for its lack of a formal placebo control group. The reasons why we chose not to include such a group are fourfold:

- (1) Our hypothesis concerned the mechanism of effect of MTX and not its clinical efficacy (we accepted the results of the published literature meta-analyses [10–12] and had no wish to repeat them).
- (2) Our controls *in vivo* were the samples taken from the same patients at different time-points, analysed contemporaneously and in a blinded fashion.
- (3) We used positive and negative controls for the *in vitro* studies.
- (4) Suitable corticosteroid-dependent asthmatic patients are scarce. A cross-over design would have been problematic because of the long 'carry-over' effect of MTX, and unmanageably large numbers of patients would have been needed for a placebo-controlled study.

We accept that, in view of this, apparent reductions in oral prednisolone requirements observed in our patients while treated with MTX might have been exaggerated because of improved compliance with existing medication in a trial context (these were certainly larger than the extent of the reduction promised by the meta-analyses). We contend that with regard to the changes in the sensitivity of the patients' peripheral blood T cells to prednisolone inhibition *ex vivo* when treated with MTX, it would be extremely unlikely that such changes would occur by chance ($P < 0.0001$) with samples analysed in a blinded, objective fashion. This fact coupled with our observations that, following MTX withdrawal, oral glucocorticoid requirements to maintain asthma stability *in vivo*, as well as IC_{50} values for prednisolone inhibition of T cell proliferation *ex vivo* showed a marked rebound effect, offer very compelling evidence that the two are causally related.

It might also be argued that our observed changes in the IC_{50} values for prednisolone inhibition of peripheral blood T cells from these patients might somehow have been a consequence of their reduced dosages of oral prednisolone. We believe this is also extremely unlikely, because (i) the median IC_{50} concentration for prednisolone inhibition of T cells in these patients showed a significant fall after the initial 12 weeks of MTX therapy, during which oral prednisolone dosages were not significantly altered, and (ii) we have observed in previous studies [20, 21] that the susceptibility of peripheral blood T cells from severe asthmatics to corticosteroid inhibition remains remarkably constant despite the presence or absence of ongoing oral corticosteroid therapy. A previous study [22] has, in addition, ruled out any effect of MTX therapy on prednisolone pharmacokinetics.

MTX therapy of the asthmatics was accompanied by a mild peripheral blood lymphopaenia. This would not have affected the prednisolone IC_{50} measurements, since they were in each case performed using constant numbers of PBMC, and normalized to a positive (no drug) control. It is theoretically possible that changes in CD4/CD8 T cell ratios, which were not measured in this study, might have contributed in part to the changes in prednisolone IC_{50} values if the susceptibility of CD4 and CD8 T cells to prednisolone inhibition had significantly differed, a possibility that has never been systematically addressed. Studies in rheumatoid arthritis suggest, however, that circulating CD4/8 ratios are modulated very little by MTX therapy (reviewed in [23]).

It would obviously be useful to be able to predict *a priori* which asthmatic patients will show a particularly favourable response to MTX in terms of a glucocorticoid-sparing effect. We investigated the possibility that the effects of MTX itself on thymidine uptake by lectin-stimulated peripheral blood T cells from these patients *in vitro* might predict this response. MTX increased the incorporation of tritiated thymidine into cellular DNA of lectin-activated T cells from these patients. This is likely because thymidine can bypass the *de novo* purine biosynthetic pathway in T cells, so that although net purine synthesis is decreased, the direct uptake of thymidine substrate is actually relatively enhanced. This phenomenon has been reported previously [24, 25]. Although there was significant variation in the concentration-response effect of MTX in enhancing tritiated thymidine uptake by lectin-stimulated peripheral blood T cells from the patients across

the time course of the study, the degree of enhancement could not be correlated in individual patients with the clinical efficacy of this drug in exerting a glucocorticoid-sparing effect *in vivo*. This phenomenon also precluded our studying the combined effects of prednisolone and MTX on T cell thymidine uptake *in vitro*, since prednisolone decreased uptake while MTX enhanced it, and the combined effects were approximately additive (data not shown). In contrast, MTX therapy *in vivo* did not significantly alter thymidine uptake in the positive control wells *in vitro*, which might have made the effects of prednisolone alone difficult to interpret. The reason for this is unexplained, but it is reminiscent of our observations, referred to above [20, 21], that prednisolone IC_{50} values measured *ex vivo* are surprisingly independent of the presence or absence of ongoing systemic prednisolone therapy. Inhibition of deoxyuridine uptake has been suggested as being a better reflection of the inhibition of cellular DNA synthesis by MTX [24, 25], but again this is unlikely to reflect its effects on *de novo* purine synthesis. Prediction of which patients will show a favourable response to immunomodulatory therapy remains an unanswered challenge.

In summary, we conclude that at least part of the corticosteroid-sparing effect of concomitant MTX therapy in chronic, severe, corticosteroid-dependent asthmatics results from increased susceptibility of their T cells to glucocorticoid inhibition. This does not exclude possible anti-inflammatory effects of MTX in its own right. A search for such effects is the subject of further ongoing, allied studies.

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