Asian Pacific Journal of Allergy and Immunology (1994) 12 : 65-71

The Inhibitory Effect of Methotrexate on PAF-Induced Neutrophil and Eosinophil Locomotion in Asthmatic Patients

Jaw-Ji Tsai[1], Ting-Fang Wang[1], and Soo-Ray Wang[2]

SUMMARY **We have tested the effect of methotrexate (MTX) on platelet activating factor (PAF)-induced neutrophil and eosinophil locomotion, neutrophil leukotriene B_4 (LTB_4) generation and mononuclear cell DNA synthesis. Neutrophils from patients treated with low dose methotrexate showed reduced PAF-induced chemotactic responses (727.8 ±72.2/10 HPF *vs* 481.9 ±87.3/10 HPF, $p < 0.05$). Both MTX and the specific PAF antagonist BN-52021 significantly inhibited PAF-induced eosinophil and neutrophil locomotion in a dose-dependent manner. MTX also reduced calcium ionophore-driven LTB_4 generation from the neutrophils of asthmatics (358.9 ±39.5 pg/10^6 cells *vs* 240.1 ±29.1 pg/10^6 cells, $p < 0.05$) and attenuated PHA-induced mononuclear DNA synthesis as shown by a reduction in ^{3}H-thymidine uptake and propidium iodide staining. These findings support the view that the beneficial effects of MTX in asthma may be due not only to its anti-mitotic effects on the proliferation of mononuclear cells but also to direct effects on granulocyte locomotion and production of LTB_4.**

Asthma is characterized by narrowing of airways in response to a variety of specific agents (eg aeroallergens) and to non-specific stimuli (eg exercise, cold air, and chemical irritants). In most patients with episodic asthma, bronchoconstriction is readily reversed by adrenergic bronchodilator agents, which exert their effect through relaxation of bronchial smooth muscle. Patients with chronic and severe clinical asthma exhibit pathological changes that are consistent with inflammation of the airways and characterized by influx of inflammatory cells, such as eosinophils, neutrophils and mononuclear cells, into the lumen of the airways and peribronchial tissue.[1] During an allergen-induced asthmatic response, the influx of inflammatory cells into the peribronchial tissues is triggered by a variety of cellular chemotactic mediators including eosinophil chemotactic factor (CEF), neutrophil chemotactic factor (NCF), leukotriene B_4 (LTB4), and platelet activating factor (PAF).[2] Activation of eosinophils and other inflammatory cells leads to the release of toxic granular proteins as well as bioactive mediators that promote enhancement of mucous secretion, bronchial edema, and bronchoconstriction.[3]

Asthma treatment strategies, therefore, include not only drugs that produce bronchodilation but also medications that control or prevent airway inflammation. Oral or topical corticosteroids are very effective for long-term control of daily asthmatic symptoms. In addition to modulating airway inflammation, corticosteroids also decrease bronchial hyperresponsiveness.[4] Despite their excellent anti-inflammatory properties long-term administration of systemic steroids is associated with significant adverse effects. For this reason, clinical investigators have begun to examine other anti-inflammatory drugs in asthma.

From the [1]Section of Allergy and Clinical Immunology, Cathay General Hospital, Taipei, [2]Section of Allergy, Immunology and Rheumatology, Veterans General Hosptial, Taipei, Taiwan.

Correspondence : Jaw-Ji Tsai, Section of Allergy and Clinical Immunology, Cathay General Hospital, Taipei, 280 Section 4, Jen-Ai Road, Taipei, Taiwan.

Medications such as azathioprine, methotrexate (MTX), colchicine, gold salts, and cyclosporin A have all been tried in asthma with variable results.[5-7] Amongst them, MTX has a more favorable safety record.

MTX has been used successfully in the therapy of cancer,[8] psoriasis,[9] psoriatic arthritis,[10] polymyositis,[11] and rheumatoid arthritis.[12] In recent studies, Mullarkey *et al*[13,14] have demonstrated that low-dose methotrexate may be useful as an adjunctive therapy to corticosteroids in patients with steroid-dependent asthma, although others have found MTX has no effect.[15] Despite the clinical benefits of MTX, the mechanisms by which MTX works in asthma remain uncertain.

PAF is a potent eosinophilotactic agent[2] and is able to enhance bronchial hyperreactivity.[16] The effects of MTX on PAF induced eosinophil locomotion have never been reported. In this study, MTX was tested for its effects on PAF-induced neutrophil-and eosinophil-locomotion, neutrophil LTB_4 production, mononuclear cell proliferation and DNA synthesis. The effect of MTX on neutrophil chemotaxis in asthmatics was also investigated after treatment.

