Differential regulation of cytokine expression after allergen exposure of sensitized rats by cyclosporin A and corticosteroids: Relationship to bronchial hyperresponsiveness

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Background: Cyclosporin A and dexamethasone exhibit different effects on allergic airway eosinophilia and bronchial hyperresponsiveness (BHR).
Objective: We determined whether these were related to alteration of cytokine expression, particularly with regard to the profile of Th1- versus Th2-derived cytokines.
Methods: Brown-Norway rats sensitized with ovalbumin were administered cyclosporine or dexamethasone before ovalbumin aerosol challenge. Bronchial responsiveness was measured 18 to 24 hours after aerosol exposure. Airway cellular influx was determined by bronchoalveolar lavage and tissue immunohistochemistry. The expression of Th1 and Th2 cytokine messenger RNA (mRNA) was analyzed by reverse transcriptase–PCR.
Results: Ovalbumin exposure induced significant BHR, with increases in eosinophils, lymphocytes, and neutrophils in bronchoalveolar lavage fluid and an increase in eosinophils, $CD2^+$ and $CD8^+$, but not $CD4^+$ T cells, in the airway submucosa. IL-2, IFN-γ, IL-4, and IL-5 mRNA expression in the lungs of sensitized ovalbumin-exposed rats was increased ($P < .05$) compared with controls. Cyclosporin A had no significant effect on BHR and neutrophil accumulation but reduced the number of bronchoalveolar lavage eosinophils ($P < .002$), airway submucosal eosinophils, and $CD4^+$ and $CD8^+$ T cells ($P < .02$). It also suppressed the induced mRNA expression of IL-2, IL-4, IL-5, and IFN-γ. By contrast, the inflammatory cell influx and mRNA expression for IL-2, IL-4, and IL-5, as well as BHR, were suppressed by dexamethasone. However, an increase in IL-10 and IFN-γ mRNA expression was found.
Conclusion: The differential activities of cyclosporin A and dexamethasone on inflammatory cell influx, particularly neutrophils, or cytokine expression such as IL-10 and IFN-γ may underlie their contrasting effects on BHR. (J Allergy Clin Immunol 1999;104:644-52.)

Key words: *Cyclosporin A, dexamethasone, bronchial hyperresponsiveness, IL-4, IL-5, IL-10, IFN-γ, neutrophil*

Abbreviations used

ACh:	Acetylcholine
$Al(OH)_3$:	Aluminum hydroxide
BHR:	Airway hyperresponsiveness
BAL:	Bronchoalveolar lavage
cDNA:	Complementary DNA
CsA:	Cyclosporin A
GAPDH:	Glyceraldehyde-3-phosphate dehydrogenase
IP:	Intraperitoneally
mRNA:	Messenger ribonucleic acid
OA:	Ovalbumin
PC_{200}:	Provocative concentration of acetylcholine needed to increase baseline lung resistance by 200%
R_L:	Lung resistance
RT-PCR:	Reverse transcriptase–PCR

Bronchial asthma is characterized by increased airway eosinophil and T-cell infiltration and bronchial hyperresponsiveness (BHR).[1,2] Recent studies have indicated that there is an increased expression of the Th2-derived cytokines, particularly IL-4 and IL-5, in the airways of patients with asthma and that this is mainly expressed in $CD4^+$ T cells.[3,4] By contrast, the expression of the Th1-derived cytokine IFN-γ was not increased,[3,4] although increased release of IFN-γ has been reported, particularly after allergen challenge.[5,6] IL-4 and IL-5 are important in isotype switching of B cells to IgE production and in increasing terminal differentiation of eosinophil precursors, respectively,[7,8] and may therefore be involved in asthma. One strategy for the treatment of asthma is to inhibit the effects of these cytokines either by preventing their synthesis and release or by neutralizing their effects. Thus preventing IL-5 effects with an anti-IL-5 antibody significantly inhibited allergen-induced BHR and airway eosinophilia,[9] whereas inhibition of the Th1 cytokine IFN-γ increases airway eosinophil infiltration.[10] Thus it is apparent that targeting the right set of cytokines may be important for effective therapy of asthma.

Cyclosporin A (CsA) is a potent immunosuppressant widely used to control rejection of allograft transplants. It suppresses calcineurin, which is important in the signal transduction pathways necessary for the expression of a

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range of cytokines, including IL-2, IL-3, IL-4, IL-5, IFN-γ, TNF-α, and GM-CSF, and thereby inhibits T-lymphocyte activation and proliferation.[11-14] Because of its capacity to inhibit the Th2 cytokines, IL-4 and IL-5, CsA has been tried in asthma, and in view of its potential side effects, it has been used to treat patients with severe asthma needing oral corticosteroid therapy to control their disease and has been shown to possess corticosteroid-sparing properties.[15,16] Although CsA has effects in inhibiting IL-4 and IL-5, it also inhibits the expression of other cytokines such as IL-2 and IFN-γ.[11-14] In animal models of IgE-sensitization followed by allergen challenge, CsA administered after primary immunization does not suppress allergen-induced BHR, despite preventing the development of lung eosinophilia,[17] whereas CsA given during the sensitization period significantly suppresses airway eosinophilia and $CD4^+$ and $CD8^+$ T-cell infiltration and also BHR in guinea pigs.[18-20]

We have re-examined the effect of CsA, and of the steroid dexamethasone, on allergen-induced inflammatory process in the airway mucosa and determined particularly its effect on T-lymphocyte subsets and Th1- and Th2-derived cytokines in the Brown-Norway sensitized rat model. We hypothesized that the contrasting effects of CsA and dexamethasone on BHR may be the result of differential effects on the expression of these cytokines.

METHODS

Animal sensitization procedures and allergen exposure

Pathogen-free inbred male Brown-Norway rats (200-250 g, 9-13 weeks old; Harlan Olac, Bicester, UK) were injected with 1 mL of 1 mg ovalbumin (OA) (grade V, salt free, Sigma, Dorset, UK) in a 100 mg aluminum hydroxide ($Al[OH]_3$) suspension in 0.9% (wt/vol) sodium chloride solution intraperitoneally (IP) on 3 consecutive days. Rats were exposed to OA aerosol (1% wt/vol, 15 minutes) with use of a 6.5-L Plexiglas acrylic plastic (Rohm and Haas, Philadelphia, Pa) chamber connected to an ultrasonic nebulizer (model No. 2512, DeVilbiss Health Care, UK, Middlesex, UK).

The study was performed in 2 parts. In the first part (CsA study) 4 groups were studied.

(1) Nonsensitized and OA-exposed animals (group NO, n = 9): Animals injected with sodium chloride solution (1 mL/kg) during the first 3 days were kept in conditions identical to those of the sensitized animals for 3 weeks before exposure to 1% OA aerosol and were studied 18 to 24 hours after the exposure.

