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Effect of topical immunomodulators on acute allergic inflammation and bronchial hyperresponsiveness in sensitised rats

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Abstract

We examined the effects of different immunomodulators administered topically on asthmatic responses in a rat model of asthma. Sensitised Brown–Norway rats were administered rapamycin, SAR943 (32-deoxorapamycin), IMM125 (a hydroxyethyl derivative of D-serine[8]-cyclosporine), and budesonide by intratracheal instillation 1 h prior to allergen challenge. Allergen exposure induced bronchial hyperresponsiveness, accumulation of inflammatory cells in bronchoalveolar lavage fluid, and also an increase in eosinophils and $CD2^+$, $CD4^+$ and $CD8^+$ T cells in the airways. Interleukin-2, interleukin-4, interleukin-5, interleukin-10, and interferon-γ mRNA expression was upregulated by allergen exposure. Budesonide abolished airway inflammation, suppressed the mRNA expression for interleukin-2, interleukin-4, and interleukin-5 ($P<0.03$), and bronchial hyperresponsiveness ($P<0.05$). IMM125 suppressed airway infiltration of eosinophils, and $CD8^+$ T cells ($P<0.02$), and prevented the upregulated mRNA expression for interleukin-4, interleukin-5, and interferon-γ ($P<0.02$). Rapamycin suppressed $CD8^+$ T cell infiltration in airway submucosa ($P<0.03$), and mRNA expression for interleukin-2 ($p<0.002$), while SAR943 suppressed interleukin-2, interleukin-4, and interferon-γ mRNA ($P<0.05$). IMM125, rapamycin and SAR943 did not alter airway submucosal $CD2^+$ and $CD4^+$ T cell infiltration, and bronchial hyperresponsiveness. $CD8^+$ T cells, in contrast to $CD4^+$ T cells, are more susceptible to the inhibition by IMM125 and rapamycin, which also caused greater suppression of Th1 compared to Th2 cytokine mRNA expression. In this acute model of allergic inflammation, differential modulation of Th1 and Th2 cytokines may determine the effects of various immunomodulators on airway inflammation and bronchial hyperresponsiveness.

Keywords: Bronchial hyperresponsiveness; Airway inflammation; Rapamycin; IMM125; SAR943; Budesonide; Th1 cytokine; Th2 cytokine

1. Introduction

$CD4^+$ T cells play a pivotal role in the pathogenesis of allergic bronchial asthma. Expression of the T helper type 2 (Th2)-derived cytokines, particularly interleukin-4 and interleukin-5, is increased in the airways of patients with asthma, particularly $CD4^+$ T cells, but the expression of the Th1-derived cytokine interferon-γ is not increased (Hamid et al., 1991; Robinson et al., 1992). In animal models, suppression of Th2 cytokines, such as interleukin-4 and interleukin-5, prevents the development of the major features of asthma, airway inflammation and bronchial hyperresponsiveness (Mauser et al., 1993; Corry et al., 1996). On the other hand, augmentation of Th1 response as induced by mycobacterial infections (Erb et al., 1998), and administration of Th1-derived cytokines, such as interferon-γ, interleukin-12, and interleukin-18 (Hofstra et al., 1998; Huang et al., 1999b) can also protect from allergen-induced airway inflammation. $CD8^+$ T cells may counteract Th2-mediated airway immune responses (Olivenstein et al., 1993; Laberge et al., 1996), probably by promoting a Th1 response (Huang et al., 1999b). Restoration of the Th1/Th2 imbalance appears to be an optimal strategy for controlling allergen-induced airway disorders.

The potential benefits from immunosuppressants, such as cyclosporin A have been studied in asthma, mainly as oral corticosteroid-sparing agents in patients with severe asthma (Alexander et al., 1992; Fukuda et al., 1995). Cyclosporin A suppresses calcineurin, which is important in the signal transduction pathways necessary for the expression of many cytokines, including interleukin-2, interleukin-3, interleu-

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kin-4, interleukin-5, interferon-γ, tumour necrosis factor-α and granulocyte macrophage-colony stimulating factor (GM-CSF), and thereby inhibits T lymphocyte activation and proliferation (Sigal and Dumont, 1992). Systemic cyclosporin A inhibited allergen-induced airway infiltration of eosinophils and lymphocytes, and suppressed mRNA expression for Th2 cytokines, but not bronchial hyperresponsiveness (Huang et al., 1999a). Rapamycin acts at a late stage in T cell activation, and inhibits proliferation of T cells, while cyclosporin A is insensitive at this stage of activation (Dumont et al., 1990). It inhibits Sephadex-induced airway inflammatory cell influx, and bronchial hyperresponsiveness of isolated bronchial strips in guinea pigs (Francischi et al., 1993). However, the effect of rapamycin in mediating Th1/Th2 balance in the airway allergic inflammation is not known.