MATERIALS AND METHODS

Patient selection

The diagnosis of bronchial asthma was confirmed by more than 15% improvement of forced expiratory volume in one second (FEV_1) following inhalation of a β_2-adrenergic bronchodilator. Patients with steroid-dependent asthma were defined as those who had low serum immunoglobulin E, negative skin test for common aeroallergen, and required an average of 15 mg of prednisolone a day taken for at least 1 year in addition to β_2-adrenergic drugs and theophylline. Twenty-one cases were selected in this study. Eight of them treated with MTX (15 mg oral use per week) for at least 6 months. After therapy the MTX-treated group required markedly reduced doses of prednisolone (15.6 ± 2.4 mg/day *vs* 4.4 ± 2.4 mg/day $p < 0.01$, $n = 8$).

Preparation and culture of peripheral blood mononuclear cells (MNC)

Human peripheral venous blood from normal healthy donors was mixed with 10 U/ml of heparin, and diluted 1:1 with RPMI-1640 containing 25 mM HEPES and 4 mM L-glutamine. Twenty millilitres of diluted blood were layered over 10 ml of Ficoll-Paque in 50 ml polypropylene tubes (Falcon-2070), and centrifuged at $1,000 \times g$ for 25 minutes at 20°C. The final MNC pellet was resuspended at a concentration of 2×10^6 cells/ml in RPMI-1640 tissue cultured medium buffered with 25 mM HEPES containing 4 mM L-glutamine, 100 U/ml penicillin and 100 μg/ml streptomycin. One millilitre of cell suspension in 12 × 75 mm tissue culture tubes was incubated with different concentrations of methotrexate (Sigma Chemical Co., St Louis, MO, USA) and 5 μg/ml phytohemagglutinin (PHA) (Sigma) for varying lengths of time in 5% CO_2 atmosphere at 37°C and 95% humidity. Cells were incubated with PHA or MTX for 24 hours, washed twice, and cultured for another 72 hours in tissue culture medium containing 10% fetal bovine serum. After incubation, the sterile and cell-free supernatants were collected by centrifugation, aliquoted and stored at -70°C until testing. Cell viability, as assessed by trypan blue dye exclusion, was greater than 90% after culture.

Neutrophil and eosinophil preparation

Heparinized (10 U/ml) peripheral venous blood from normal healthy donors (five parts) was mixed with one part of dextran and sedimented at 37°C for 30 minutes. For neutrophil preparation, the leukocyte-rich supernatant was layered over Ficoll-Paque and centrifuged at $1,000 \times g$ for 20 minutes. After removal of the cells from the interface, the pellet cells were lysed with lysis buffer (NH_4Cl 8.2 gm and $KHCO_3$ 1.0 gm in 1.0 l distilled water, pH 7.3) at 4°C. Neutrophils were washed twice in RPMI-1640 at 4°C, and counted in a modified hemocytometer using Kimura stain.[17] Only cell suspensions containing > 95% neutrophils were used for experiments. Human eosinophils were obtained and separated from patients attending a routine allergy clinic with either allergic rhinitis or bronchial asthma, who were found to have an eosinophilia between 5% to 20% and total eosinophil count $> 500/mm^3$, or from in-patients with hypereosinophilia admitted to our medical ward. For eosinophil preparation, leukocyte-rich supernatant was removed and washed twice in RPMI-1640, 1 ml aliquots, each containing the cells from 10 ml blood, were layered onto discontinuous metrizamide gradients in 15 ml conical tubes. The gradient consisted of 2 ml aliquots of 25, 23, 22, 21, 20 and 18% w/v metrizamide in Tyrodes/gelatin buffer.[18] Tubes were centrifuged at $1,200 \times g$ for 45 minutes at 20°C, and the eosinophils were recovered from the 23/25% interface (metrizamide densities 1.123 to 1.129 g/ml) with a purity greater than 85%. The cells were washed twice in RPMI-1640 and reconstituted to 4×10^6 cells/ml. Only normal density eosinophils were used for assay.