(2) Sensitized and sodium chloride solution–exposed animals (group SS, n = 7): Animals injected with 1 mL of 1 mg OA/100 mg $Al(OH)_3$ in 0.9% sodium chloride suspension for 3 consecutive days were exposed to sodium chloride aerosol 3 weeks later and then studied 18 to 24 hours thereafter.

(3) Sensitized CsA vehicle-treated and OA-exposed animals (group SOO, n = 9): Animals injected with 1 mL of 1 mg OA/100 mg $Al(OH)_3$ in 0.9% sodium chloride suspension for 3 consecutive days received, 17 days after the final injection, 1 mL of olive oil (the vehicle for CsA) by daily oral gavage for 5 days. Then on the fifth day, 6 to 8 hours after last dose of olive oil, the rats were exposed to 1% OA aerosol for 15 minutes, followed with studies 18 to 24 hours later.

(4) Sensitized CsA-treated and OA-exposed animals (group SOC, n = 10): The procedures were the same as for group SOO, except the 5 days of oral gavage was with 50 mg/kg CsA (Sandimmun, Sandoz, Basel, Switzerland) in olive oil.

In the second part (dexamethasone study), another 3 groups were studied.

(1) Sensitized and sodium chloride solution–exposed animals (group SS, n = 7): The protocol was the same as above for the CsA study.

(2) Sensitized vehicle-treated and OA-exposed animals (group SO, n = 7): The protocol was the same as for group SOO for the CsA study above except that the treatment was vehicle for dexamethasone (sodium chloride solution 0.5 mL IP, 3 days).

(3) Sensitized dexamethasone-treated and OA-exposed animals (group SOD, n = 7): The procedures were the same as for group SO, except the treatment was 3 days IP injection with 0.5 mg/kg/day dexamethasone in sodium chloride solution.

Measurement of airway responsiveness to acetylcholine

Airway responsiveness was measured as previously described.[21] In brief, anesthetized, tracheostomized, and ventilated rats were monitored for airflow with a pneumotachograph (model F1L, Mercury Electronics, Glasgow, UK) connected to a transducer (model FCO40, 20 mm H_2O, Furness Controls, Sussex, UK) and for transpulmonary pressure with a transducer (model FCO40, 1000 mm H_2O, Furness Controls). Lung resistance (R_L) was calculated with software (LabView, National Instruments, Austin, Tex) on a Macintosh II computer (Apple Computer). Aerosol generated from increasing half $\log_{10}$ concentrations of acetylcholine chloride (ACh) (Sigma) was administered by inhalation (45 breaths). The concentration of ACh needed to increase the R_L 200% above baseline (PC_{200}) was calculated by interpolation of the log concentration–lung resistance curve.

Bronchoalveolar lavage and cell counting

Bronchoalveolar lavage and cell counting are also described in detail elsewhere.[22] Briefly, after rats were given an overdose of anesthetic, lavage was performed with 20 mL of 0.9% sterile sodium chloride solution through the endotracheal tube. Total cell counts, viability, and differential cell counts from cytospin preparations stained by May-Grunwald stain were determined.

Collection of lung tissues

After the thoracic cavity was opened and the lungs were removed, the right lung was cut into pieces and snap-frozen in liquid nitrogen (BOC, Luton, UK) and then stored at –80°C. The left lung was inflated with 3 mL of sodium chloride solution–ornithine carbamoyltransferase (1:1). Two blocks of half cubic centimeters were cut from the left lung around the major bronchus, embedded in ornithine carbamoyltransferase medium (Raymond A. Lamb, London, UK), and snap-frozen in melting isopentane (BDH, Dorset, UK) and liquid nitrogen. Cryostat sections (6 μm) of the tissues were cut, air-dried, fixed in acetone, and then air-dried again, wrapped in aluminum foil, and stored at –80°C.

Immunohistochemistry

For detection of eosinophils, sections were incubated with a mouse IgG1 mAb against human major basic protein, BMK-13[23] (1:50 dilution, 30 minutes at room temperature). After labeling with the second antibody, rabbit antimouse IgG, positively stained cells were visualized with the alkaline phosphatase–anti-alkaline phosphatase method. For staining of $CD2^+$, $CD4^+$, and $CD8^+$ T lymphocytes, sections were incubated with mouse antirat CD2 (pan T-cell marker), antirat CD4, and anti-CD8 antibodies (Pharmingen, Cambridge Bioscience, Cambridge, UK) at a dilution of 1:500 for 1 hour. Biotin goat antimouse antibody (Pharmingen) and avidin