We, therefore, examined the differential effects of rapamycin and one of its newly developed analogues, SAR943 or 32-deoxorapamycin, administered by intratracheal instillation on allergen-induced airway asthmatic responses in the Brown–Norway rat model, and compared their actions to those of a corticosteroid, budesonide, and the compound IMM125, which is a hydroxyethyl derivative of D-serine[8]-cyclosporine (Donatsch et al., 1992; Hiestand et al., 1992). We have studied the effects of these different immunosuppressants on allergic inflammation and bronchial hyperresponsiveness. In previous studies (Elwood et al., 1992; Huang et al., 1999a), the effects of cyclosporin A and corticosteroids have been studied following systemic administration and in the present study, we examined the effects of these and related drugs by topical administration to the airways.

2. Methods

2.1. Sensitisation and allergen exposure

Pathogen-free inbred male Brown–Norway rats (Harlan Olac Bicester, UK; 200–250 g, 9–13 weeks old) were injected with 1 ml of 1 mg ovalbumin (Grade V, salt-free, Sigma, Dorset, UK) in 100 mg $Al(OH)_3$ (BDH, Dorset, UK) suspension in 0.9% (wt/vol) saline intraperitoneally (i.p.) for 3 consecutive days. Ovalbumin aerosol exposure (15 min; 1% ovalbumin) to rats was performed in a 6.5-l Plexiglas chamber connected to a DeVilbiss PulmonSonic nebuliser (model No. 2512, DeVilbiss Health Care, Middlesex, UK), that generated an aerosol mist pumped into the exposure chamber by the airflow supplied by a small animal ventilator (Harvard Apparatus, Kent, UK) set at 60 strokes/min with a pumping volume of 10 ml.

2.2. Protocol

The following groups of rats were studied:

(1) Saline-treated and saline-exposed animals (group SA, $n=8$): animals injected with 1 ml of 1 mg ovalbumin/100 mg $Al(OH)_3$ in 0.9% saline suspension for 3 consecutive days received saline (100 μl) by intratracheal instillation under light anaesthesia 17 days after last injection. Animals were allowed to recover for 2–3 h before exposure to saline aerosol for 15 min, and then studied 18–24 h thereafter.

(2) Saline-treated and ovalbumin-exposed animals (group OA, $n=7$): the procedures were the same as group SA above, except the aerosol exposure was with 1% ovalbumin aerosol.

(3) Budesonide-treated and ovalbumin-exposed animals (group BUD, $n=7$): the procedures were the same as for group OA. Budesonide was administered intratracheally (1 mg/kg in 100 μl saline).

(4) IMM125-treated and ovalbumin-exposed animals (group IMM, $n=7$): the procedures were the same as for group OA. IMM125 (10 mg/kg in 100 μl saline), a new analogue of cyclosporin A, was administered intratracheally.

(5) Rapamycin-treated and ovalbumin-exposed animals (group RAP, $n=8$): the procedures were the same as for group OA. Rapamycin (1 mg/kg in 100 μl saline) was administered intratracheally.

(6) SAR943-treated and ovalbumin-exposed animals (group SAR, $n=7$): the procedures were the same as for group OA. SAR943 (10 mg/kg in 100 μl saline) was administered intratracheally.

All rats were studied 18–24 h after exposure to either 1% ovalbumin or 0.9% NaCl aerosol.

2.3. Measurement of airway responsiveness to acetylcholine

Airway responsiveness was measured as previously described (Elwood et al., 1992). In brief, anaesthetised, tracheostomised, paralysed, and ventilated rats were monitored for airflow with a pneumotachograph (model F1L, Mercury Electronics, Glasgow, Scotland) connected to a transducer (model FCO40; ± 20 mm H_2O, Furness Controls, Sussex, UK), transpulmonary pressure via a transpleural catheter connected to a transducer (model FCO40; ± 1000 mm H_2O, Furness Controls) and blood pressure via carotid artery catheterisation. Lung resistance (R_L) was simultaneously calculated using a software (LabView, National Instruments, Austin, TX, USA) on a Macintosh II computer (Apple Computer, CA, USA). Aerosol generated from increasing half $\log_{10}$ concentrations of acetylcholine chloride (Sigma) was administered by inhalation (45 breaths of 10 ml/kg stroke volume) with the initial concentration of $10^{-3.5}$ mol/l and the maximal concentration of 0.1 mol/l, through a different circuit from the one for R_L measurements. The concentration of acetylcholine needed to increase R_L 200% above baseline (PC_{200}) was calculated by interpolation of the log concentration–lung resistance curve.