Cell proliferation assay and cell cycle analysis

Proliferation was assessed by incubating 100 ml of 2×10^5 MNC in triplicate using 96-well, round-bottomed microtitre plates (Flow Laboratories, Rickmansworth,

Herts, UK). Tritiated thymidine (^{3}H-TdR, 0.66 μCi/well) was added during the final 6 hours of incubation. The cells were collected on GF/C, glass fibre paper (Ilacon Ltd., Tonbridge, UK) using a multi-harvester apparatus. Radioactivity was determined in a liquid scintillation beta-counter. Cell cycle analysis was performed on a Counter FACS analyser (Coulter Electronic Inc, FL, USA). Cells, after culture, were stained for DNA with propidium iodide 50 μg/ml in 0.15 M PBS containing 0.1% triton-X 100 for 20 minutes at 37°C, 5% CO_2 incubator, then analysed with FACS analyser.

LTB_4 production from neutrophils

Neutrophils 2×10^6/ml were pretreated with MTX (10^{-5}M) or RPMI-1640 for 90 minutes at 37°C, then washed twice with RPMI-1640. Cells were stimulated with calcium ionophore (4 μg/ml) for 30 minutes at 37°C without agitation. The cell-free supernatants were collected by centrifugation (400 × g, 10 minutes, 4°C) and stored at -80°C prior to LTB_4 assay.

Radioimmunoassay of LTB_4

LTB_4 was assayed in duplicate with a double antibody radioimmunoassay using LTB_4 assay kits (NEN Research products, Boston, MA, USA). Samples and standards were diluted in buffer (100 μl), mixed with 50 μl of a dilution of antiserum and then 50 μl of tracer (14, 15-^{3}H-LTB_4 in assay buffer). Tubes were incubated overnight at 4°C on an orbital shaker prior to the addition of 400 μl of charcoal in buffer, and then incubated on ice for another 2 hours, centrifuged at 2,000 g for 15 minutes at 4°C, and 300 μl of the supernatent was removed and transferred to scintillation vials together with 3 ml of optiphase scintillation fluid (LKB-Pharmacia, Milton Keynes, Bucks, UK). Samples were "counted" on a beta-counter, and compared to a standard curve constructed with known concentrations of LTB_4.

Extraction of LTB_4 and identification with reverse phase HPLC

Samples were extracted with C18 Sep-pak columns (Waters Associates, MA, USA), then resuspended in HPLC running buffer and separated on a 5 μg Nucleosil C18 column (Macherey-Nagal, Duren, germany) eluted at 1.0 ml/minute with running buffer [methanol:water:acetic acid: orthophosphoric acid (70:29:0.07: 0.03), pH 5.4] for 30 min, followed by 20 minutes gradient to washing buffer [methanol: water:orthophosphoric acid:acetic acid (95:4.9:0.07: 0.03)]. Each HPLC fraction was dried on the Speed-Vac concentrator and reconstituted for assay of LTB_4 immuno-reactivity.

Chemotaxis assay

Chemotaxis was assayed using a modified Boyden Chamber as previously described.[19] A 48 blind well chemotaxis chamber (Nuclepore filtration product Costar Co., Cambridge, MA, USA) and polycarbonate filters (Sartorius membrane, 3 μm pore size) were used in the assay. Neutrophils (purity >95%) were suspended in Hanks balanced salt solution (Gibco Ltd) containing 0.4% ovalbumin (type IV Sigma Chemical Co.). PAF and control buffer (25 μl) were placed in the lower compartment of the chemotaxis chamber, and the neutrophil suspensions (with or without MTX treatment, 25 μl at 5×10^6 cells/ml) were placed in the upper compartment. Following incubation at 37°C for 90 minutes the filters were removed, fixed and stained as previously described.[19] Neutrophils that had migrated through the entire thickness of the filter were counted and the results were expressed as the total number of cells in 10 random high-power fileds (HPF). Samples were assayed blind in triplicate with an intra-assay variation of $< 20\%$. In eosinophil chemotaxis experiments, the filters were washed in normal saline, fixed in saturated mercuric chloride/ethanol (50:50) for 60 minutes, then stained with hematoxylin for 20 minutes and Chromotrope 2R for 30 seconds.

Statistical analysis

The modulatory effect of MTX on PAF-induced eosinophil and neutrophil locomotion and calcium ionophore induced LTB_4 production was compared using two-tailed paired Student's t test. The chemotactic responses of neutrophils between asthmatics with and without MTX treatment were compared using non-paired Student's t test.

RESULTS

The effect of MTX on PHA-stimulated MNC proliferation and DNA synthesis

MNC proliferation was inhibited by MTX in a dose-dependent manner ranging from 2×10^{-4} to 2×10^{-11}M (after 4-day culture) as determined by ^{3}H-TdR uptake (Fig. 1A). There was a transient increase of ^{3}H-TdR uptake after 2 and 3 days of culture. Cell cycle analysis using propidium iodide staining technique showed that the proportion of cells in G_2-M phase in PHA stimulated MNC were $16.97 \pm 1.00\%$, which reduced to $5.45 \pm 0.55\%$ in the presence of MTX and PHA. The reduced proportion of cells in G_2-M phase was comparable with the reduction in ^{3}H-TdR uptake (Table 1).

