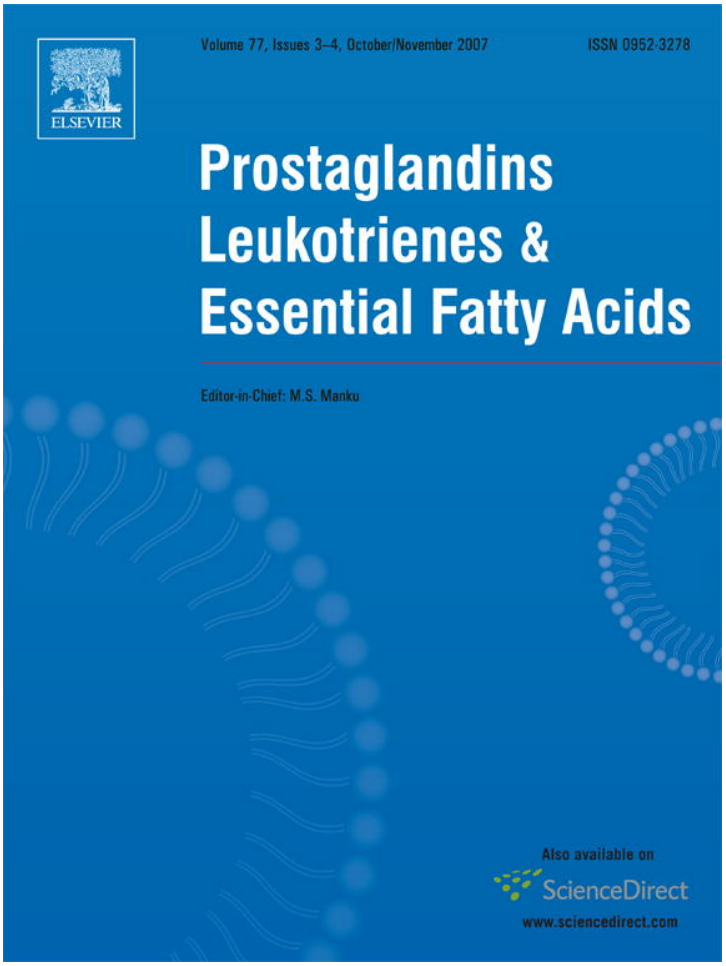

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Prostaglandins, leukotrienes and PAF selectively modulate lymphocyte subset and eosinophil infiltration into the airways in a murine model of asthma

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Abstract

The effects of inhibitors of prostaglandins synthesis, indomethacin and nimesulide, or of receptor antagonists of cysteinyl-leukotrienes, MK571 or of platelet activating factor (PAF), WEB2170, were studied on the infiltration of lymphocytes (T$\gamma\delta$, NKT, CD_4, CD_8 and B cells) and eosinophils into the bronchoalveolar lavage fluid (BALF) in two mouse strains (C57Bl/6 and BALB/c) as well as on bronchial hyperreactivity and mucus production. It was found that indomethacin and nimesulide strongly reduced the number of all cell types analyzed in both mouse strains. MK571 did not affect T$\gamma\delta$ or CD_4 lymphocytes but reduced the other populations. WEB2170 reduced all lymphocyte subpopulations in both mouse strains. Moreover, the relative numbers of the lymphocyte subsets in the airways and their response to PAF antagonist were strain-dependent. The intensity of bronchoconstriction and mucus production did not correlate with BALF cell types or numbers. The cysteinyl-leukotriene receptor antagonist inhibited eosinophil infiltration and bronchial hyperreactivity, without affecting the T$\gamma\delta$ cell subset. Since T$\gamma\delta$ cells play a major role in mucosa protection and resolution of lung inflammation, this would represent an additional benefit of cysteinyl-leukotrienes antagonism in asthma.

1. Introduction

Allergic asthma is a chronic inflammatory disease associated with the migration of T lymphocytes predominantly of the Th2 profile. Lung biopsies have revealed that in human asthma, eosinophilic and lymphocytic infiltrations consistently occur in the epithelium and lamina propria. The T$\gamma\delta$ and NKT cells are commonly located in mucosal and epithelial sites where they are present in small numbers but exert important immunoregulatory functions. T$\gamma\delta$ and NKT cells do not recognize antigen by the conventional MHC/peptide way. The T$\gamma\delta^+$ binds to several ligands (MHC-like molecules, phospholipids, heat shock proteins) expressed by epithelial cells following trauma or stress. Substantial increase in pulmonary T$\gamma\delta$ cells was observed after prolonged airway stimulation with antigen [1]. Following *in vivo* allergen provocation of asthmatic patients, the number of T$\gamma\delta$ lymphocytes significantly increased [2]. The T$\gamma\delta^+$ cells appear to play a major role in the protection of the mucosa by eliminating damaged epithelial cells and thus, contribute to the remarkable resistance of the lung to environmental stimuli [3]. It has been recently suggested that the T$\gamma\delta$ helps to restore the number of macrophages to homeostatic levels contributing to the resolution of lung inflammation [4].

NKT cells are members of another cell population that is normally present in small numbers in human lung interstitium [5] and is increased in asthmatic patients [6]. The activity of these cells is increased after bronchial

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allergen challenge in asthmatic subjects [7]. Mouse NK1.1$^+$ cells represent a small population of NK cells that express T cell receptor, a characteristic feature of NKT cells [8]. A human counterpart of mouse NKT cells has been identified [9] but the role of these cells in allergic lung diseases has never been investigated.