2.4. Bronchoalveolar lavage and cell counting

This is also described in detail elsewhere (Haczku et al., 1997). Briefly, after an overdose of anaesthetic, rats were

lavaged with a total of 20 ml 0.9% sterile saline via the endotracheal tube. Total cell counts, viability and differential cell counts from cytospin preparations stained by May–Grünwald–Giemsa stain were determined under an optical microscope (Olympus BH2, Olympus Optical, Tokyo, Japan). At least 500 cells were counted and identified as macrophages, eosinophils, lymphocytes and neutrophils according to standard morphology under $\times 400$ magnification.

2.5. Collection of lung tissues

After opening the thoracic cavity and removal of the lungs, the right lung without major vascular and connective tissues was cut into pieces and snap-frozen in liquid nitrogen (BOC, Luton, UK), and then stored at -80 °C for later assays for mRNA expression. The left lung was inflated with 3 ml saline/O.C.T. tissue embedding medium (1:1). Two half-cubic centimeter blocks were cut from the left lung around the major bronchus, embedded in O.C.T. medium (Raymond A Lamb, London, UK), and snap-frozen in melting isopentane (BDH) and liquid nitrogen. Cryostat sections (6 μm) of the tissues were cut, air-dried, fixed in acetone, and then air-dried again, wrapped in aluminium foil and stored at -80 °C for later immunohistochemical studies.

2.6. Immunohistochemistry

For detection of eosinophils, we used a mouse immunoglobulin (Ig)G1 monoclonal antibody against human major basic protein, clone BMK-13 (Monosan®, Bradsure Biologicals, Leicestershire, UK), which is both sensitive and specific for staining rat eosinophils in frozen sections. The cryostat sections were incubated with BMK-13 at a dilution of 1:50 for 30 min at room temperature. After labelling with the second antibody, rabbit anti-mouse IgG, positively stained cells were visualised with alkaline phosphatase-anti-alkaline phosphatase method. For staining of $CD2^+$, $CD4^+$ and $CD8^+$ T lymphocytes in tissues, sections were incubated with mouse anti-rat monoclonal antibodies (Pharmingen, Cambridge Bioscience, Cambridge, UK), anti-rat CD2 (pan T cell marker), anti-rat CD4 and anti-CD8 antibodies at a dilution of 1:500 for 1 h. Biotin goat anti-mouse antibody (Pharmingen) and avidin phosphatase (DAKO, High Wycome, UK) at a dilution of 1:200 were applied for 30 min in turn.

For all tissue sections, alkaline phosphatase was developed as a red stain after incubation with Naphthol AS-MX phosphate in 0.1 M trismethylamine–HCl 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. Slides were read in a coded randomised blind fashion, under a microscope. Cells within 175 μm beneath the basement membrane were counted in all airways. Submucosal area was quantified with the aid of a computer-assisted graphic tablet visualised by a sidearm attached to the microscope. Counts were expressed as cells per square millimeter of cross-sectional subepithelial area.