The effect of MTX on PAF-induced eosinophil and neutrophil chemotactic activity.

The chemotactic response of neutrophils to PAF (10^{-6}M) was inhibited by MTX in a dose-dependent manner ranging from 10^{-3} to 10^{-7}M (Fig. 1B). The dose of MTX causing fifty percent inhibition of neutrophil chemotaxis was 10^{-5}M (Fig. 1B). When the neutrophils were obtained from MTX-treated

Table 1. The effect of MTX on MNC proliferation and DNA synthesis.

	RPMI-1640	MTX	PHA	PHA + MTX
G_0-G_1	90.31 ±1.95%	89.05 ±1.97%	73.54 ±1.48%	88.15 ±1.03%
S	4.62 ±1.28%	5.69 ±1.46%	9.95 ±0.82%	6.67 ±0.70%
G_2-M	5.07 ±0.83%	5.26 ±0.99%	16.97 ±1.00%	*5.45 ±0.55%
^{3}H-TdR uptake (cpm)	498.1 ±121.7	ND	20,156.2 ±2797.6	*8,454.7±1468.1

*$p < 0.01$, in comparison with PHA group (n=16)
ND = not determined

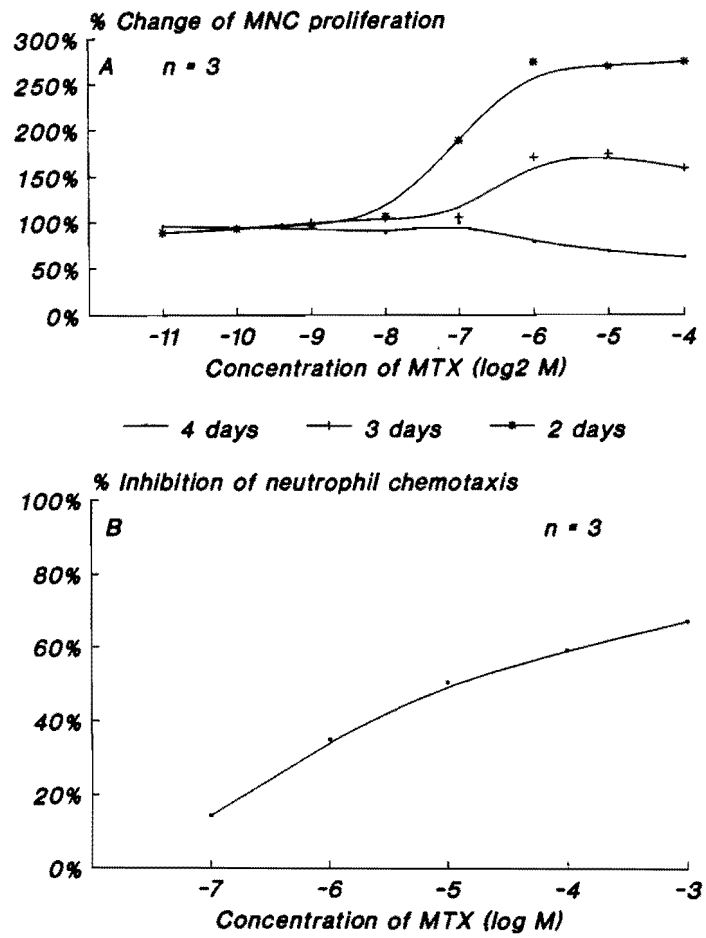

Fig. 1. The dose response of MTX to MNC proliferation and neutrophil chemotaxis. Fig. 1A: MNC cultured with PHA and MTX for varying lengths of time. The MNC prolieration was detected by ^{3}H-TdR uptake. Data represent mean of three experiments. Fig. 1B: Neutrophils pretreated with varying concentrations of MTX then tested for its chemotactic activity in response to PAF. Data represent the mean of three experiments.

patients, the chemotactic response was 481.9 ± 87.3/10 HPF (Fig. 2B). This chemotactic response was significantly reduced when compared with that of MTX-untreated group (727.8 ± 70.2/10 HPF) (Fig. 2A).