Experimental evidence suggests that the T$\gamma\delta$ and NKT cells produce Th2 cytokines and may be critical in induction/regulation of Th2 response. Studies using T$\gamma\delta$ deficient mice showed that these cells are required for inducing allergen specific IgE and IgG1 isotypes and consequently affect the Th2-mediated lung inflammation [10].

Lipid mediators such as prostaglandins, leukotrienes and platelet activating factor (PAF) are involved in the pathogenesis of asthma where they contribute to early events such as inflammatory cell infiltration, bronchial hyperreactivity and mucus secretion [11]. However, it is not known if these mediators affect the recruitment of lymphocytes, particularly the T$\gamma\delta$ cells and NKT subsets. We have previously shown, in a murine model of lung inflammation, a clear increase in the number of TCD_4^+, TCD_8^+, B, T$\gamma\delta^+$ and NK1.1$^+$ in the bronchoalveolar lavage fluid (BALF) of immunized mice following antigen challenge [12]. In the present study, using the same model, we investigated the effect of inhibitors or receptor antagonists of the lipid mediators, prostaglandins, leukotrienes and PAF, on the number of these lymphocytes subsets and eosinophils in the BALF. In parallel, we investigated the effect of these treatments on two pathological events observed in experimental asthma, bronchial hyperreactivity and mucus secretion, in an attempt to see if alterations in lymphocyte subsets would correlate with the intensity of the disease parameters. These studies were performed in two mouse strains which are commonly used in asthma research, BALB/c and C57Bl/6 mice. BALB/c is considered a Th2 strain producing high levels of antigen-specific IgE antibodies whereas C57Bl/6 mice produce much lower levels of this antibody isotype [13].

2. Materials and methods

2.1. Animals

Male C57Bl/6 and BALB/c mice weighing 20–25 g, 6–8 weeks old, from our own animal facilities were housed in a room with 12 h light–dark cycle with water and food ad libitum. Animal care and research protocols were in accordance with the principles and guidelines adopted by the Brazilian College of Animal Experimentation (COBEA) and approved by the Biomedical Sciences Institute/USP–Ethical Committee for Animal Research (CEEA).

2.2. Immunization protocol

Mice were sensitized on days 0 and 7 by an intraperitoneal injection of a mixture containing 50 μg of ovalbumin and 1 mg of $Al(OH)_3$ in saline (a total volume of 0.2 ml). At 14 and 21 days after the first immunization the animal were challenged by exposure to an aerosol of ovalbumin (grade III, Sigma) generated by an ultrasonic nebulizer (ICEL US-800, SP, Brazil) delivering particles of 0.5–10 μm diameter at approximately 0.75 ml min^{-1} for 20 min. The concentration of ovalbumin in the nebulizer was 2.5% (w/v).

The control group consisted of animals immunized as described, and challenged with saline solution. Groups of animals where treated 30 min before each challenge with effective doses of a cysteinyl-leukotriene receptor antagonist (MK 571, 2 mg kg^{-1} i.p.), a selective COX2 inhibitor (Nimesulide, 5 mg kg^{-1} i.p), a cycloxygenase inhibitor (indomethacin, 4 mg kg^{-1} i.p.) or a PAF receptor antagonist (WEB 2170, 5 mg kg^{-1} i.p.).

2.3. Bronchoalveolar lavage

The animals were killed by injection of ketamine/xylazine (50 μl of a 100 mg ml^{-1} solution, i.p.) 24 h after exposure to the second aerosol challenge. A tracheal cannula was inserted via a midcervical incision and the airways were lavaged twice with 1 ml of phosphate buffered saline (PBS; pH 7.4 at 4 °C).

2.4. Total and differential cell counts

The BALF was centrifuged at 170*g* for 10 min at 4 °C, the supernatant was removed, and the cell pellet was resuspended in 0.5 ml of PBS. One volume of a solution containing 0.5% crystal violet dissolved in 30% acetic acid was added to nine volumes of the cell suspension. The total number of cells was determined by counting in a hematocytometer. Differential cell counts were performed after cytocentrifugation and staining with hematoxylin–eosin (Hema 3).

2.5. Flow cytometric analyses of lymphocytes

Phenotypic analysis of lymphocyte subpopulations were assessed by three color FACS, using a Facscalibur Cytometer equipped with Cell Quest software (Becton & Dickinson, San Jose, CA, USA), using gates defined by forward and side light scatter properties. Bronchoalveolar lavage cells were incubated with fluorescein isothiocyanate (FITC), R-phycoerythrin (PE) or Cytochrome-labeled monoclonal antibodies anti-CD_4 (clone H129.19), anti-CD_8 (clone 53–6.7), anti-CD45R/B220 (clone RA3-6B2), anti-T$\gamma\delta$ (clone GL3) or anti-NK-1.1 (clone PK136) and adjusted to 1×10^5 cells/ml in PBS

supplemented with 5% fetal bovine serum and sodium azide (0.1%).

2.6. Evaluation of airway reactivity

Mice were immunized and submitted to two antigen aerosol exposures as described above. Twenty-four hours after the second antigen challenge, mice received an injection of ketamine/xylazine (50 μl of a 100 mg ml^{-1} solution, i.p.); the peritoneal cavity was cut open and animals were exsanguinated by section of the abdominal aorta. The thoracic cavity was then opened, and the pulmonary artery was cannulated and perfused with 10 ml of Krebs solution at 10 ml min^{-1}. A cannula was then inserted in the trachea; the lungs were removed carefully and perfused (5 ml min^{-1}) through the trachea with Krebs solution (37 °C, 95% O_2 and 5% CO_2). A small incision was made in the lower end of each lobe to permit the outflow of the perfusate. The perfusion pressure was recorded in a Beckman R511A using Gould P23DB pressure transducers. Increases over basal levels of perfusion pressure following bolus injection of methacholine were taken as a measure of constriction of the airways. Increase in perfusion pressure (cm H_2O) as a function of the dose (μg) of methacholine was determined for the entire recording period. The areas under the curve were calculated, and the results were expressed as mean area under the curve (mm^2).