2.7. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA from lung tissue of each rat was extracted separately according to the method of Chomczynski and Sacchi (1987). The yield of RNA was measured by optical density at 260 nm in a spectrophotometer. The RNA was analysed on a 1.5% agarose/formaldehyde gel in order to check for degradation, and stored at -80 °C until later use. After denaturing at 70 °C for 5 min, 1 μg of total RNA was used for reverse transcription in a 20 μl reaction volume containing $1\times$ Avian Myeloblastosis Virus buffer (50 mM Tris–HCl, pH 8.3, 50 mM KCl, 10 mM MgCl2, 10 mM dithiothreitol, 0.5 mM spermidine), 1 mM of four deoxynucleotide triphosphates (dNTP), including deoxyadenosine triphosphate (dATP), deoxycytidine triphosphate (dCTP), deoxyguanosine triphosphate (dGTP) and thymidine 5′-triphosphate (dTTP), ribonuclease inhibitor 32 U, 0.2 μg random primer pd(N)6 sodium salt (Pharmacia, Milton Keynes, UK), 8 U AMV reverse transcriptase (all apart from the random primer from Promega, Southampton, UK) at 42 °C for 60 min. 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 $1\times$ KCl or NH_4 buffer with 1.5 mM $MgCl_2$, 0.2 mM dNTP, 0.2 μg each of sense and anti-sense primers, and 1 U Taq polymerase (Bioline, London, UK) in a thermal cycler. The primers were designed according to published sequences as described in our previous study (Huang et al., 1999a). The PCR reagents were overlaid with mineral oil and amplification was carried out using a multi-well thermal cycler through 20–40 cycles of denaturation at 94 °C for 30 s, annealing at individual temperature for 30 s and extension at 72 °C for 30 s, followed by final extension at 72 °C for 10 min. The optimal PCR conditions, in terms of suitable buffer, annealing temperature and number of cycles, were determined by PCR with pooled cDNA from all samples. Annealing temperatures were 62 °C for GAPDH, interleukin-4 and interferon-γ, 58 °C for interleukin-5, and 65 °C for interleukin-2 and interleukin-10. Serial sampling every two cycles through 20–42 cycles was used to determine the exponential phase of the product amplification curve. The cycle numbers we used for PCR were 26 for GAPDH, 36 for interleukin-2, interleukin-4, and interleukin-5, and 34 for interleukin-10 and interferon-γ.

2.8. Southern blotting and Cerenkov counting

Each PCR product (10 μl) of was size-fractionated and visualised with ethidium bromide (Sigma) on 1.5% agarose

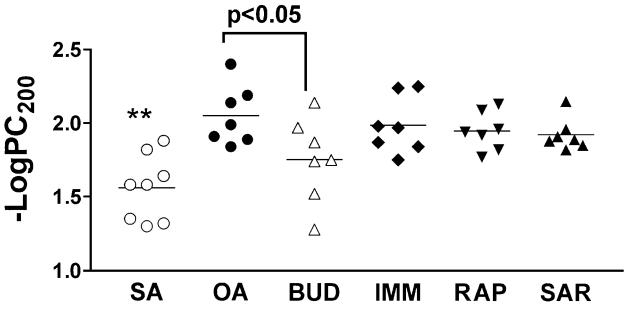

Fig. 1. Individual and mean-logPC_{200}, which is the negative logarithm of the provocative concentration of acetylcholine (ACh) needed to increase baseline lung resistance by 200%. Rats of six groups were all ovalbumin-sensitised. SA: ○, saline-treated and ovalbumin-exposed, $n=8$; OA: ●, saline-treated and 1% ovalbumin-exposed, $n=7$; BUD: □, budesonide-treated and ovalbumin-exposed, $n=7$; IMM: ◆, IMM125-treated and ovalbumin-exposed, $n=7$; RAP: ▼, rapamycin-treated and ovalbumin-exposed, $n=8$; SAR: ▲, SAR943-treated and ovalbumin-exposed, $n=7$. Ovalbumin exposure of sensitised rats induced bronchial hyperresponsiveness as reflected by an increase in-logPC_{200}, which was significantly reduced by budesonide. IMM125, rapamycin, or SAR943 did not have a significant effect. * $P<0.05$ as compared to SS group; ** $P<0.01$ as compared to other groups except group BUD.

gel electrophoresis, followed by Southern blotting to Hybond-N membrane (Amersham, Bucks, UK) and hybridisation to the appropriate cloned cDNA in order to confirm the identity of the product and, because all primer pairs cross at least one intron, to check for possible genomic contamination. Hybridisations were carried out at 65 °C overnight with the appropriate cloned cDNA, which had been ^{32}P labelled, in 6 × standard saline citrate, 10 × Denhardt's solution (0.2% w/v each of bovine serum albumin, ficol and polyvinylpyrolidone), 5 mM EDTA, 0.5% sodium dodecyl sulphate and 0.2% sodium pyrophosphate, 100 μg/ml sonicated salmon sperm DNA. In addition, 5 μl of each PCR reaction was dot-blotted onto Hybond-N membrane and also hybridised to cDNA probe. Dot blots were excised and radioactivity measured by Cerenkov counting. All measurements were made below the saturation level of a Packard 1900CA liquid scintillation analyser (Packard Instrumentation, Groningen, Netherlands). Results were generated from the counting of dot blots and expressed as a ratio of cytokine to GAPDH count, the latter used as an internal control.