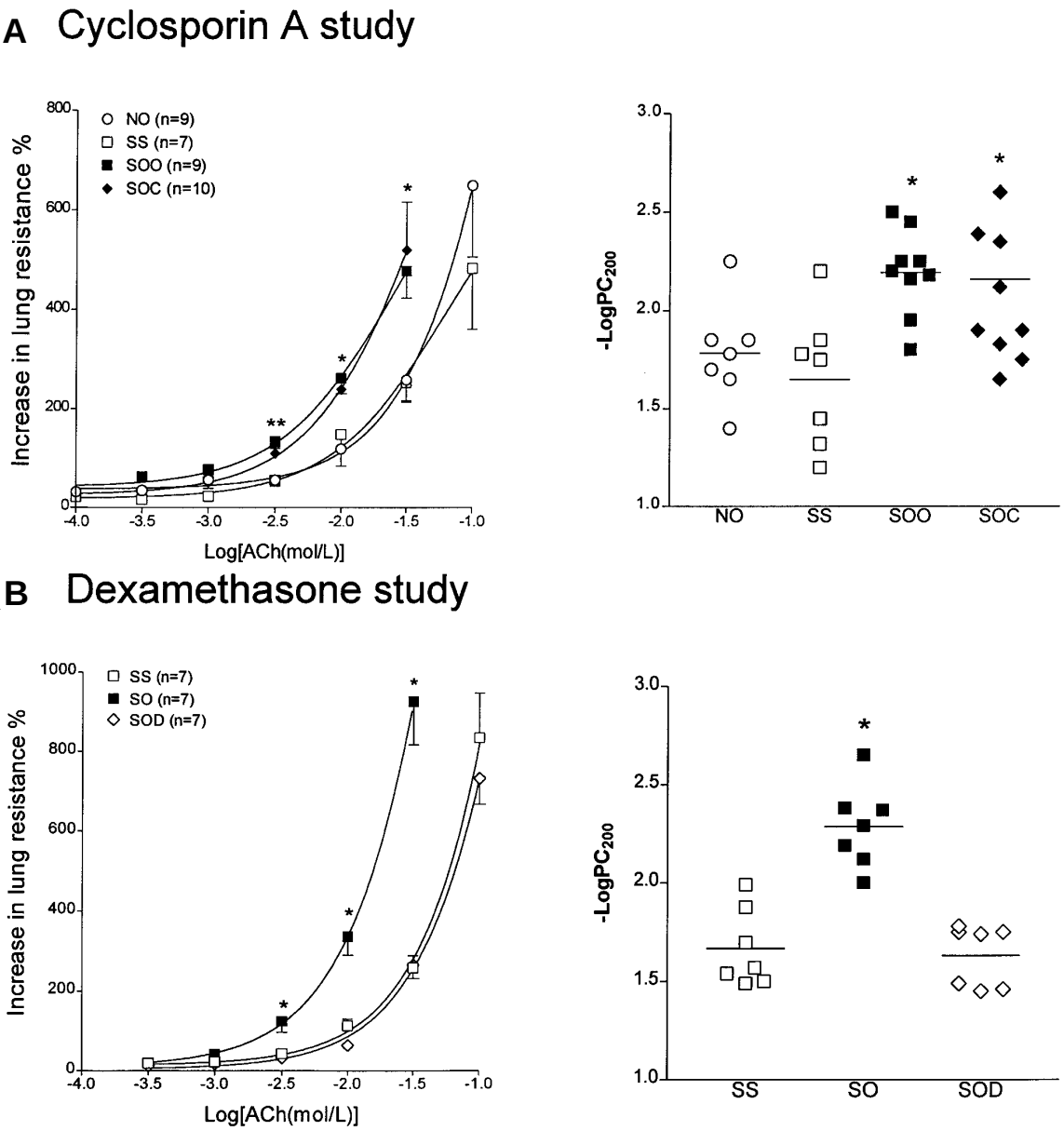

FIG 1. Bronchial responsiveness in CsA study **(A)** and dexamethasone study **(B).** *Left,* Mean percent increase in R_L to increasing concentrations of ACh for different groups of rats. **A,** CsA study: *NO,* nonsensitized and OA exposed, n = 9, *open circles; SS,* OA sensitized and sodium chloride solution exposed, n = 7, *open squares; SOO,* sensitized, olive oil treated, and 1% OA exposed, n = 9, *solid squares;* and *SOC,* sensitized, CsA treated and OA exposed, n = 10, *solid diamonds.* **B,** Dexamethasone study: *SS,* OA sensitized and sodium chloride solution exposed, n = 7, *open squares; SO,* sensitized, sodium chloride solution treated, and 1% OA exposed, n = 7, *solid squares;* and *SOD,* sensitized, dexamethasone treated, and OA exposed, n = 7, *open diamonds.* Concentration-response curves are significantly shifted leftward for groups SOO, SOC, and SO. *Right,* Mean $-\log PC_{200}$, which is negative logarithm of provocative concentration of ACh needed to increase baseline lung resistance by 200%, for groups of rats as detailed above. Dexamethasone, but not CsA, inhibited allergen-induced increase in $-\log PC_{200}$. *Asterisk, P* < .05 as groups SOO and SOC compared with SS in **A** or SO compared with SS and SOD in **B;** *two asterisks, P* < .001 as SOO compared with NO and SS. Data shown as mean ± SEM.

phosphatase (DAKO, High Wycome, UK) at a dilution of 1:200 were applied for 30 minutes in turn. Alkaline phosphatase was developed as a red stain after incubation with Naphthol AS-MX phosphate in 0.1 mol/L TRIS–hydrochloric acid buffer (pH 8.2) containing levamisole to inhibit endogenous alkaline phosphatase and 1 mg/mL Fast Red-TR salt (Sigma). Then sections were counterstained with Harris hematoxylin (BDH) and mounted in Glycergel (DAKO). System and specificity controls were carried out for

all staining. Cells within 175 μm beneath the basement membrane were counted in a coded, randomized, blind fashion. The submucosal area was quantified with the aid of a computer-assisted graphic tablet.

Reverse transcriptase–PCR and Southern blotting

Reverse transcriptase–PCR (RT-PCR) was performed as previously described.[24] Total RNA from lung tissue was extracted[24] and denatured at 70°C for 5 minutes. Total RNA (1 μg) was used for reverse transcription in a 20-μL reaction volume containing 1× AMV buffer, 4 deoxynucleotide triphosphate (dNTP, 1 mmol/L), including deoxy-ATP, deoxycytidine triphosphate, deoxyguanosine triphosphate, and thymidine 5´-triphosphate, ribonuclease inhibitor 32 U, 0.2 μg of random primer pd(N)6 sodium salt (Pharmacia, Milton Keynes, UK), and 8 U AMV reverse transcriptase (all apart from the random primer from Promega, Southampton, UK) at 42°C for 60 minutes. Complementary DNA (cDNA) product was diluted to 100 μL in water. PCR was performed on 5 μL of diluted cDNA product in a total volume of 25 μL with a final concentration of 1× potassium chloride or ammonium hydroxide buffer with 1.5 mmol/L magnesium chloride, 0.2 mmol/L deoxynucleotide triphosphate, 0.2 μg each of sense and antisense primers, and 1 U of Taq polymerase (Bioline, London, UK) with use of a Hybaid Omnigene thermal cycler (Hybaid, Teddington, UK). The sense and antisense primers used have been previously described.[24] Amplification was carried out with use of a multiwell thermal cycler through 20 to 40 cycles of denaturation at 94°C for 30 seconds, annealing at individual temperatures for 30 seconds, and extension at 72°C for 30 seconds, followed by final extension at 72°C for 10 minutes. Annealing temperatures were 62°C for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), IL-4, and IFN-γ; 58°C for IL-5; and 65°C for IL-2 and IL-10. Serial sampling every 2 cycles through 20 to 40 cycles was used to determine the exponential phase of the product amplification curve. The cycle numbers we used for PCR were 26 for GAPDH and 33 to 38 for IL-2, IL-4, IL-5, IL-10, and IFN-γ.

Each PCR product (10 μL) was size fractionated and visualized with ethidium bromide on 1.5% agarose gel electrophoresis, followed by Southern blotting to Hybond-N membrane (Amersham) and hybridization. Hybridizations were carried out at 65°C overnight with the appropriate cloned cDNA, which had been labeled with phosphorus 32, in 6× standard saline citrate, 10 × Denhardt's solution (0.2% wt/vol each of bovine serum albumin, Ficoll, and polyvinylpyrrolidone) (Sigma), 5 mmol/L EDTA, 0.5% SDS, 0.2% sodium pyrophosphate, and 100 μg/mL sonicated salmon sperm DNA. Each PCR reaction (5 μL) was dot-blotted on to Hybond-N membrane and also hybridized to cDNA probe. Dot blots were excised and radioactivity measured by Cerenkov counting. Results were generated from the counting of dot blots and expressed as a ratio of cytokine to GAPDH count.

Data analysis

Data were presented as mean ± SEM. For multiple comparison of the 4 groups, the Kruskal-Wallis test for analysis of variance was used. For comparison of individual groups, we used the Mann-Whitney *U* test. Data analysis was performed with use of SPSS for Windows statistical software package (SPSS, Chicago, Ill). A *P* value of <.05 was considered to be significant.