2.7. Histological analysis of mucus

Lungs were dissected after the recovery of the BALF, perfused via the right ventricle with 10 ml PBS to remove residual blood, immersed in 10% phosphate-buffered formalin for 24 h and then kept in 70% ethanol until embedding in paraffin. Tissues were sliced (5 μm sections) and stained with hematoxylin/eosin for light microscopy examination or with periodic acid-Schiff (PAS)/hematoxylin for evaluation of mucus producing cells. The intensity of mucus production was evaluated in each preparation and scores from 0 to 3 were attributed: 0 when none of the bronchi showed any sign of mucus; 1, 2 or 3 when 25%, 50% or more than 50% of the bronchial epithelium was covered by mucus, respectively. Values shown for each lung represent the sum of 10 bronchi scored randomly at 250 × magnification.

2.8. Extraction and quantification of eicosanoids

The lung tissues were homogenized, centrifuged and the supernatants were acidified to pH 3.4–3.6 with HCl 1 N and passed slowly through an octadecylsilyl silica column (Sep Pak C 18 column), pre-washed with 10 ml of ethanol and 10 ml of water. After washing the column with 10 ml water and 1 ml ethanol (35%), the eicosanoids were eluted from the column with 2 ml absolute ethanol and the samples dried under a stream of nitrogen. LTC_4/D_4 and PGE_2 concentrations were determined by using EIA kits (Cayman Chemical Co., MI, USA) according to the method of Pradelles et al. [14]. Briefly, dilutions of the supernatants were incubated with the conjugated eicosanoid–acetylcholinesterase complex and with the specific antiserum in 96-well plates precoated with anti-rabbit immunoglobulin G antibodies. After an overnight incubation at 4 °C, the plates were washed and the enzyme substrate (Ellman's reagent) was added for 60–120 min at 25 °C. The optical density of the samples was determined at 412 nm in a microplate reader, and the concentration of eicosanoids was calculated from a standard curve.

2.9. Drugs and reagents

The following drugs and chemicals were used: leukotriene receptor antagonist MK 571, prostaglandin synthesis inhibitor nimesulide (Cayman Chemical, Ann Arbor, MI, USA); PAF receptor antagonist WEB 2170 (Boehringer-Ingelheim, Frankfurt, Germany); indomethacin, ovalbumin grade III, methacholine and fetal bovine serum (Sigma Chemical Co., St. Louis, MO, USA); monoclonal antibodies to lymphocyte markers (BD Pharmingen, San Diego, CA, USA); Hema 3 (Biochemical Sciences Inc., Swedesboro, NJ, USA) and aluminum hydroxide gel "Rehydragel" (Reheis Inc., Berkley Heights, NJ, USA).

2.10. Statistical analysis

Data are expressed as the mean ± S.E.M. Statistical evaluation of the data was carried out by analysis of variance (ANOVA) and sequential analysis of differences among means was done by Tukey's contrast analysis. A P-value lower than 0.05 was considered to be significant. $P<0.05$, $P<0.01$ and $P<0.001$ were marked with one, two or three asterisks, respectively.

3. Results

3.1. Effect of lipid mediator inhibitors/antagonists on lung cell infiltration

Mice immunized with ovalbumin were submitted to two ovalbumin aerosol challenges and bronchoalveolar lavage was performed 24 h after the second aerosol challenge. Control group consisted of immunized and saline challenged mice. As shown in Fig. 1A and B (C57Bl/6 mice), the number of eosinophils present in the BALF of the control group was very low (less than 1 cell/ml). In the immunized and antigen challenged