Neutrophils obtained from asthmatics and normal subjects could both be inhibited by MTX *in vitro*. The percentage of inhibition was 67.9 ± 4.1% in MTX-untreated group, 56.9 ± 3.8% in MTX-treated group and 59.9 ± 2.3% in normal subjects. The potency of inhibition was comparable with BN 52021 (Fig. 2).

The PAF-induced eosinophil chemotactic response was also reduced markedly by MTX. The inhibitory effect of MTX was comparable to that of the specific PAF antagonist BN 52021 (Fig. 2D).

The effect of MTX on neutrophil LTB_4 production from asthmatics

Both calcium ionophore and PAF induced LTB_4 release from neutrophils. The LTB_4 production from neutrophils reduced with MTX in both asthmatic and normal subjects. However, only the effect of calcium ionophore induced LTB_4 release in asthmatics reached a statistically significant level. The inhibitory effect of MTX on PAF induced LTB_4 release was trivial in both asthmatic patients and normal (Fig. 3).

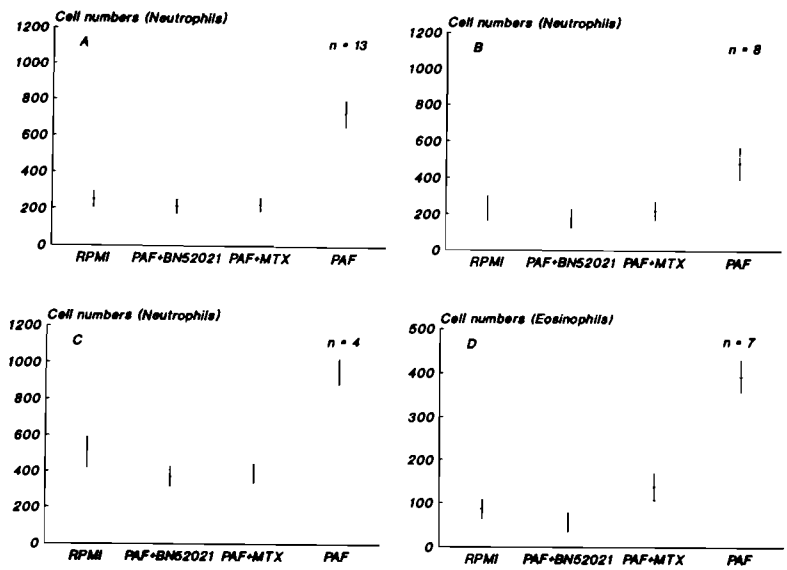

Fig. 2. Effect of MTX on granulocyte locomotion: neutrophils from MTX-untreated asthmatic (A), MTX-treated asthmatics (B), normal subjects (C) and normal density eosinophils from hypereosinophilic subjects (D) were tested for the PAF-induced chemotactic activity. Both MTX and BN 52021 were used to inhibit granulocyte locomotion. Data were expressed as cell numbers/10 HPF. The results represent the mean ± sem.

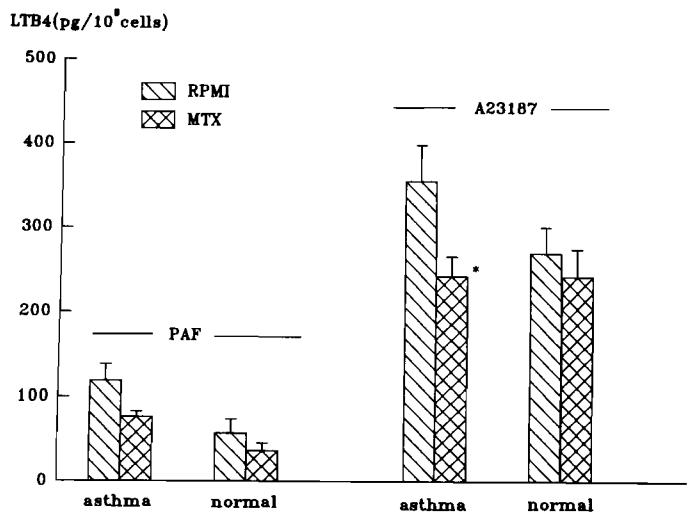

Fig. 3. Effect of MTX on PAF- and calcium ionophore-induced LTB_4 release from neutrophils of asthmatics (n=9) and normal subjects (n=9). MTX was used to reduce LTB_4 release. The results represent mean ± sem of 9 experiments.* $p<0.05$, comparing MTX and RPMI treated A23187-induced LTB_4 release from neutrophils of asthmatics.

The identity of LTB_4 immunoreactivity generated by neutrophils was confirmed by both RP-HPLC and assay LTB_4 immunoreactivity in the fractions. The peak of immunoreactivity was coeluted with synthetic LTB_4 (Fig. 4).