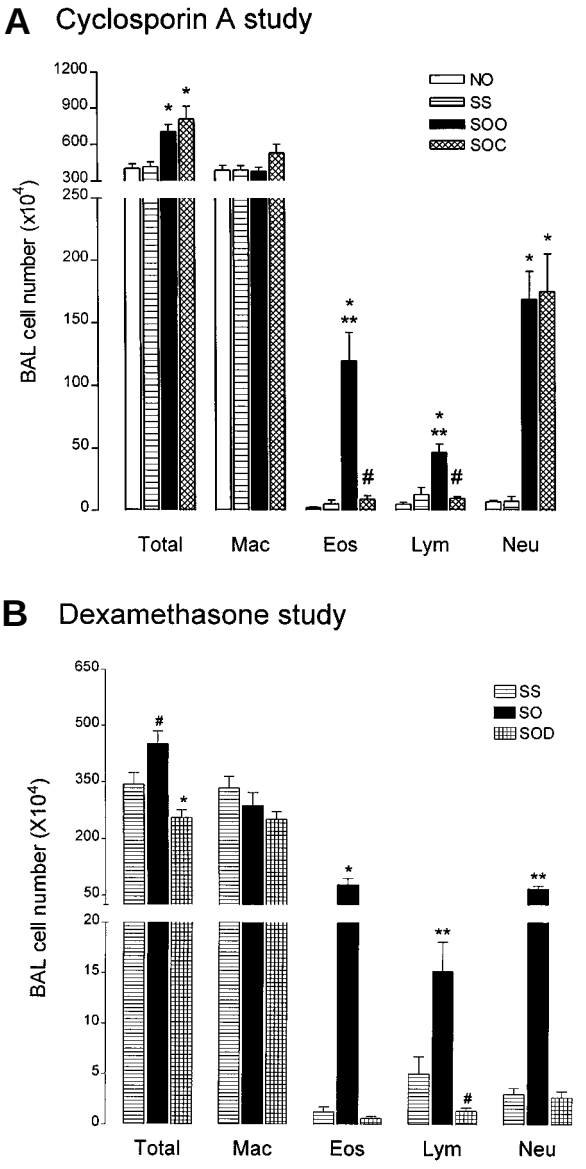

FIG 2. Mean cell numbers of total cell, macrophage *(Mac),* eosinophil *(Eos),* lymphocyte *(Lym),* and neutrophil *(Neu)* in BAL fluid in CsA study **(A)** and dexamethasone study **(B)** for groups of rats as specified in Fig 1. CsA treatment suppressed increase in eosinophil and lymphocyte counts induced by allergen exposure of sensitized rats in BAL fluid but had no effect on neutrophil influx. Dexamethasone also suppressed induced increases in eosinophil, lymphocyte, and neutrophil influx. **A,** *Asterisk, P* < .005 compared with NO and SS groups; *two asterisks, P* < .001 compared with SOC group; *pound sign, P* < .04 compared with NO group. **B,** *Asterisk, P* < .05 compared with other groups; *two asterisks, P* < .02 compared with groups SS and SOD; *pound sign, P* < .04 compared with group SS. Data shown as mean ± SEM.

RESULTS

Bronchial responsiveness to ACh

There was no significant difference in baseline R_L between the groups. CsA or dexamethasone alone had no effect on the baseline responsiveness to ACh. Sensitized vehicle-treated and OA-exposed rats showed a significant increase in mean logPC_{200} compared with nonsensitized OA-exposed or sensitized sodium chloride solu-