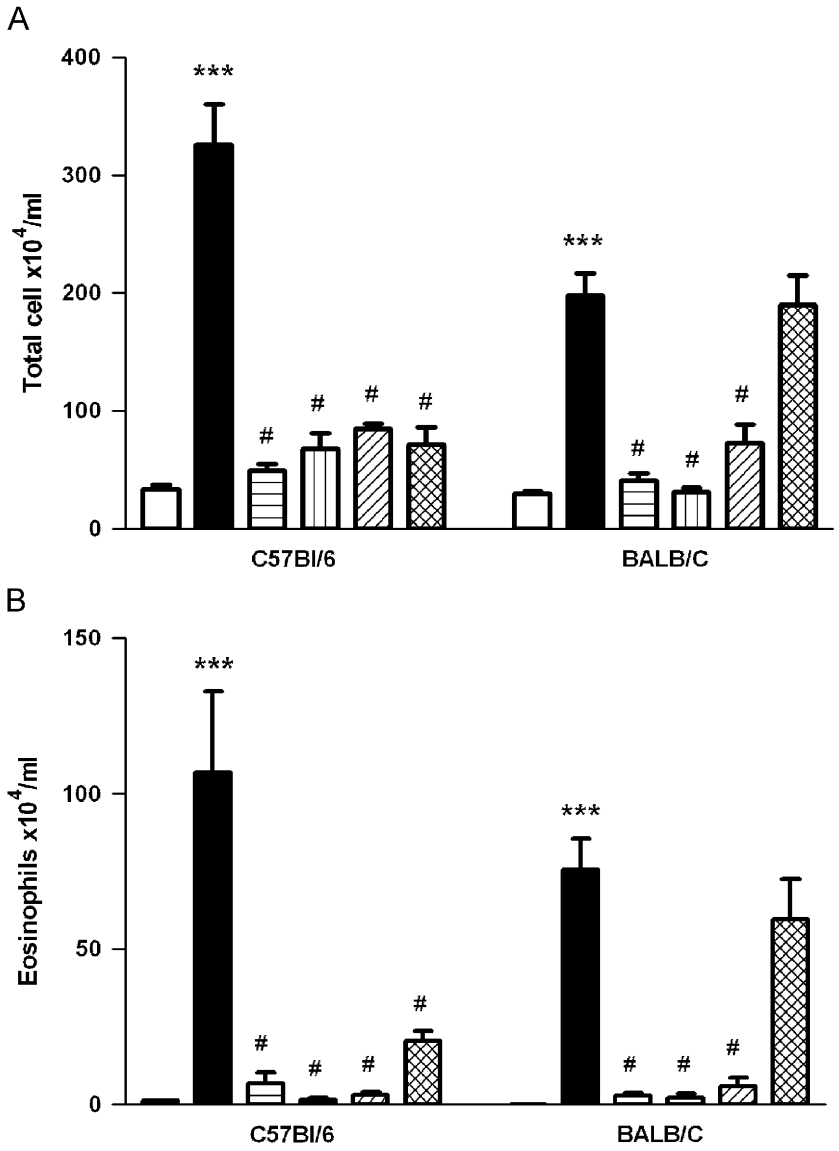

Fig. 1. Effects of indomethacin, nimesulide, MK 571 and WEB 2170 on the number of cells in the BALF. C57Bl6 and BALB/c mice were immunized with OVA and challenged twice with OVA aerosol, one week apart (■). Control group consisted of immunized mice that received saline aerosol (□). Twenty-four hours after the second challenge, bronchoalveolar lavage was performed and the cells counted. Eosinophils were counted in preparations stained with hematoxylin/eosin. Drugs were given i.p 30 min before each aerosol challenge at the following doses: 4 mg kg^{-1} indomethacin (▤); 5 mg kg^{-1} nimesulide (▥); 2 mg kg^{-1} MK571 (▨); 5 mg kg^{-1} WEB2170. (▩). Results are the mean ± S.E.M. of 7–8 animals per group. $^{***}P<0.001$ in comparison with the control group and $^{\#}P<0.01$ in comparison with the OVA challenged group.

lungs, there were marked increases in total cell (33.7 ± 3.8–326.3 ± 34.3 cells $\times 10^4$ ml^{-1}) and eosinophil (0.9 ± 0.2–106.3 ± 30 cells $\times 10^4$ ml^{-1}) counts in the BALF. As shown in Fig. 1A and B, the number of eosinophils present in the BALF of the control group of BALB/c was also very low (less than 1 cell/ml). In the immunized and antigen-challenged lungs, there were also marked increases in total cells number (29.8 ± 1.9–198.1 ± 19 cells $\times 10^4$ ml^{-1}) and eosinophils (0.05 ± 0.2–75.3 ± 10 cells $\times 10^4$ ml^{-1}) in the BALF. Eosinophils were the predominant cell type in the experimental groups accounting for 32% (C57Bl/6) and 38% (BALB/c) of the total cells in the BALF. It is also noteworthy that the total cell infiltration is lower in BALB/c compared to C57Bl/6 mice.

Groups of immunized mice received intraperitoneal injections of selected effective doses of prostaglandin synthesis inhibitors (indomethacin, 4 mg kg^{-1} or nimesulide, 5 mg kg^{-1}), or antagonists of cysteinyl-leukotrienes (MK 571, 2 mg kg^{-1}) or PAF receptors (WEB 2170, 5 mgkg^{-1}), 30 min before each of the aerosol antigen challenges. Fig. 1A and B shows that all treatments significantly decreased the number of total cells and eosinophils in the BALF in C57Bl/6

