

The immunomodulatory drugs cyclosporin A, mycophenolate mofetil, and sirolimus (rapamycin) inhibit allergen-induced proliferation and IL-5 production by PBMCs from atopic asthmatic patients

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We have used an optimized, physiologically relevant in vitro assay system to show that in a concentration-dependent fashion the immunomodulatory drugs cyclosporin A, mycophenolate mofetil, and sirolimus (rapamycin), as well as the glucocorticoid dexamethasone, inhibit allergen-driven T-cell proliferation and IL-5 production in PBMCs from allergen-sensitized atopic asthmatic individuals at physiologic concentrations. This effect of cyclosporin A might at least partially account for its established clinical efficacy in sparing systemic glucocorticoid therapy while improving lung function in chronic, severe, glucocorticoid-dependent asthma. The data are also compatible with the hypothesis that the newer immunomodulatory drugs mycophenolate mofetil and sirolimus exert similar effects, perhaps with a more favorable benefit/risk ratio. (*J Allergy Clin Immunol* 2001;108:915-7.)

Key words: Asthma, allergen, T cell, interleukin-5, dexamethasone, cyclosporin A, sirolimus, mycophenolate mofetil, therapy

The eosinophil-active cytokine IL-5 is considered to play a key role in the regulation of asthma severity. IL-5 mRNA and protein, principally of T-cell origin, are produced excessively in the asthmatic airway to a degree that correlates consistently with impairment of pulmonary function.^{1,2} At least some of this IL-5 production is probably allergen-driven, because allergen-stimulated T cells isolated directly from the airways of individuals with atopic asthma secrete elevated quantities of IL-5 in comparison with controls in vitro,³ a property that we have

Abbreviation used

IC₅₀: Inhibitory concentration producing 50% of control response

demonstrated to be reflected in PBMCs using an optimized system.⁴ In this same system, we have also demonstrated that IL-5 gene expression is suppressed by topical antiasthma glucocorticoids,⁵ which is consistent with the widely held view that glucocorticoid suppression of T-cell activation and cytokine production is a key feature of the activity of these drugs.

Some asthmatic patients are relatively refractory to glucocorticoid therapy and suffer debilitating symptoms despite treatment with systemic glucocorticoids. For these patients, alternative T-cell immunomodulators have been advocated as glucocorticoid-sparing therapy. Some, such as cyclosporin A, have been subjected to clinical trials in asthma whereas other, newer drugs have not.

To clarify the rationale for the established use of cyclosporin A and the possible use of the newer T-cell immunomodulatory drugs mycophenolate mofetil and sirolimus in glucocorticoid-refractory asthma, we examined the inhibitory effects of these drugs on allergen-induced proliferation and IL-5 production by PBMCs from atopic asthmatic subjects, comparing these effects with those of the glucocorticoid dexamethasone, used as a positive inhibitory control.

SUBJECTS, MATERIALS, AND METHODS

With Ethics Committee approval and written, informed consent, we obtained 100 mL of peripheral venous blood from 6 atopic, glucocorticoid-naïve asthmatic patients sensitized to the allergen in the house dust mite *Dermatophagoides pteronyssinus*. Asthma was defined as a clear history of relevant symptoms, reversible airflow limitation ($\geq 15\%$ reversibility in FEV₁), and bronchial hyperreactivity (histamine provocative concentration causing a 20% fall in baseline FEV₁ ≤ 8 mg/mL). Sensitization to the *D pteronyssinus* allergen was defined by a history of allergen sensitivity together with a positive skin prick test result or a RAST (CAP system, Pharmacia, Uppsala, Sweden) score of more than 0.7 IU/mL to the *D pteronyssinus* allergen.