DISCUSSION

This study has demonstrated that MTX could inhibit PAF-induced eosinophil- and neutrophil-chemotaxis *in vitro* and the neutrophil chemotactic response to PAF *in vivo*. MTX also reduced calcium ionophore induced LTB_4 release in asthmatics *in vitro*. In addition to the inhibition of granulocyte function, MTX could also suppress PHA-stimulated MNC proliferation and DNA synthesis.

It has been reported previously that MTX can inhibit neutrophil chemotaxis induced *in vitro* by $LTB_4$and C_{5a}.[20-22] In the present study, MTX also inhibited neutrophil chemotaxis induced by PAF. These results indicated that the inhibitory effect of MTX on neutrophil chemotaxis may not be exerted through a PAF specific receptor on neutrophils. Since PAF-induced neutrophil- and eosinophil-chemotaxis were both inhibited by MTX and granulocyte viability was over 85% after MTX treatment, the inhibitory effect of MTX does not appear to be due to cytotoxicity. More detailed investigation of the subcellular pathway of activation is needed to clarify the precise mechanism of inhibition.

PAF has shown to play an important role in the pathogenesis of bronchial asthma.[23,24]. PAF not only induces eosinophil locomotion,[2] but also enhances bronchial hyperresponsiveness after inhalation in man.[17] We have demonstrated that MTX, as well as the PAF antagonist BN 52021, could inhibit PAF-induced eosinophil chemotaxis, suggesting that MTX might play a therapeutic role in

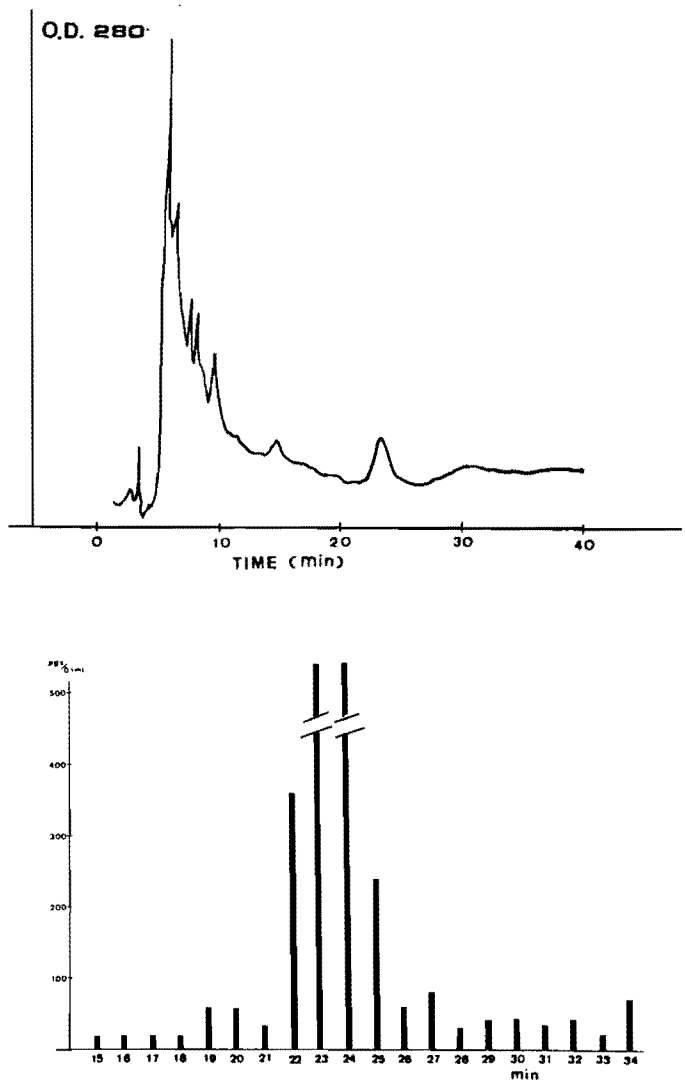

Fig. 4. Confirmation of the identity of LTB_4 released by neutrophils, using a combination of RP-HPLC and RIA. Cell-free supernatants were extracted using C-18 Sep-Pak columns and the concentrated polar lipid mediators were sequentially fractionated on RP-HPLC. Each fraction was assayed for immunoreactive LTB_4. Synthetic LTB_4 eluted in the 23 and 24 minutes fractions.