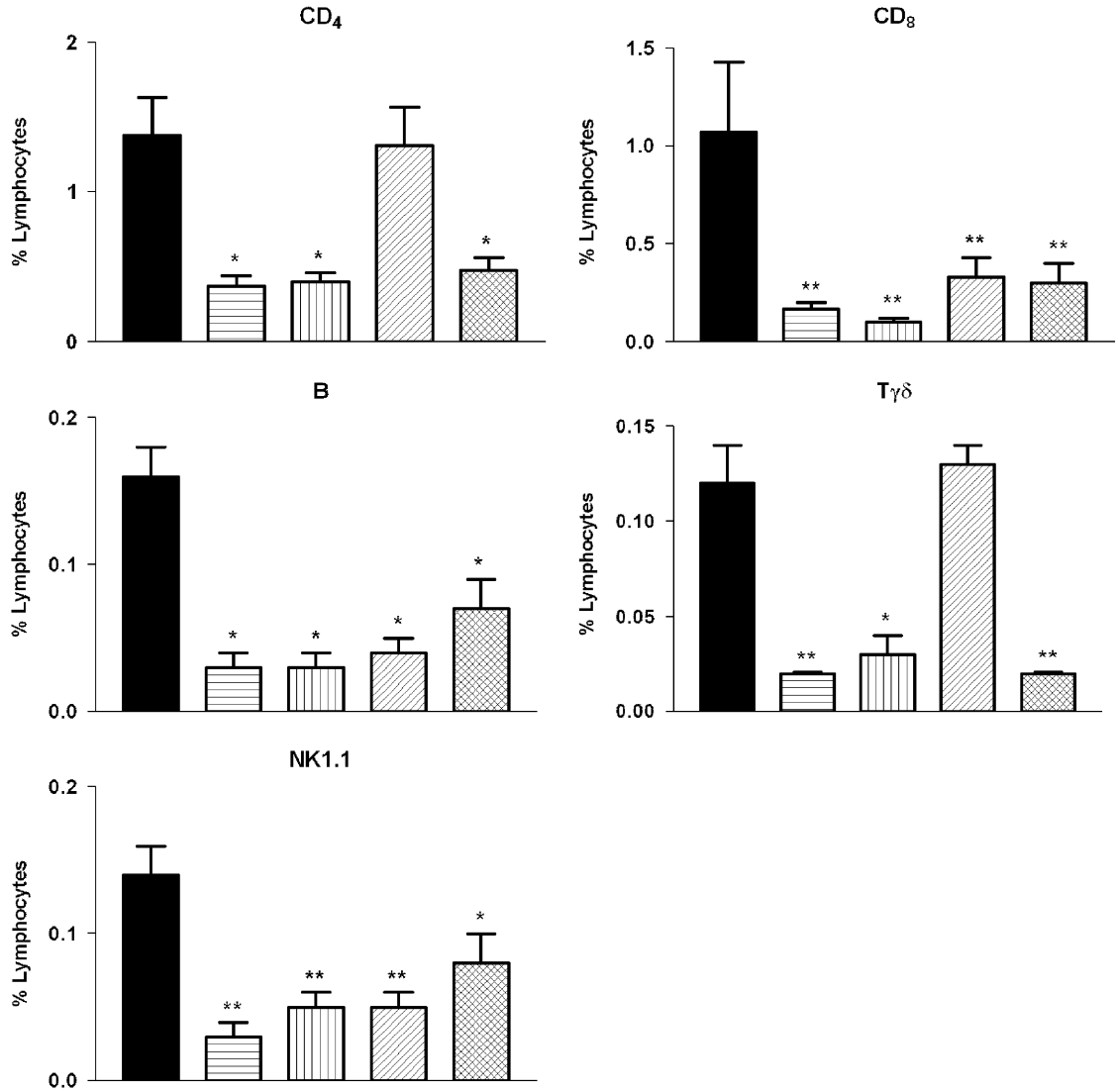

Fig. 2. Effects of indomethacin, nimesulide, MK 571 and WEB 2170 on BALF lymphocyte subpopulations in C57Bl/6 mice. Mice were immunized, challenged and treated as described in Fig. 1. Bronchoalveolar lavage was performed 24 h after the second challenge. The cells were incubated with fluorochrome-labeled monoclonal antibodies to NK1.1, T$\gamma\delta$, CD_4, CD_8, and B220, submitted to FACS analysis. (■) immunized/OVA; (▤) immunized/OVA + indomethacin; (▥) immunized/OVA + nimesulide; (▨) immunized/OVA + MK 571 and (▩) immunized/OVA + WEB 2170. Results are the mean ± S.E.M. of 6–8 animals per group. $^{*}P<0.05$ and $^{**}P<0.01$ in comparison with OVA challenged group.

mice although the treatment with cysteinyl-leukotriene receptor antagonist did not reduce the cell infiltration in BALB/c mice.

In a separate series of experiments, BALF cells were incubated with monoclonal antibodies to the cell markers NK 1.1^+, T$\gamma\delta^+$, CD_4^+, CD_8^+, $B220^+$ as described in Section 2 and submitted to flow cytometry analyses. Fig. 2 shows that whereas these cell populations are either undetectable or found in very low number in the control group (immunized animals given saline aerosol), significant increases in all cell populations were observed in the immunized and challenged C57Bl/6 mice. Treatment with indomethacin or nimesulide or with WEB 2170 before each of the antigen challenges significantly decreased T$\gamma\delta^+$, CD_4^+ $NK1.1^+$, CD_8^+ and $B220^+$ cells in the BALF. Treatment with MK 571 had no effect on T$\gamma\delta^+$ and CD_4^+ lymphocytes but reduced the other cell types. Similar profile of drug inhibition was observed in BALB/c mice (Fig. 3).

Tissue levels of prostaglandins and leukotrienes C_4/D_4 were significantly higher in the animals which were sensitized and challenged than in the control group (405.7 ± 13.3–42.304 ± 727.6 pg/ml for prostaglandin E_2 and 23.7 ± 3.8–64.31 ± 7.9 pg/ml for leukotrienes). The concentration of indomethacin and nimesulide used in our studies reduced by approximately 88% the levels of prostaglandin E_2 in the lung tissue.