2.9. Data analysis

Data were presented as mean ± S.E.M. For multiple comparison of different groups, Krüskal–Wallis test for analysis of variance was used. If the Krüskal–Wallis test for analysis of variance was significant, Mann–Whitney *U*-test was used for comparison between two selected groups. Data analysis was performed utilising SPSS for Windows statistical software package. A P value of <0.05 was considered to be significant.

3. Results

3.1. Bronchial responsiveness to acetylcholine

There was no significant difference in baseline R_L between the groups. Sensitised, saline-treated and ovalbumin-exposed rats had a significant increase in mean-logPC_{200} compared to sensitised saline-exposed rats ($P<0.002$, Fig. 1). The drugs had no effect on the baseline responsiveness

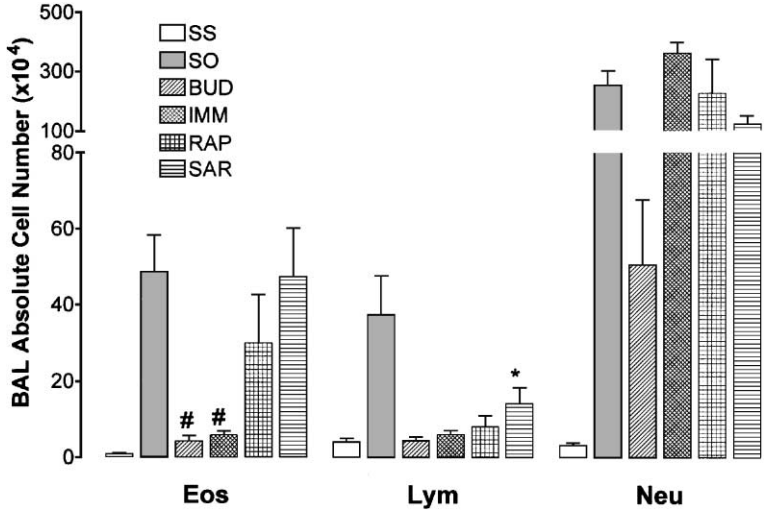

Fig. 2. Mean cell numbers of eosinophils (Eos), lymphocytes (Lym) and neutrophils (Neu) in bronchoalveolar lavage fluid. Groups of rats as specified in Fig. 1. The counts of eosinophils, lymphocytes, and neutrophils were significantly increased in sensitised rats exposed to ovalbumin aerosol. Budesonide treatment suppressed the increase in counts of all three cell types. IMM125 suppressed the allergen-induced increase in eosinophil and lymphocyte influx, but had no effect on neutrophil counts, while rapamycin and SAR943 reduced the increase in lymphocyte counts only. * $P<0.01$ as compared to SS group; # $P<0.003$ as compared to group SO. Data shown as mean ± S.E.M.

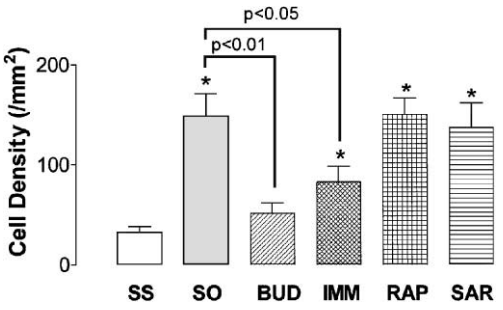

Fig. 3. Mean eosinophil counts in airway submucosa. Groups of rats as detailed in Fig. 1. There was a significant increase in eosinophils in the airway submucosa following allergen challenge of sensitised rats, which was suppressed by pre-treatment with budesonide and IMM125. * $P<0.01$ as compared to group SS. Data shown as mean ± S.E.M.

to acetylcholine. Budesonide significantly suppressed allergen-induced bronchial hyperresponsiveness ($P<0.05$ compared to ovalbumin-sensitised and exposed rats treated with saline), but rapamycin, SAR943, and IMM125 had no effect.

3.2. Inflammatory cell responses

3.2.1. Bronchoalveolar lavage

There was a significant increase in the numbers of total cells, eosinophils, lymphocytes and neutrophils recovered in bronchoalveolar lavage fluid of sensitised rats exposed to ovalbumin compared to sensitised rats exposed to saline ($P<0.005$, Fig. 2). Ovalbumin exposure or treatment with drugs had no effect on the numbers of macrophages in bronchoalveolar lavage fluid, while ovalbumin-induced increase in total cell counts was significantly reduced by budesonide only ($P<0.03$, data not shown). The increase of inflammatory cells in bronchoalveolar lavage fluid induced by ovalbumin exposure of sensitised rats was suppressed by budesonide ($P<0.01$, Fig. 2). IMM125 suppressed the increased numbers of eosinophils and lymphocytes ($P<0.003$, Fig. 2) but not neutrophils in bronchoalveolar lavage fluid, while rapamycin and SAR943 reduced lymphocyte influx only ($P<0.02$, Fig. 2).