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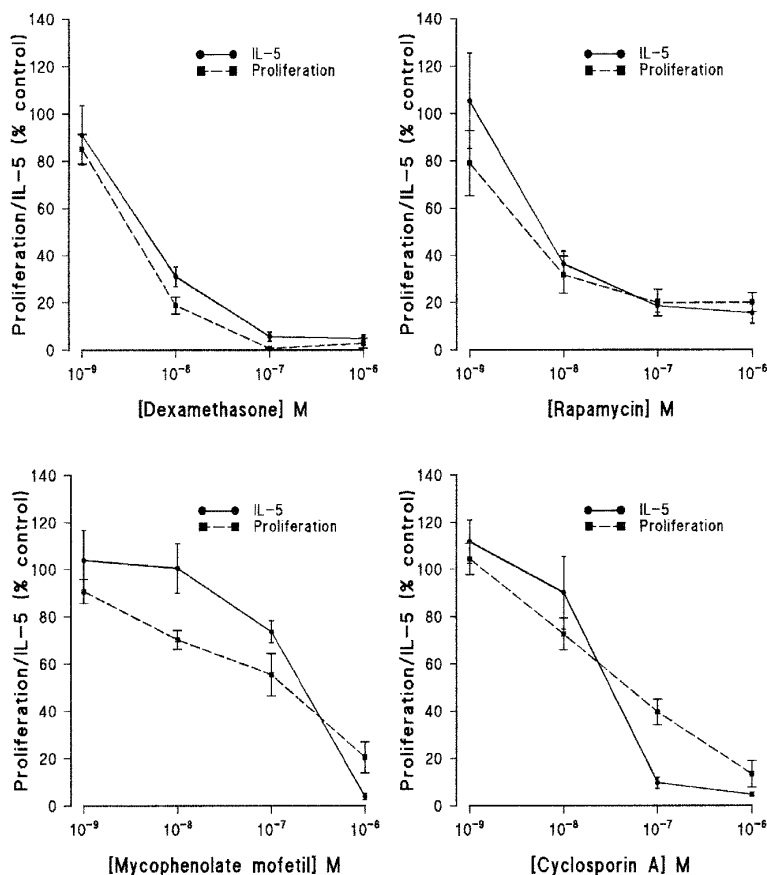


FIG 1. Concentration-response curves for inhibition of allergen-induced T-cell proliferation and IL-5 production by dexamethasone, sirolimus, mycophenolate mofetil, and cyclosporin A. Data are expressed as the mean $\pm$ SEM ($n = 6$) percentages of the thymidine uptakes and supernatant IL-5 concentrations in the vehicle control (no drug) wells, which were $25,530 \pm 2976$ counts/min above background and 360.3 ± 36.3 pg/mL, respectively. $P < .01$ for all curves by 1-way parametric ANOVA.

Cell isolation and culture

PBMCs were isolated from heparinized peripheral venous blood, as described previously,⁴ and then resuspended in RPMI-1640 medium supplemented with 2.0 mmol/L L-glutamine, 5% v/v human AB serum, and antibiotics (all from Sigma, Poole, United Kingdom), subsequently referred to as "complete medium."

Stock solutions of dexamethasone, cyclosporin A, mycophenolate mofetil, and sirolimus obtained from commercial sources were prepared by dissolving the drugs in 100% ethanol at 10^{-2} mol/L and filtering sterile. Serial 10-fold dilutions of these stocks were made in complete medium before each experiment. The cell culture conditions were as previously optimized.⁴ To measure IL-5 production, PBMCs were cultured (5×10^6 cells/mL) in the presence of serial dilutions of drug and 2.5 μ g/mL *D pteronyssinus* allergen extract ("Aquagen," kindly provided by ALK, Horsholm, Denmark) in 200- μ L total volumes in round-bottomed 96-well plates in triplicate. Positive (allergen and diluent vehicle) and negative (vehicle only) control wells were also used. After 6 days of culture (at 37°C in a humidified 5% CO₂ atmosphere), supernatants were harvested from the wells, pooled, and stored at -20°C pending measurement of cytokine concentrations.

To measure T-cell proliferation, the PBMCs were cultured in triplicate at 0.5×10^6 cells/mL in the presence of 25 μ g/mL of the *D pteronyssinus* allergen. Proliferation was measured on day 7 by adding 0.5 μ Ci tritiated thymidine (Amersham, Little Chalfont, United King-

dom) for the final 16 hours of culture and assessing label incorporation by means of liquid scintillation spectroscopy. After subtraction of background counts (negative control vehicle only), results were expressed as the geometric means of triplicate counts per minute.