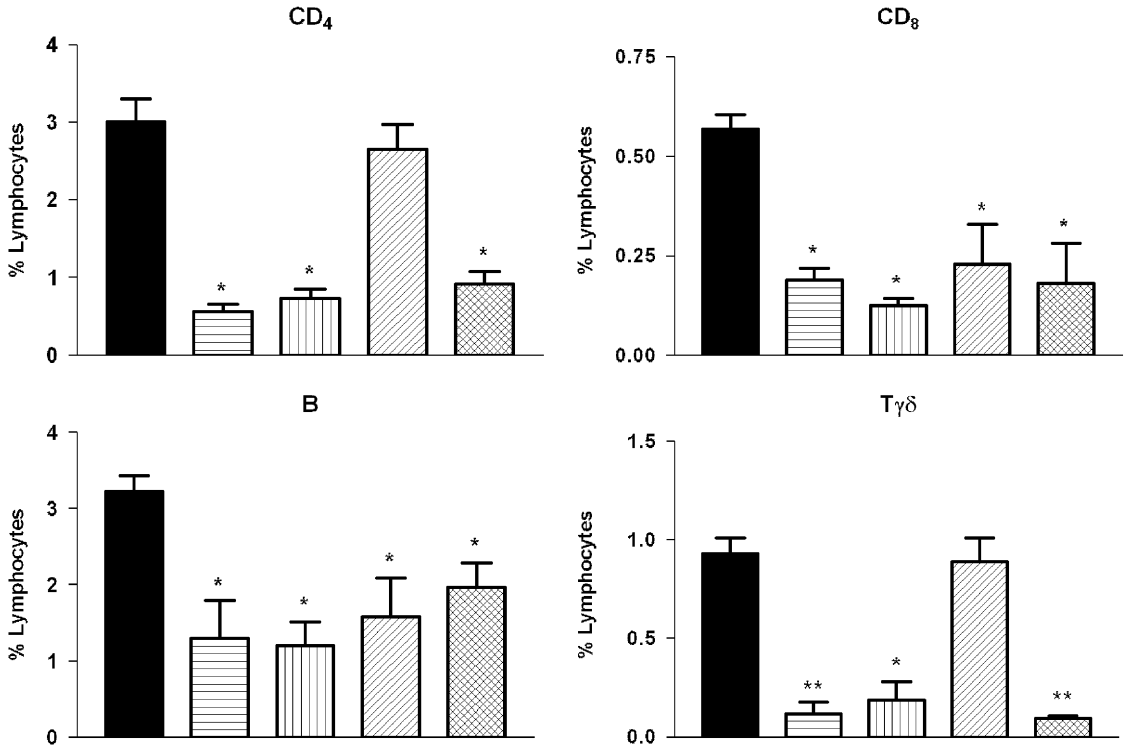

Fig. 3. Effects of indomethacin, nimesulide, MK 571 and WEB 2170 on the bronchoalveolar lavage lymphocyte subpopulations in BALB/c mice. Mice were immunized, challenged and treated as described in Fig. 1. Bronchoalveolar lavage was performed 24 h after the second challenge. The cells were incubated with fluorochrome-labeled monoclonal antibodies to T$\gamma\delta$, CD_4, CD_8, and B220, submitted to FACS analysis. (■) immunized/OVA; (▤) immunized/OVA + indomethacin; (▥) immunized/OVA + nimesulide; (▨) immunized/OVA + MK 571; and (▩) immunized/OVA + -WEB2170. Results are the mean ± S.E.M. of 6–8 animals per group. $^{*}P<0.05$ and $^{**}P<0.01$ in comparison with OVA challenged group.

3.2. Effects of lipid mediator inhibitors/antagonists on airway hyperreactivity and mucus

The lungs of immunized mice that were administered two aerosol ovalbumin challenges were dissected 24 h later and perfused through the trachea as described in Section 2. Bolus injections of increasing doses of methacholine (0.1–100 μg) caused bronchoconstrictions as measured by the increases in perfusion pressure. Fig. 4A shows that the reactivity of the airways to methacholine of the challenged group was significantly higher than that of the control group (around 30% higher comparing areas under the curves).

The bronchoconstriction induced by the antigen challenge in C57Bl/6 mice was significantly reduced by the pre-treatment with indomethacin (97%), nimesulide (41%) and MK 571 (95%). However, pre-treatment with WEB 2170 did not affect the bronchoconstriction (Fig. 4A). In BALB/c mice the bronchoconstriction was also significantly reduced by pre-treatment with indomethacin (74%), nimesulide (80%) and MK 571 (72%).

In another series of experiments, the intensity of mucus production was evaluated in the bronchi ($n = 10$) and scored from 0 to 3 as described in Section 2. In the control group none of the bronchi contained mucus (score 0) in contrast to the immunized group where the scores averaged 1.5. Mucus plugs were not observed in any group. It can be seen in Fig. 4B that lung pre-treatments with indomethacin or nimesulide significantly inhibited mucus production in the C57Bl/6 bronchi (65% and 55%, respectively) and BALB/c bronchi (66% and 58%, respectively). However, the pre-treatment with MK 571 or WEB 2170 did not significantly affect mucus production.

4. Discussion

Our results showed that the use of prostaglandin synthesis inhibitors, cysteinyl-leukotriene receptor antagonist or PAF receptor antagonist reduced significantly the influx of total cells and eosinophils in the BALF of sensitized C57Bl/6 mice, 24 h after the second antigen challenge. In BALB/c mice however, the PAF antagonist did not reduce the cell infiltration. Previous studies showed an important role of these lipid mediators in asthma and suggested that eosinophils are the main cells that migrate into the lungs and are responsible for the production of these mediators in selected asthma models [15]. The production of prostaglandins in the airways is well known and documented, but it is not clear whether these mediators play a