3.2.2. Airway tissues

Allergen exposure of sensitised rats caused a significant increase in eosinophil infiltration in airway submucosa ($P<0.002$, Fig. 3). The eosinophilia was significantly reduced by budesonide and IMM125 ($P<0.01$ and 0.05, respectively as compared to ovalbumin-sensitised and exposed, sham-treated rats, Fig. 3). Allergen exposure of sensitised rats caused a significant increase in $CD2^+$ T cell, $CD4^+$ T cells, and $CD8^+$ T cell counts ($P<0.05$, Fig. 4). Budesonide reduced the allergen-induced increases in all three subsets of T cells, but the reduction in $CD4^+$ T cells was not statistically significant. The increases in $CD8^+$ T cell counts induced by allergen exposure was also significantly reduced by pre-treatment with IMM125 and rapamycin ($P<0.03$, Fig. 4). Rapamycin, SAR943, and IMM125 had no significant effect on the counts of submucosal $CD2^+$ and $CD4^+$ T lymphocytes.

3.3. Cytokine expression in lungs

In sensitised rats, ovalbumin exposure induced a significant increase in interleukin-2, interleukin-4, interleukin-5, interleukin-10, and interferon-γ mRNA expression ($P<0.03$, Fig. 5). Budesonide suppressed ovalbumin-induced increase in interleukin-2, interleukin-4, and interleukin-5 mRNA expression ($P<0.03$, Fig. 5). IMM125 reduced allergen-induced increase in interleukin-4, interleukin-5, and

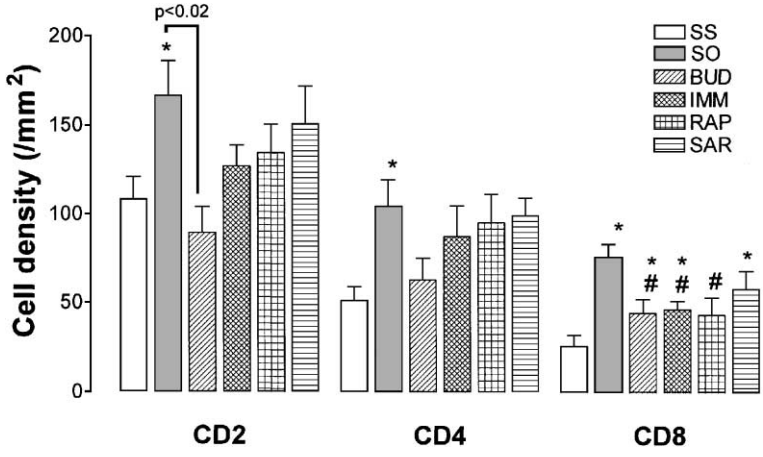

Fig. 4. Mean CD_2^+, CD_4^+ and CD_8^+ T cell counts in airway submucosa. Groups as detailed in Fig. 1. Allergen challenge caused a significant increase in total number of $CD2^+$, $CD4^+$, and also $CD8^+$ T cells. Budesonide significantly reduced $CD2^+$ and $CD8^+$ T cells, but the reduction in $CD4^+$ T cells was statistically non-significant in comparison to ovalbumin-sensitised and challenged group SO. Intratracheal administration with IMM125 and rapamycin also significantly reduced the number of $CD8^+$ T cells. * $P<0.05$ as compared to group SS; #$P<0.03$ as compared to group SO. Data shown as mean ± S.E.M.