PAF induced eosinophil accumulation in asthma. Eosinophils are heterogeneous, consisting of both normal and light density cells which may differ in functional properties. Several experiments using normal- and light-density eosinophils from the same donors have shown that locomotion is greater in light-density than in normal-density eosinophils (data not shown). The eosinophils used in our study were obtained from patients with a considerable eosinophilia and were predominantly of light density. However, only highly purified eosinophils of normal density were used for the chemotaxis assay to avoid preactivation of eosinophils.

LTB_4 is a potent neutrophil activator, which is generated by neutrophils after calcium ionophore stimulation.[25] The reduction by MTX of LTB_4 release from neutrophils has been suggested as the principal mechanism of the therapeutic effect of MTX in rheumatoid arthritis.[26] Our results showed that MTX could reduce the ability of neutrophils release LTB_4 in asthmatics, when stimulated with calcium ionophore. These results indicated that the therapeutic efficacy of MTX in bronchial asthma may be through the reduction of LTB_4 release from neutrophils.

It has been reported that MTX may increase cellular avidity for uptake of thymidine. It may therefore be preferable to use alternative markers of DNA synthesis, such as propidium iodide.[27] We also observed an increase in PHA-induced thymidine uptake after 2 and 3 days of culture, however, thymidine uptake decreased after 4 days of culture even though MTX had been removed after 24 hours. Cell viabilities were all above 85% at the end of 4 days culture. The decrease in DNA synthesis was confirmed by FACS using the propidium iodide DNA staining technique. These results suggest that MTX also has profound effect on mononuclear cell proliferation.

Given the increasing recognition of the long-term severe morbidity and mortality of steroid-dependent bronchial asthma, our results showing reduced neutrophil locomotion after MTX therapy might provide a good rationale for that therapy. The results of the *in vitro* study further indicate that MTX is not only an antimetabolic agent, but also a potent antiinflammatory agent. The potent anti-inflammatory effect of MTX and its clinical benefit in asthma lend support to the concept of the inflammatory basis of asthma.

ACKNOWLEDGEMENTS

The authers wish to thank Dr. AJ Frew for critically reading the

manuscript. This study was supported by Grant NSC 81-04-1-B075 513 from National Science Council, DOH83-HR-211 from Department of Health and partially from Clinical Research Center VGH, Institute of Biomedical Science, Academia Sinica, ROC.