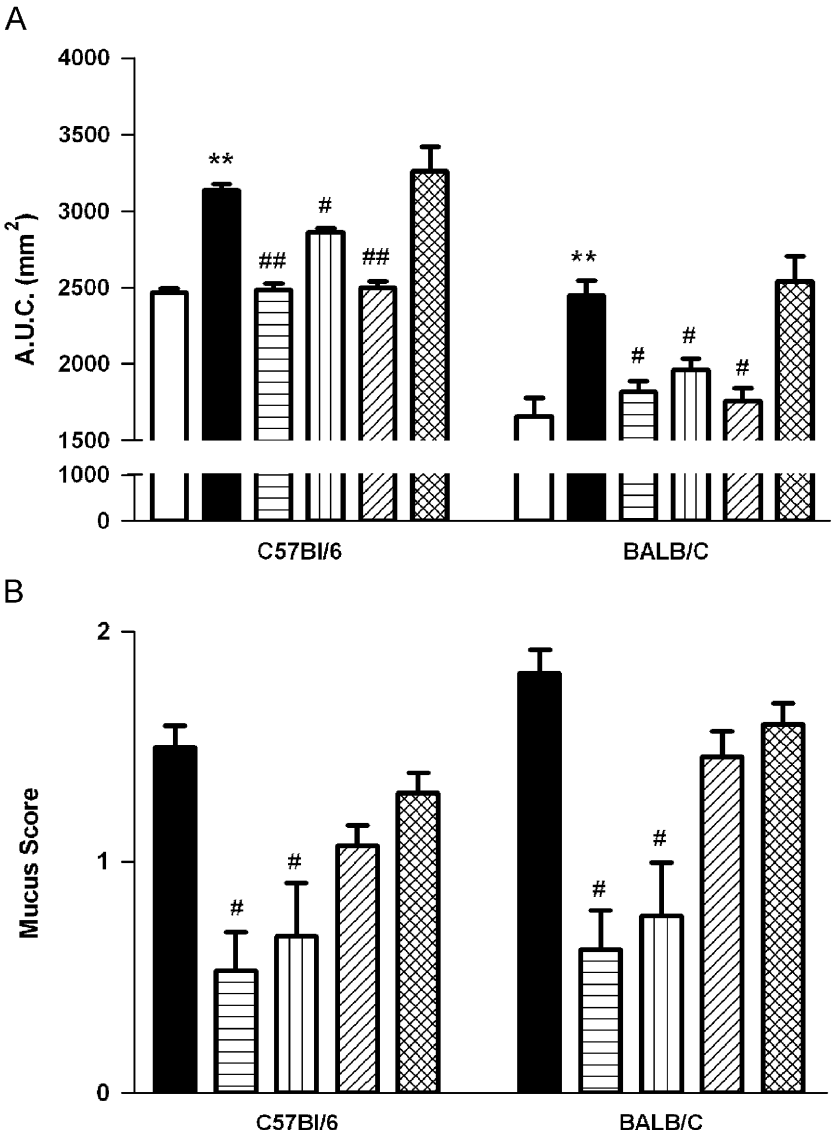

Fig. 4. Effects of indomethacin, nimesulide, MK 571 and WEB 2170 on airway reactivity to methacholine and mucus production in mice. Mice were immunized, challenged and treated as described in Fig. 1. Twenty-four hours after the second challenge, the lungs were removed, perfused via the trachea and increases in perfusion pressure to bolus injection of methacholine were recorded (A), or lungs were removed, embedded in parathion, sliced and stained with PAS/hematoxylin for evaluation of mucus (B). (■) immunized/OVA; (▤) immunized/OVA + indomethacin; (▥) immunized/OVA + nimesulide, (▨) immunized/OVA + MK 571 and (▩) immunized/OVA + WEB 2170. Results are expressed as mean area under the curve ± S.E.M. of 6–7 animals per group. $^{**}P<0.01$ in comparison with the saline challenged group and $^{\#}P<0.05$ and $^{\#\#}P<0.001$ in comparison with the OVA challenged group.

deleterious or a beneficial role in airway diseases. PGE_2 was shown to have a protective effects on allergen-induced [16] and aspirin-induced asthma [17], but other studies has shown an inverse correlation between eosinophilic airway inflammation and PGE_2 concentration in induced sputum from asthmatic subjects, which supported the possible anti-inflammatory role of PGE_2 [18]. On the other hand, other studies suggested that PGE_2 may enhance eosinophil survival, providing a potential proinflammatory activity [19,20].

Another prostanoid produced in high concentration in lungs in response to allergic stimulation is the PGD_2 which is mostly derived from mast cells. PGD_2 potentiates the vascular events of inflammation such as vasodilatation and edema and causes bronchodilation through its action on DP1 receptors. It is also a potent bronchoconstrictor, an effect mediated by the TP receptor expressed in bronchial smooth muscle, and this effect seems to predominate, masking the DP1-mediated bronchodilation. PGD_2 also potentiates cell recruitment, predominantly of eosinophils and Th2 lymphocytes and this effect seems to be mediated by the CRTH2 (orphan chemoattractant-like receptor) which is expressed in these cell types. There is now considerable pre-clinical evidence that DP1 and CRTH2 receptors, acting together, have a key role in allergic disease such as asthma [21].

The cysteinyl-leukotrienes were shown to be released in experimental asthma and in humans, and their inhibition with selected receptor antagonists had marked anti-inflammatory effects, including inhibition of airway eosinophil influx, secretion of mucus and cytokines [22]. PAF is also released in several asthma models and has a number of effects including the recruitment and activation of eosinophils [23], the release of mediators such as leukotriene C_4 from eosinophils, and the stimulation of mucus secretion [24]. However, previous studies with PAF receptor antagonists have failed to show significant beneficial effects on the allergen-induced response in asthmatic subjects, casting doubt on the importance of PAF as a major mediator in asthma [25–27]. Interestingly, in our study, the PAF antagonist reduced cell infiltration in C57Bl/6 but not in BALB/c mice. This could either suggest that BALB/c does not produce or produce less PAF in response to antigen stimulation or that this strain expresses lower levels of PAF receptor or receptors with lower affinity for PAF. The second hypothesis appears more likely since previous studies showed that PAF induced a much lower increase of vascular permeability in BALB/c mice than in C57Bl/6 mice [28].