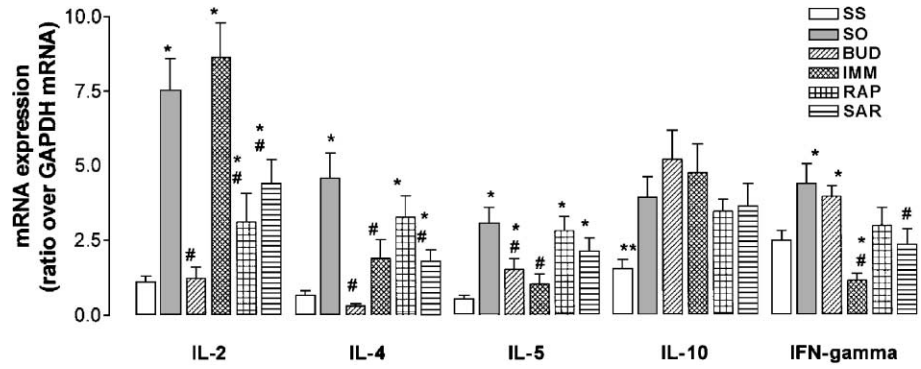

Fig. 5. Mean interleukin (IL)-2, IL-4, IL-5, IL-10 and interferon (IFN)-γ mRNA expression. Expression in rat lung is expressed as a ratio to GAPDH mRNA as determined by reverse-transcription polymerase chain reaction, followed by Southern blot analysis. The expression was obtained on a radioactive probe-hybridised dot blot of the polymerase chain reaction products. An increase in the mRNA expression for all five cytokines was noted after ovalbumin exposure of sensitised rats (group SO). Budesonide significantly reduced mRNA expression of IL-2, IL-4 and IL-5, while IMM125 reduced the allergen-induced increase in mRNA expression for IL-4, IL-5, and IFN-γ. Pre-treatment with rapamycin suppressed the increase in IL-2 mRNA expression, while its analogue, SAR943, reduced the increase in mRNA expression for IL-2, IL-4, and IFN-γ. * $P<0.05$ as compared to group SS; ** $P<0.05$ as compared to other groups; # $P<0.05$ as compared to group SO. Data shown as mean ± S.E.M. of lungs from each animal in each experimental group.

interferon-γ mRNA expression ($P<0.02$). Rapamycin and its analogue, SAR943, suppressed the increase in interleukin-2 mRNA induced by allergen exposure of sensitised rats ($P<0.05$), while SAR943 also showed a suppressive effect on the expression of interleukin-4 and interferon-γ mRNA ($P<0.04$). Rapamycin had no significant effects.

4. Discussion

We have shown that the topical administration of the cyclosporin A analogue, IMM125, attenuated bronchoalveolar lavage and tissue eosinophil and lymphocyte but not neutrophil influx, while reducing airway submucosal accumulation of $CD8^+$ T cells and lung mRNA expression for interleukin-4, interleukin-5, and interferon-γ induced by allergen exposure. The selective suppression of $CD8^+$ T cells, together with reduced bronchoalveolar lavage lymphocyte influx, was also observed in rats treated with rapamycin or with its analogue SAR943. Intratracheal rapamycin and SAR943 reduced the allergen-induced upregulation of interleukin-2 mRNA induced by allergen exposure, while SAR943 also significantly inhibited interleukin-4 and interferon-γ mRNA expression. Both rapamycin and SAR943 did not inhibit allergen-induced eosinophilia in bronchoalveolar lavage or in the airway tissue. Despite these effects on cellular influx and cytokine expression, the intratracheally administered immunomodulators did not inhibit allergen-induced bronchial hyperresponsiveness. By contrast, topical administration of budesonide successfully reduced airway inflammatory cell recruitment, bronchial hyperresponsiveness, and mRNA expression for interleukin-2, interleukin-4 and interleukin-5.

IMM125 did not inhibit interleukin-2 mRNA expression, but reduced interleukin-5 mRNA expression to a greater extent than budesonide, whereas rapamycin and its analogue, SAR943, inhibited interleukin-2 but not interleukin-5 mRNA expression. SAR943 also inhibited interleukin-4 expression. IMM125 inhibited allergen-induced eosinophilia, while rapamycin and SAR943 did not, indicating that the expression of interleukin-5 may be important for the induction of allergen-induced eosinophilia. Interleukin-2 expression was not related to allergen-induced airway infiltration of eosinophils, despite the fact that interleukin-2 administration to the Brown–Norway rat can induce an airway inflammatory response with eosinophil, lymphocyte and mast cell infiltration (Renzi et al., 1992). By contrast, none of these three immunomodulators inhibited allergen-induced bronchial hyperresponsiveness. In terms of the modulation of cytokine profile, it is possible that the reduction of interferon-γ particularly by IMM125 and by SAR943 may have contributed to this lack of suppression of bronchial hyperresponsiveness since interferon-γ in our model is a modulator of allergen-induced bronchial hyperresponsiveness and partly mediates Th1 inhibition of Th2-mediated bronchial hyperresponsiveness (Huang et al., 1999b, 2001). Budesonide inhibited bronchial hyperresponsiveness (Huang et al., 1999c) associated with persistent enhancement of interferon-γ expression. In addition, budesonide inhibited interleukin-2 and interleukin-4 expression to the greatest extent, together with a reduction in interleukin-5 after allergen challenge.