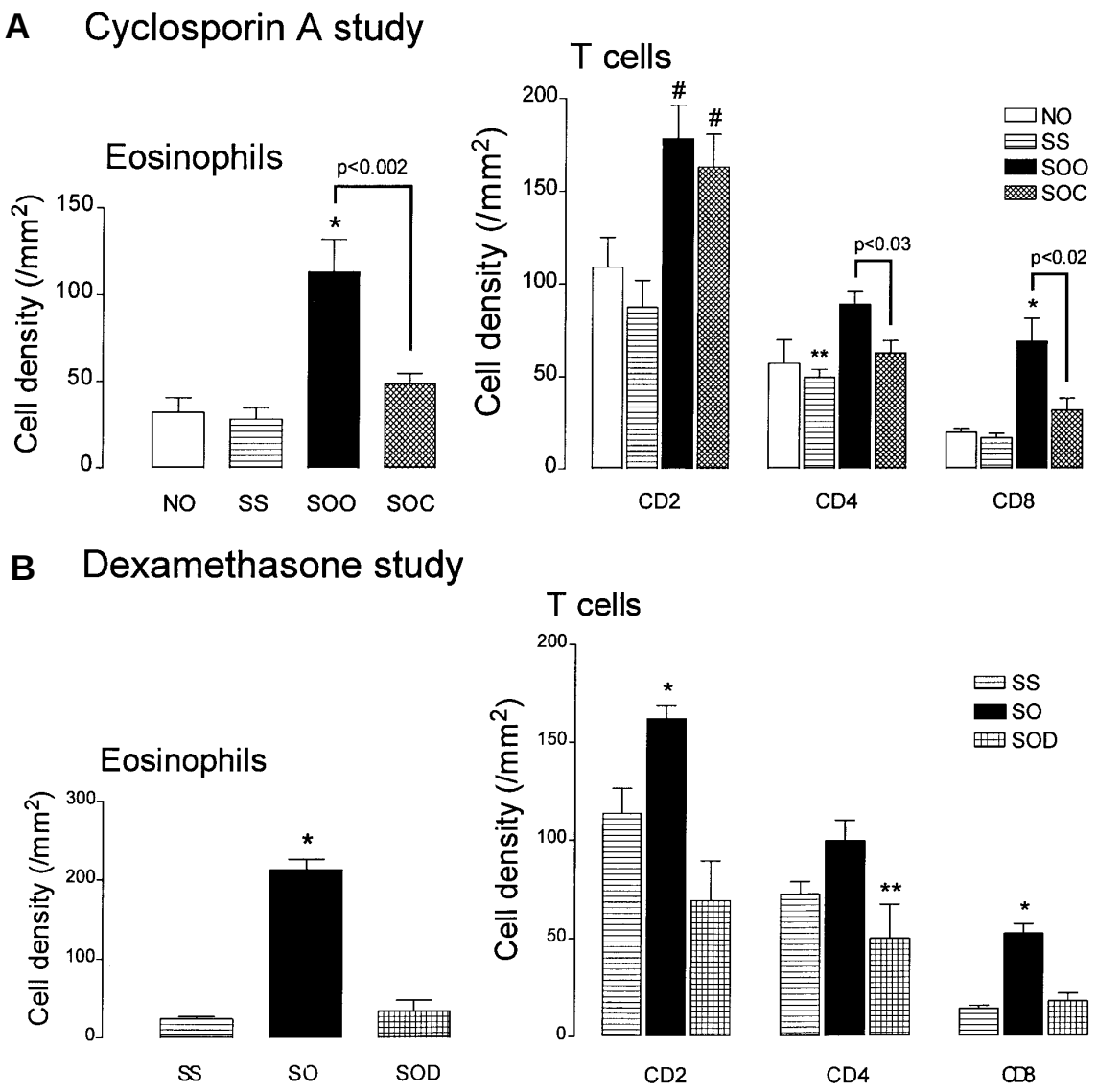

FIG 3. Mean eosinophil *(left)* and T-lymphocyte subsets ($CD2^+$, $CD4^+$, and $CD8^+$, *right*) counts in airway submucosa expressed as square millimeter in CsA study **(A)** and dexamethasone study **(B)** with groups as detailed in Fig 1. There was significant increase in eosinophils in airway submucosa after allergen challenge of sensitized rats, which was suppressed by pretreatment with CsA and dexamethasone. Allergen challenge led to significant increase in total number of T lymphocytes expressing pan T-cell marker $CD2^+$ and also $CD8^+$ T-lymphocytes. Although CsA did not significantly reduce $CD2^+$ T cells, there was significant inhibition of increase in $CD4^+$ T cells and $CD8^+$ T cells induced by allergen exposure. In contrast, dexamethasone significantly inhibited increase in all 3 subsets of T lymphocytes. *Asterisk, P* < .05 compared with other groups; *two asterisks, P* < .03 compared with group SOO or SO; *pound sign, P* < .03 compared with NO and SS groups in CsA study. Data shown as mean ± SEM.

tion–exposed rats ($P < .02$, Fig 1). CsA had no effect on the allergen-induced BHR, whereas dexamethasone suppressed BHR ($P < .002$ compared with sensitized OA-exposed rats treated with sodium chloride solution only). This suppression was reflected by the nearly identical R_L-ACh response curves for sensitized sodium chloride solution–exposed rats and sensitized dexamethasone-treated OA-exposed animals (Fig 1, *B*).

Inflammatory cell response

Bronchoalveolar lavage. There was a significant increase in the numbers of total cells, eosinophils, lymphocytes, and neutrophils recovered in BAL fluid of sensitized rats exposed to OA compared with sensitized rats exposed to sodium chloride solution ($P < .04$, Fig 2). CsA significantly reduced the numbers of eosinophils and lymphocytes ($P < 0.001$) but had no effect on neu-

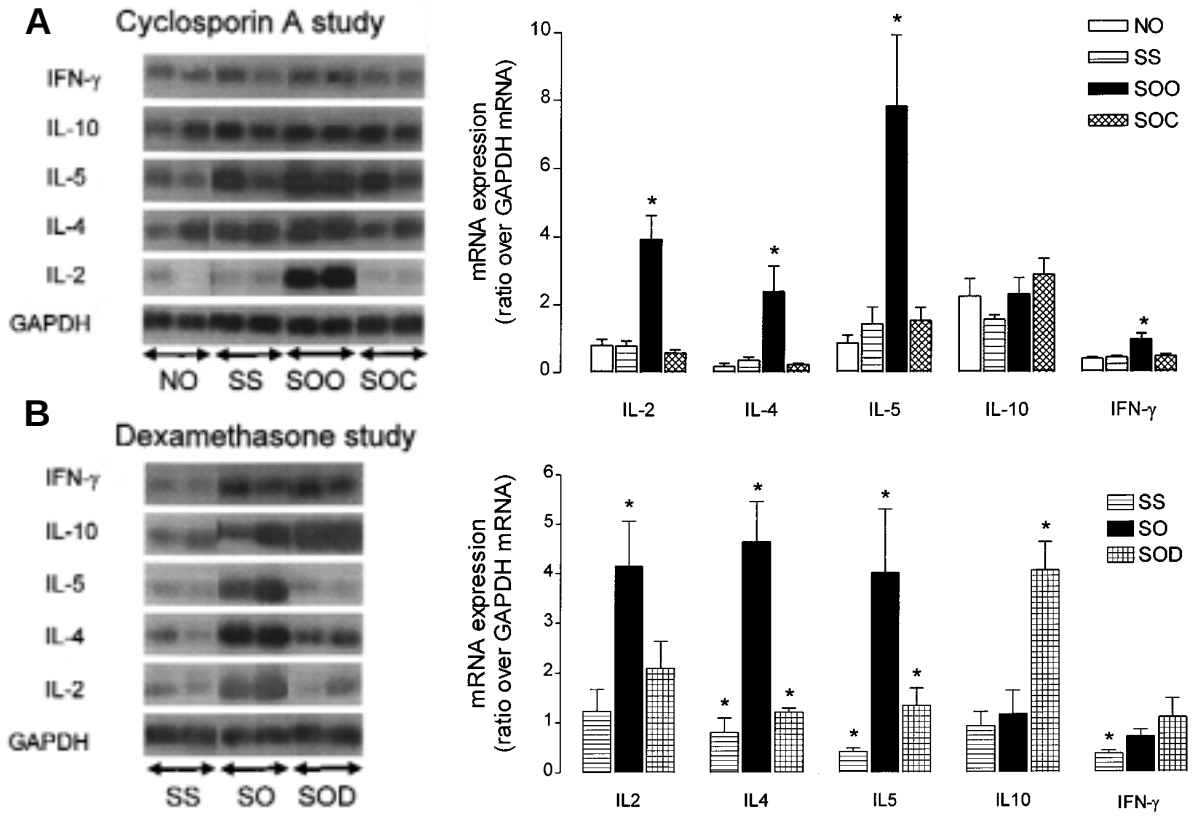

FIG 4. Mean IL-2, IL-4, IL-5, IL-10, and IFN-γ mRNA expression in rat lung expressed as ratio to GAPDH mRNA, as determined by RT-PCR, followed by Southern blot analysis. Expression was obtained as radioactivity count of dot-blot analysis of PCR products. Representative bands are shown on *left* (2 rats from each group), and mean mRNA expression with SEM is shown on *right.* Groups of rats were as for Fig 1. There was significant increase in expression of IL-2, IL-4, IL-5, and IFN-γ after allergen exposure of sensitized rats (groups SOO and SO), an effect that was inhibited by CsA. Dexamethasone suppressed expression of IL-2, IL-4, and IL-5 mRNA but increased IL-10 expression, together with nonsignificant further increase in IFN-γ mRNA expression. *Asterisk, P* < .05 compared with other groups.

trophils in BAL fluid. In contrast, the increase of inflammatory cells in BAL fluid induced by OA exposure of sensitized rats was suppressed by dexamethasone. There was a further reduction of lymphocyte count by dexamethasone to a level significantly lower than that of sensitized rats exposed to sodium chloride solution ($P <$ .02).