Our results have also showed a clear increase in the number of $T\gamma\delta^+$ and $NK1.1^+$ as well as of CD_4^+, CD_8^+, and B-lymphocytes in the BALF of immunized BALB/c and C57Bl/6 mice following antigen challenge; this data confirmed previous findings from our laboratory in the C57Bl/6 mice strain [12]. However, the distribution of the various subsets was clearly distinct: the percentage of CD_4^+ and B-lymphocytes in BALB/c was $3\times$ and $10\times$ higher than in C56Bl/C, respectively. Similarly, the percentage of $T\gamma\delta$ was much higher ($10\times$) in BALB/c. We were unable to measure the $NK1.1^+$ cell population in BALB/c because the antibody used reacts with NK1.1 surface antigen expressed in cells from C57Bl/6 but not from BALB/c mice [29]. Mouse $NK1.1^+$ cells represent a population of NK cells that express TCR and are thus NKT cells.

These results are in agreement with the results of Spinozzi and Bertotto [30], who showed that following an *in vivo* allergen provocation of asthmatic patients the number of $T\gamma\delta^+$ cells was significantly higher in asthmatics than in controls. It has also been shown that the activity of $NK1.1^+$ cells was increased after bronchial allergen challenge in asthmatic subjects [7]. Depletion of NK 1.1^+ cells before the immunization was shown to inhibit the lung eosinophilia in a mice model of allergic asthma [31]. Studies using $T\gamma\delta^+$ deficient mice suggested that these cells were required for inducing allergen specific IgE and IgG1 isotypes production [10]. More recently, it was shown that human NKT cells, which are the counterpart of mouse $NK1.1^+$ cells, are potent source of IL-4 [9]. However, despite the evidence which suggests that the NKT and $T\gamma\delta^+$ cells play a role in priming Th2 responses, the role of these cells in asthma symptoms remains to be determined.

Our results also showed that the treatment with the prostaglandin synthesis inhibitors or with PAF antagonist reduced all lymphocyte subset counts from the BALF. However, treatment with cysteinyl-leukotrienes receptor antagonist preserved the CD_4^+ and $T\gamma\delta^+$ lymphocytes populations, despite inhibiting the other cell types. This was observed in both mouse strains studied. In humans, the treatment with Zafirlukast, a cysteinyl-leukotriene receptor antagonist, was also shown to decrease the number of total lymphocytes, basophils but not eosinophils in the BALF [32].

Previous studies using mice knock out of $T\gamma\delta$ cells suggested that these cells down regulate the bronchial hyperreactivity [33]. Moreover, Zuany-Amorim et al. [10] reported that the allergic airway inflammation is more intense in $T\gamma\delta$-gene knock out mice compared to the inflammation seen in the $T\gamma\delta$-gene normal mice of the same strain. This suggests that the $T\gamma\delta$ cells have an inhibitory effect on lung inflammation. In our experiments, the cysteinyl-leukotriene antagonist reduced the hyperreactivity to methacholine without affecting the relative number of $T\gamma\delta$ cells in BALF. The hyperreactivity was also significantly reduced by treatment with prostaglandin synthesis inhibitors, but in this case the relative numbers of $T\gamma\delta$ were also reduced. Pretreatment with the PAF antagonist did not have any effect on the hyperreactivity although it did reduce the $T\gamma\delta$ subset. From these results it does not appear that there is a correlation between the number of $T\gamma\delta$ cells and bronchial hyperreactivity in our model of asthma.

Involvement of cysteinyl-leukotrienes in airway obstruction caused by mucus hypersecretion was suggested by studies where treatment with a *cys*-leukotrienes receptor antagonist Montelukast significantly reduced the hyperplasia of goblet cells, mucus plugging and fibrosis in a mouse model of asthma [34]. In the particular asthma model used in the present study, in which mucus production was measured after the second antigen challenge, mucus production was neither affected by the cysteinyl-leukotrienes antagonist nor by the PAF antagonist. In contrast, prostaglandin inhibitors were extremely effective to reduce mucus in this model. In brief, the inhibition of prostaglandins synthesis reduced the counts of the various cell types, the bronchial hyperreactivity and the mucus secretion whereas the leukotriene-receptor antagonist neither affected CD_4 and $T\gamma\delta$ infiltration nor mucus secretion, but reduced hyperreactivity. The PAF-receptor antagonist reduced cell infiltration without affecting hyperreactivity or mucus secretion. Thus, it does not appear to have a correlation between the cell infiltration into the BALF, the bronchial hyperreactivity and the mucus

production, at least in the experimental conditions employed in this study.

The T$\gamma\delta^+$ cells were shown to have an important role in the protection of the mucosa by eliminating damaged epithelial cells [3]. The T$\gamma\delta$ cells may also help to restore macrophage numbers to homeostatic levels contributing to the resolution of lung inflammation [4]. It is possible to speculate that the absence of effects of the cysteinyl-leukotriene antagonists on the T$\gamma\delta$ cell population may preserve the capacity of lung repair, in addition to reducing inflammatory cell infiltration and bronchial hyperreactivity.

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