Despite suppressing the infiltration of eosinophils and $CD8^+$ T cells, IMM125 did not alter allergen-induced bronchial hyperresponsiveness, which is similar to the effects observed with cyclosporin A administered systemically (Huang et al., 1999b; Elwood et al., 1992). Rapamycin and SAR943 did not inhibit allergen-induced eosinophilia and bronchial hyperresponsiveness. The dissociation of bronchial hyperresponsiveness and eosinophilic infiltration has been observed in other studies. For example, adoptive transfer of ovalbumin-specific $CD4^+$ T cells to recipient

Brown–Norway rats induces both eosinophilia and bronchial hyperresponsiveness, while ovalbumin-specific $CD8^+$ T cells induces a mild degree of eosinophilia without bronchial hyperresponsiveness (Haczku et al., 1997). Systemic administration of blocking antibodies against leukocyte adhesion molecule CD11b inhibited allergen-induced bronchial hyperresponsiveness without affecting bronchoalveolar lavage eosinophilia (Sun et al., 1994). This dissociation suggests that other mechanism(s) parallel to or independent of inflammatory cell recruitment exists.

$CD8^+$ T cells in airway tissue and total T cell counts in bronchoalveolar lavage fluid were suppressed by all the immunosuppressants used in our study, while $CD4^+$ Th2 cells were only inhibited by IMM125 and budesonide, but not by rapamycin or SAR943, as reflected in the alteration in cytokine mRNA expression. Rapamycin has a strong antiproliferative effect on T cells stimulated via the CD3/TCR or CD28 pathways, and on activated T cells stimulated by interleukin-2, interleukin-4, or interleukin-12 (Dumont et al., 1990; Luo et al., 1993; Bertagnolli et al., 1994), and a preferential inhibition of $CD8^+$ T cell expansion, and of $CD4^+$ Th1- but not Th2-associated cytokines is reported in a graft-versus-host model (Bertagnolli et al., 1994). Rapamycin may also upregulate Th2 cytokines, interleukin-10 and interleukin-4, but not interleukin-2, in transplantation tolerance and prevention of autoimmune diseases (Ferraresso et al., 1994), indicating different effects on different disease processes. Our data indicates that its predominant effect in a model of allergic inflammation is to decrease $CD8^+$ T cell numbers. Nagai et al. (1997) also reported a lack of inhibition of allergen-induced airway eosinophilia and bronchial hyperresponsiveness by rapamycin in a mouse model, despite an inhibition of the immunoglobulin E response.

Previous studies have shown that cyclosporin A may enhance the production of interleukin-13 from $CD4^+$ and $CD8^+$ T cells, but inhibit interleukin-4 (Van der Pouw Kraan et al., 1996), and that in in vivo studies, it may increase IgE levels and pulmonary eosinophilia (Wang et al., 1993). Cyclosporin A administered after sensitisation of Brown–Norway rats to trimellitic anhydride did not reduce IgE antibody production (Pullerits et al., 1997). IMM125, and rapamycin or SAR943 may favour the development of Th2 response by the enhancement of T cell production of certain Th2 promoting cytokines, such as interleukin-13, which may induce interleukin-4-independent IgE synthesis (Punnonen et al., 1993), or by inhibiting Th1 and $CD8^+$ T cells. Our results are compatible with this speculation in that IMM125 inhibited interleukin-4 and interleukin-5 mRNA expression without accompanying suppression of bronchial hyperresponsiveness, and in that rapamycin and SAR943 selectively suppressed submucosal accumulation of $CD8^+$ T cells and mRNA expression for interleukin-2 and interferon-γ. Thus, IMM125, rapamycin and SAR943 may induce a more sustained Th2 response in asthma, and this also supports the hypothesis that allergen-induced airway inflammation is Th2-mediated.

The model that we have studied represents an acute allergic response to a single allergen exposure, and since the immunomodulators that we have studied have a chronic effect on T cells, the model may not be entirely appropriate for studying the effects of the immunomodulators. Thus, their effects may not have been observed. A more chronic model of allergic inflammation needs to be studied.

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