REFERENCES

1. De Monchy JGR, Kauffman HF, Venge P, *et al.* Bronchoalveolar eosinophilia during allergen-induced late asthmatic reactions. Am Rev Respir Dis 1985; 131 : 373-6.
2. Wardlaw AJ, Moqbel R, Cromwell O, Kay AB. Platelet-activating factor a potent chemotactic and chemokinetic factor for human eosinophils. J Clin Inest 1986; 78 : 1701-6.
3. Gleich GJ, Flavahan NA, Fujisawa T, Vanhoutte PM. The eosinophil as a mediator of damage to respiratory epithelium: A model for bronchial hyperreactivity. J Allergy Clin Immunol 1988; 81 : 776-81.
4. Ryan G, Latimer KM, Juniper EF, Roberts RS, Hargreave FE. Effect of beclomethasome dipropionate on bronchial responsiveness to histamine in controlled nonsteroid-dependent asthma. J Allergy Clin Immunol 1985; 75 : 25-30.
5. Barnes PJ. A new approach to the treatment of asthma: drug therapy. N Engl J Med 1989; 321 : 1517-27.
6. Cott GR, Cherniack RM. Steroids and "steroid-sparing" agents in asthma (editorial). N Engl J Med 1988; 318 : 634-6.
7. Alexander AG, Barnes NC, Kay AB. Trial of cyclosporin in corticosteroid-dependent chronic severe asthma. Lancet 1992; 339 : 324-8.
8. Chabner BA, ed: Pharmacologic principles of cancer treatment philadelphia : WB Saunders 1982; 229-55.
9. Weinstein GD. Methotrexate. Ann Intern Med 1977; 86 : 199-204.
10. Black RL, O'Brien WM, VanScott EJ, *et al.* Methotrexate therapy in psoriatic arthritis. JAMA 1964; 189 : 743-7.
11. Clements PJ, Davis J. Cytotoxic drug: their clinical application to the rheumatic diseases. Semin Arthritis Rheum 1986; 14 : 231-54.
12. Williams HJ, Willkens RF, Samuelson Co, *et al.* Comparison of low-dose oral pulse methotrexate and placebo in the treatment of rheumatoid arthritis. Arthritis Rheum 1985; 28 : 721-30.
13. Mullarkey MF. Webb DR, Pardee NE. Methotrexate in the treatment of steroid-dependent asthma. Ann Allergy 1986; 56 : 347-50.
14. Mullarkey MF, Blumenstein BA, Andrade WP, Bailey GA, Olason I, Wetzel CE. methotrexate in the treatment of corticosteroid-dependent asthma. A double-blind crossover study. N Engl J Med 1988; 318 : 603-7.
15. Erzunum SC, Leff JA, Ackerson LM. Lack of benefit of methotrexate in severe, steroid-dependent asthma. Ann Int Med 1991; 114 : 353-60.
16. Cuss FM, Dixon CM, Barnes PJ. Effects of inhale platelet activating factor on pulmonary function and bronchial responsiveness in man. Lancet 1986; II : 189-92.
17. Kimura I, Moritani Y, Tanisaki Y. Basophils in bronchial asthma with reference to reagin-type allergy. Clin Allergy 1973; 3 : 195-202.
18. Vadas MA, Nicola NA, Metcalf D. Activation on antibody-dependent cell-mediated cytotoxicity of human neutrophils and eosinophils by separate colony-stimulating factors, J Immunol 1983; 130 : 795-9.
19. Maestrelli P, Tsai JJ, Cromwell O, Kay AB. The identification and partial characterization of a human mononuclear cell-derived neutrophil chemotactic factor apparently distinct from IL-1, IL-2, GM-CSF, TNF, and IFN-gamma. Immunology 1988; 64 : 219-25.
20. Van DE Kerkhof PCM, Bauer FW, Maassen-DE Grood RM. Methotrexate inhibits the leukotriene B_4 induced intraepidermal accumulation of polymorphonuclear leukocytes. Br J Dermatol 1985; 113 : 251a-5a.
21. Suarez CR, Pickett WC, Bell DH, *et al.* Effect of low dose methotrexate on neutrophil chemotaxis induced by leukotriene B_4 and complement C5a. J Rheumatol 1987; 14 : 9-11.
22. Johnson JD, Summersgill JT, Raff MJ. The *in vitro* effects of methotrexate on the phagocytosis and intracellular killing of *Staphylococcus aureus* by human neutrophils. Cancer 1986; 57 : 2343-5.
23. Barnes PJ, Chung KF, Page CP. Inflammatory mediators and asthma. Pharmacol Rev 1988; 40 : 49-84.
24. Barnes PJ, Chung KF. PAF closely mimics pathology of asthma. Trends Pharmacol Sci 1987; 8 : 285-97.
25. Borgeat P, Samuelsson B. Arachidonic acid metabolism in polymorphonuclear leukocytes: effects of ionophore A 23187. Proc Natl Acad Sci USA 1979; 76 : 2148-52.
26. Sperling RI, Coblyn JS, Larkin JK, Benincaso AI, Austen KF, Weinblatt ME. Inhibition of leukotriene B_4 synthesis in neutrophils from patients with rheumatoid arthritis by a single oral dose of methotrexate. Arthritis Rheum 1990; 33 : 1149-55.
27. Olsen NJ, Callabhan LF, Pincus T. Immunologic studies of rheumatoid arthritis patients treated with methotrexate. Arthritis Rheum 1987; 30 : 481-8.

12TH ASIA PACIFIC CANCER CONFERENCE
Towards Total Cancer Control

DID YOU KNOWN...
...that the next Asia Pacific Cancer Conference will be held in sunny Singapore in 1995 ?

AND DID YOU KNOW...
...that this will be an exciting international gathering of practitioners, scientists, nurses as well as para-medical people ?

...that we have organised an interesting Scientific Programme that will cater to your interests, and of course, a list of internationally renowned seakers.

IF...
...you are in the midst of an interesting research which you cannot wait to share with your counterparts, make it a point to be in Singapore to present your findings at the Scientific forum.

OR...
...if you just want to know more about the latest and current thinking on cancer control and treatment, come to Singapore to attend the Conference.

MORE INFORMATION NOW THAT YOU ARE INTERESTED ?
Simple. Just write or fax to the Singapore Cancer Society at :

15 Enggor Street
Realty Centre #04-01 to 04
Singapore 0207
Tel: (65) 221-9577
Fax: (65) 222-7424

and we will send you the registration form and a brochure on this meeting.

REMEMBER THE DATES ...

17 to 20 October 1995 in Singapore, the little fast-moving Asian city that has all the comforts of modern living and yet Asian enough to provide the thrills of many cultures existing side by side.

SEE YOU THERE !