Airways. Allergen exposure of sensitized rats caused a significant increase in eosinophil, $CD2^+$ T-cell, and $CD8^+$ T-cell counts ($P <$ .03, Fig 3) in both protocols. The increase in submucosal $CD4^+$ T cells observed after allergen challenge in the cyclosporine protocol was significant ($P <$ .03) but not in the dexamethasone study. The eosinophil, $CD8^+$, and $CD4^+$ T-cell count increases induced by allergen exposure were significantly reduced by both CsA and dexamethasone treatment ($P <$ .03).

Cytokine expression in lungs

In sensitized rats OA exposure induced a significant increase in IL-2, IL-4, IL-5, and IFN-γ messenger RNA (mRNA) expression ($P <$ .05, Fig 4), which was significantly inhibited by CsA ($P <$ .04). Neither OA exposure nor CsA treatment had an effect on IL-10 mRNA expression. In the dexamethasone study the increase in IL-2, IL-4, and IL-5 mRNA expression was significantly suppressed by dexamethasone ($P <$ .05), which also increased expression of IL-10 mRNA ($P <$ .01) with a nonsignificant enhancement of the OA-induced increase in IFN-γ mRNA expression.

DISCUSSION

CsA did not inhibit allergen-induced BHR but decreased the infiltration of eosinophils and lymphocytes in airways, whereas dexamethasone suppressed allergen-induced airway inflammation, BHR. CsA significantly reduced the increased numbers of eosinophil and lymphocyte recovered in BAL fluid and in submucosal tissues after allergen challenge, as has been previously observed,[17,18] but not neutrophil in BAL fluid. By contrast, dexamethasone inhibited BHR with the suppression of

the influx of all inflammatory cell types, including neutrophils. Although both CsA and dexamethasone inhibited the Th2 cytokines IL-4 and IL-5, there were significant differences between these agents regarding the expression of other cytokines, whereas CsA inhibited IFN-γ mRNA expression and had no significant effect on IFN-γ expression. Therefore the differential effects of CsA and dexamethasone on the cytokine profile expression and on the neutrophilic inflammation may underlie the divergent effects of these drugs on allergen-induced BHR.

CsA is a potent inhibitor of the synthesis of lymphokines such as IL-2, IFN-γ, IL-3, IL-4, IL-5, and TNF-α from T cells activated after recognition of antigen by the T-cell receptor–CD3 complex.[11-14] The induced mRNA expression of cytokines, such as IL-4, IL-5, IL-2, and IFN-γ, by allergen challenge was inhibited by CsA, whereas dexamethasone inhibited induced expression of IL-2, IL-4, and IL-5 but not of IFN-γ. The inhibitory effects of cyclosporine and corticosteroids on the expression and synthesis of IL-2, which is produced early during T-cell activation, particularly the $CD4^+$ Th1 subset, have been previously evaluated.[14,25] IL-2 up-regulates IL-2 receptor[28] and the production of other lymphokines, including IFN-γ.[27] IL-2 facilitates clonal expansion of both $CD4^+$ and $CD8^+$ T-cell subsets.[28] In the OA-sensitized Brown-Norway rat IL-2 increases the late-phase response to allergen challenge and causes an inflammatory response around the airways with eosinophil, lymphocyte, and mast cell infiltration.[29] In addition, IL-2 is a potent chemoattractant of eosinophils in vivo.[30] Treatment with CsA at 10 to 17 days after sensitization of Brown-Norway rats to trimellitic anhydride does not, however, reduce IgE antibody production[31] and it is unlikely that any of the observed effects in our study was the result of IgE suppression. CsA and corticosteroids inhibit anti-CD3–induced production of IL-4 and IL-5 from a murine Th2-type cell clone.[32] Therefore inhibition of induced IL-2, IL-5, and also possibly IL-4 mRNA expression by CsA and dexamethasone is important in the suppression of the cellular influx of inflammatory cells after allergen challenge.

By contrast, there was differential modulation of IL-10 and IFN-γ by CsA and dexamethasone. IL-10 appears to possess a dual role in allergen-induced inflammation. IL-10 was originally characterized as a factor generated by mouse Th2 cells that inhibit cytokine synthesis by Th1 cells.[33] However, IL-10 can also prevent allergic airway inflammation induced by Th2 cells.[34] IL-10 expression was not altered by allergen challenge or by CsA treatment but was markedly enhanced by corticosteroid treatment of allergen-exposed rats. This increase of IL-10 mRNA expression may additionally contribute to the effects of corticosteroids in suppressing allergen-induced inflammation. There was a small but significant increase in IFN-γ expression after allergen challenge, an effect that was inhibited by CsA but not significantly affected by dexamethasone. A similar increase of IFN-γ has been demonstrated in allergen-exposed sensitized mice,[35] and corticosteroid treatment increased IFN-γ mRNA expression in bronchial biopsy specimens of asthmatic subjects.[36] IFN-γ is produced by Th1 cells and exerts inhibitory effects on Th2 cells and has anti-inflammatory effects on allergic inflammation. Thus it inhibits antigen-induced eosinophil recruitment in the mouse[10,37] and IL-4–induced IgE synthesis by B cells.[7,38] In the sensitized Brown-Norway rat model exogenous administration of IFN-γ attenuates allergen-induced BHR and eosinophilia, and inhibition of endogenously released IFN-γ with an anti-IFN-γ antibody enhanced these effects.[39] Treatment with exogenous IFN-γ also prevents the development of BHR in sensitized mice.[40,41] Thus an increase in IFN-γ expression may also contribute to the anti-inflammatory effects of dexamethasone and possibly to BHR.

Although dexamethasone and CsA had marked effects in inhibiting the eosinophilic and lymphocytic inflammation induced by allergen exposure, only dexamethasone suppressed BHR, confirming previous data.[17] The persistence of IFN-γ expression together with the induction of IL-10 mRNA and the inhibition of neutrophilic inflammation with corticosteroids may underlie the suppression of BHR. Neutrophils have been previously implicated in the induction of BHR,[42,43] and IL-10 has inhibitory effects on neutrophil chemotaxis and in resolving neutrophilic inflammation by promoting apoptosis of these cells.[44,45] In addition, in OA-sensitized mice exogenous IL-10 administration inhibits neutrophil and eosinophil recruitment into the airways, and neutralization of endogenous IL-10 with an anti-IL-10 monoclonal antibody increases allergen-induced neutrophil and eosinophil accumulation in the BAL fluid.[34] Therefore the enhancement of IL-10 mRNA expression in OA-exposed rats by corticosteroid treatment in our study may contribute to the inhibition of allergen-induced neutrophil influx, which is not suppressed by CsA treatment.