Cytokine measurements

IL-5 concentrations were measured in cell culture supernatants through use of a commercial ELISA (R&D Systems, Abingdon, United Kingdom) according to the manufacturer's instructions. All samples were analyzed in triplicate in a single assay. Cytokine concentrations below the level of sensitivity of the assay were regarded as zero.

RESULTS

Results are summarized in Fig 1. Like the positive control inhibitor dexamethasone, sirolimus, cyclosporin A, and mycophenolate mofetil all inhibited allergen-induced IL-5 production in a concentration-dependent fashion (inhibitory concentration producing 50% of control response [IC₅₀]: 8.0×10^{-9} mol/L, 1.9×10^{-8} mol/L, 4×10^{-8} mol/L, and 1.3×10^{-7} mol/L, respectively).

Furthermore, like dexamethasone, sirolimus, cyclosporin A, and mycophenolate mofetil inhibited allergen-induced T-cell proliferation in a concentration-dependent fashion

(IC₅₀: 4.2×10^{-9} mol/L, 7.0×10^{-9} mol/L, 5.5×10^{-8} mol/L, and 1.1×10^{-7} mol/L, respectively).

Unlike cyclosporin A, sirolimus, and dexamethasone, mycophenolate mofetil was relatively more potent at inhibiting T-cell proliferation than at inhibiting IL-5 production.

DISCUSSION

We have demonstrated that cyclosporin A, sirolimus, and mycophenolate mofetil suppress IL-5 production and T-cell proliferation in an optimized in vitro assay system driven by specific allergen relevant to the clinical sensitivity of atopic asthmatic donors, in which IL-5 production correlates with the severity of the donors' asthma.⁴

Our findings on the inhibitory effects of dexamethasone and cyclosporin A on allergen-driven PBMC activation in this study extend previous reports that these drugs inhibit IL-5 gene expression in mitogen-activated T cells,⁶ and the data are consistent with our previous observations of IL-5 suppression by topical antiasthma glucocorticoids.⁵ The effects of cyclosporin A might partially account for its clinical efficacy in sparing oral glucocorticoids while improving lung function in severe, glucocorticoid-dependent asthma⁷ and in attenuating allergen-induced, late-phase bronchoconstriction and associated upregulation of IL-5 mRNA expression in the bronchial mucosa of individuals with atopic asthma.⁸

The known activities of sirolimus and mycophenolate mofetil differ fundamentally from those of cyclosporin A. Both drugs inhibit T-cell mitosis—sirolimus through inhibition of phosphorylation/activation of the kinase p70s6 and the enzymatic activity of the cyclin-dependent kinase cdk2-cyclin E complex, and mycophenolate mofetil through inhibition of the enzyme inosine monophosphate dehydrogenase, critical for de novo nucleic acid synthesis in T cells. The question of whether these actions are relevant to reduction of allergen-induced IL-5 synthesis must be addressed in future studies. Clearly, reduced IL-5 production by T cells need not always be simply a consequence of reduced cellular proliferation, as evidenced preliminarily by our data with mycophenolate mofetil and by other findings of our group⁵ and others.

Our data nevertheless suggest that sirolimus and mycophenolate mofetil might prove efficacious, at least in theory, for the treatment of severe asthma in individuals refractory or resistant to glucocorticoid therapy. We have previously shown that whereas T cells from these

patients are resistant to inhibition by concentrations of glucocorticoids likely to be achieved in a therapeutic setting, they remain sensitive to inhibition by cyclosporin A, mycophenolate mofetil, and sirolimus at therapeutic concentrations.^{9,10} Mycophenolate mofetil and sirolimus are presently in routine clinical use, principally for prevention of allograft rejection, and early data suggest that they might have fewer serious unwanted effects. Further clinical experience with these drugs might form a framework for investigation of their possible therapeutic role in chronic severe asthma, inasmuch as cyclosporin A and other immunomodulatory drugs investigated in this context, such as gold salts and methotrexate, are associated with rather poor benefit/risk ratios in clinical practice.

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