It is also probable that the differential effects of CsA and dexamethasone on IgE development also contribute to the dissociation between inhibition of BHR and eosinophilia. CsA treatment 10 to 17 days after sensitization of Brown-Norway rats with trimellic anhydride had no effect on the production of specific antibodies, whereas treatment with corticosteroid attenuated the IgE and IgG responses.[31] This differential effect of CsA and dexamethasone on IgE responses also may result from their differing effects on IFN-γ expression because IFN-γ can inhibit IgE synthesis from B cells.[7,38] IFN-γ administered after the period of sensitization does not inhibit the specific IgE level in sensitized mice exposed to OA while inhibiting BHR.[40,41] Although we did not measure specific IgE levels, CsA and dexamethasone could have shown differential effects on IgE levels.

In summary, although allergen-induced eosinophilic inflammation is suppressed by both CsA and dexamethasone, only dexamethasone inhibits neutrophilia and BHR, an effect probably related to the enhancement of

IL-10 mRNA expression and to the lack of inhibition of IFN-γ mRNA expression by corticosteroids. Our data indicate that inhibition of selected Th2 cytokines, IL-4 and IL-5, is important for eosinophil accumulation and that the selective enhancement of the Th2 cytokine, IL-10, accompanies suppression of BHR.

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REFERENCES

1. Chung KF. Role of inflammation in the hyperresponsiveness of the airways in asthma. Thorax 1986;41:657-62.
2. Azzawi M, Bradley B, Jeffery PK, Frew AJ, Wardlaw AJ, Knowles G, et al. Identification of activated T lymphocytes and eosinophils in bronchial biopsies in stable atopic asthmatics. Am Rev Respir Dis 1990;142:1407-11.
3. Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, et al. Expression of mRNA for interleukins in mucosal bronchial biopsies from asthma. J Clin Invest 1991;87:1541-6.
4. Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant Th2-like bronchoalveolar T-lymphocyte population in atopic asthma. N Engl J Med 1992;326:298-304.
5. Calhoun WJ, Hinton KL, Friedenheim RE. Simultaneous Th1 and Th2 lymphocyte activation in allergic asthma following segmental allergen challenge (SAC) [abstract]. Am J Respir Crit Care Med 1995;151:A778.
6. Cembrzynska-Nowak M, Szklarz E, Inglot AD, Teodorczyk-Injeyan. Elevated release of tumor necrosis factor-alpha and interferon-gamma by bronchoalveolar leukocytes from patients with bronchial asthma. Am Rev Respir Dis 1993;147:291-5.
7. Romagniani S. Regulation and deregulation of human IgE synthesis. Immunol Today 1990;11:316-21.
8. Sanderson CJ. Interleukin-5, eosinophils and disease. Blood 1992;79:3101-9.
9. Mauser PJ, Pitman A, Ferendez X, Zurcher J, Watnick A, Egan RW, et al. The effects of anti-IL-5 on antigen-induced airway hyperreactivity and pulmonary eosinophilia in guinea pigs [abstract]. Am Rev Respir Dis 1992;145:A859.
10. Iwamoto I, Nakajima H, Endo H, Yoshida S. Interferon γ regulates antigen-induced eosinophil recruitment into the mouse airways by inhibiting the infiltration of CD4+ cells. J Exp Med 1993;177:573-6.
11. Di Padova FE. Pharmacology of cyclosporin (Sandimmune), V: pharmacological effects on immune functions: in vitro studies. Pharmacol Rev 1989;41:373-405.
12. Rolfe FG, Valentine JE, Sewell WA. Cyclosporin A and FK506 reduce interleukin-5 mRNA abundance by inhibiting gene transcription. Am J Respir Cell Mol Biol 1997;17:243-50.
13. Sano T, Nakamura Y, Matsunaga Y, Takahashi T, Azuma M, Okano Y, et al. FK506 and cyclosporin A inhibit granulocyte/macrophage colony-stimulating factor production by mononuclear cells in asthma. Eur Respir J 1995;8:1473-8.
14. Sigal NH, Dumont FJ. Cyclosporin A, FK-506, and rapamycin: pharmacological probes of lymphocyte signal transduction. Annu Rev Immunol 1992;10:519-60.
15. Lock SH, Kay AB, Barnes NC. Double-blind, placebo-controlled study of cyclosporin A as a corticosteroid-sparing agent in corticosteroid-dependent asthma. Am J Respir Crit Care Med 1996;153:509-14.
16. Fukuda T, Asakawa J, Motojima S, Makino S. Cyclosporin A reduces T lymphocyte activity and improves airway hyperresponsiveness in corticosteroid-dependent chronic severe asthma. Ann Allergy Asthma Immunol 1995;15:65-72.
17. Elwood W, Lotvall JO, Barnes PJ, Chung KF. Effect of dexamethasone and cyclosporin A on allergen-induced airway hyperresponsiveness and inflammatory cell response in sensitized Brown-Norway rats. Am Rev Respir Dis 1992;145:1289-94.
18. Norris AA, Jackson DM, Eady RP. Protective effect of cyclophosphamide, cyclosporin A and FK506 against antigen-induced lung eosinophilia in guinea-pigs. Clin Exp Immunol 1992;89:347-50.
19. Lapa e Silva JR, Ruffié C, Vargaftig BB, Pretolani M. Modulation of the bronchial inflammation in sensitized guinea-pigs by FK-506, nedocromil sodium and dexamethasone. Eur Respir J 1995;8:1321-7.
20. Fukuda T, Akutsu I, Motojima S, Makino S. Inhibition of allergen-induced late asthmatic response and bronchial hyperresponsiveness by cyclosporin and FK 506. Int Arch Allergy Appl Immunol 1991;94:259-61.
21. Elwood W, Lotvall JO, Barnes PJ, Chung KF. Characterisation of allergen-induced inflammation and bronchial hyperresponsiveness in sensitized Brown-Norway rats. J Allergy Clin Immunol 1991;88:951-60.
22. Haczku A, Moqbel R, Jacobson M, Kay AB, Barnes PJ, Chung KF. T-cells subsets and activation in bronchial mucosa of sensitized Brown-Norway rats after single allergen exposure. Immunology 1995;85:591-7.
23. Huang T-J, MacAry PA, Kemeny DM, Chung KF. Effect of CD8+ T cell depletion on bronchial hyperresponsiveness and inflammation in sensitised and allergen-exposed Brown-Norway rats. Immunology 1999;96:416-23.
24. Chomczynski P, Sacchi N. Single step method of RNA isolation by acid guanidium thiocyanate–phenol chloroform extraction. Anal Biochem 1987;162:156-9.
25. Arya SK, Wong-Staal F, Gallo RC. Dexamethasone mediated inhibition of human T-cell growth factor and γ-interferon messenger RNA. J Immunol 1984;133:273-6.
26. Malek TR, Ashwell JD. Interleukin 2 upregulates expression of its receptor on a T-cell clone. J Exp Med 1985;161:1575-80.
27. Howard M, Matis L, Malek TR, Shevach E, Kell W, Cohen D, et al. Interleukin 2 induced antigen-reactive T-cell lines to secrete BCGF-1. J Exp Med 1983;158:2024-39.
28. Erard F, Corthesy P, Nabholz M, Lowenthal JW, Zaech P, Plaetinck G, et al. Interleukin 2 is both necessary and sufficient for the growth and differentiation of lectin-stimulated cytolytic T lymphocyte precursors. J Immunol 1985;134:1644-52.
29. Renzi PM, Sapienza DUT, Waserman R, Olivenstein R, Martin JG. Effects of interleukin-2 on the acute and late phase response to ovalbumin in the rat. Am Rev Respir Dis 1992;146:163-9.
30. Rand TH, Silberstein DS, Kornfeld H, Weller PF. Human eosinophils express functional interleukin 2 receptors. J Clin Invest 1991;88:825-32.
31. Pullerits T, Dahlgren U, Skoogh B, Lotvall J. Development of antigen-specific IgE after sensitisation with trimellitic anhydride in rats is attenuated by glucocorticoids and cyclosporin A. Int Arch Allergy Immunol 1997;112:279-86.
32. Schmidt J, Fleissner S, Heimann-Weitschat I, Lindstaedt R, Szelenyi I. The effect of different corticosteroids and cyclosporin A on interleukin-4 and interleukin-5 release from murine Th2-type T-cells. Eur J Pharmacol 1994;260:247-50.
33. Fiorentino DF, Bond MW, Mosmann TR. Two types of mouse helper T-cells, IV: Th2 clones secrete a factor that inhibits cytokine production by Th1 clones. J Exp Med 1989;170:2081-95.
34. Zuany-Amorim C, Hailé S, Leduc D, Dumarey C, Huerre M, Vargaftig BB, et al. Interleukin-10 inhibits antigen-induced cellular recruitment into the airways of sensitized mice. J Clin Invest 1995;95:2644-51.
35. Keane-Myers A, Gause WC, Linsley PS, Chen S-J, Wills-Karp M. B7-CD28/CTLA-4 costimulatory pathways are required for the development of T helper cell 2–mediated allergic airway responses to inhaled antigens. J Immunol 1997;158:2040-9.
36. Bentley AM, Hamid Q, Robinson DS, Schotman E, Meng Q, Assoufi B, et al. Prednisolone treatment in asthma: reduction in the number of eosinophils, T-cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5, and interferon-gamma cytokine gene expression within the bronchial mucosa. Am J Respir Crit Care Med 1996;153:551-6.
37. Li X-M, Chopra RK, Chou T-Y, Schofield BH, Wills-Karp M, Huang S-K. Mucosal IFN-γ gene transfer inhibits pulmonary allergic responses in mice. J Immunol 1996;157:3216-9.
38. Snapper CM, Paul WE. Interferon-γ and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science 1987;236:944-7.
39. Huang TJ, MacAry PA, Wilke T, Kemeny DM, Chung KF. Inhibitory effects of endgenous and exogenous interferon-γ on bronchial hyperresponsiveness, allergic inflamation, and T-helper 2 cytokines in Brown Norway rats. Immunology 1999. In press.
40. Hofstra CL, Van Ark I, Hofman G, Nijkamp FP, Jardieu PM, Van Oosterhout AJ. Differential effects of endogenous and exogenous interferon-gamma on immunoglobulin E, cellular infiltration, and airway responsiveness in a murine model of allergic asthma. Am J Respir Cell Mol Biol 1998;19:826-35.

41. Nagai H, Maeda Y, Tanaka H. The effect of anti-IL-4 monoclonal antibody, rapamycin and interferon-γ on airway hyperreactivity to acetylcholine in mice. Clin Exp Allergy 1997;27:218-24.
42. O'Byrne PH, Walters EH, Gold BD, Aizawa HA, Fabbri LM, Alpert SE, et al. Neutrophil depletion inhibits airway hyperresponsiveness induced by ozone exposure. Am Rev Respir Dis 1984;130:214-9.
43. Murphy KR, Wilson MC, Irvin CG, Glezen LS, Marsh WR, Haslett C, et al. The requirement for polymorphonuclear leukocytes in the late asthmatic response and heightened airway reactivity in an animal model. Am Rev Respir Dis 1986;134:62-8.
44. Driscoll KE, Carter JM, Howard BW, Hassenbein D, Burdick M, Kunkel SL, et al. Interleukin-10 regulates quartz-induced pulmonary inflammation in rats. Am J Physiol 1998;275:L887-94.
45. Cox G. IL-10 enhances resolution of pulmonary inflammation in vivo by promoting apoptosis of neutrophils. Am J Physiol 1996;271:L566-71.

Correction

The following correction applies to "The Editors' Choice" that appeared in volume 104, number 1, p 1-2, 1999, of the Journal. On p 1, the figure illustrating the article by Molet et al was incorrect. The correct figure appears below.

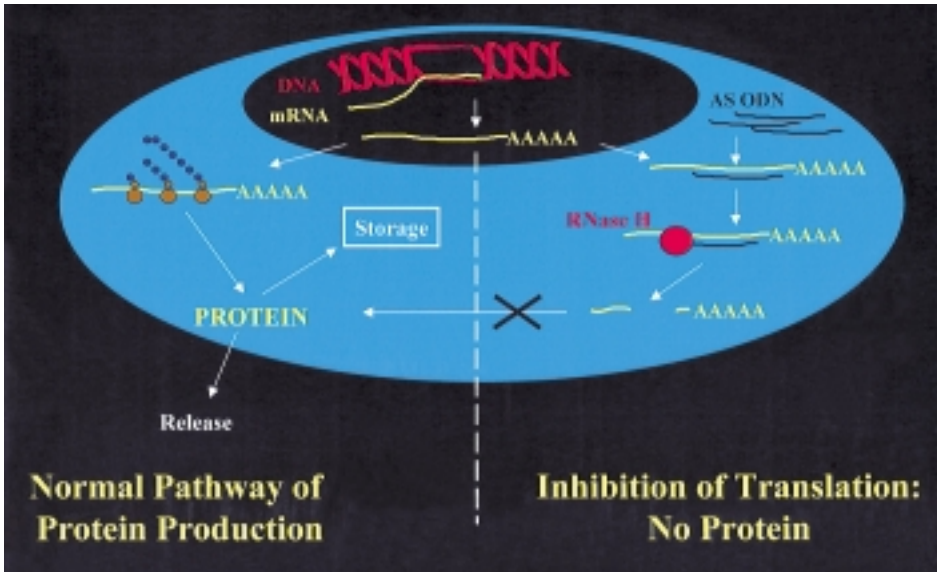

FIG 1. Mechanisms of antisense oligodeoxynucleotide antisense (ODN AS) action.