

Stimulation of dispersed nasal polyp cells by hyperosmolar solutions

Françoise Souques, B. Pharm,^a Louis Crampette, MD,^b
Michel Mondain, MD,^b Antonio M. Vignola, MD,^c Pascal Chanez, MD,^a
Jean Bousquet, MD,^a and Alison M. Campbell, PhD^a Montpellier, France,
and Palermo, Italy

It has been suggested that hyperosmolarity may be one of the stimuli that provoke exercise-induced asthma and rhinitis. We investigated whether changes in osmolarity could result in increased levels of mediator release from nasal cells. Cells were dispersed from nasal polyps by enzymatic digestion and were incubated for 15 minutes with solutions of varying osmolarity obtained by the addition of mannitol to Hanks' balanced salt solution. After incubation was performed, cell supernatants were removed, and the release of 15-hydroxyeicosatetraenoic acid, prostaglandin₂, leukotriene B₄, and fibronectin was measured. Lactate dehydrogenase was measured to assess cell viability. Epithelial cells made up 40% to 60% of cells and mononuclear cells 40% to 65%. At 900 mOsm/kg H₂O, which has been suggested as the osmolarity of the fluid lining the airways during exercise, we observed a significant increase (Wilcoxon W test) in the release of 15-hydroxyeicosatetraenoic acid ($p < 0.008$), leukotriene B₄ ($p < 0.008$), and prostaglandin₂ ($p < 0.008$), but no significant increase in the release of fibronectin was seen. No significant increase was seen between lactate dehydrogenase and 15-hydroxyeicosatetraenoic acid release, suggesting that the increase in mediator levels was not caused by cell death. These results show that hyperosmolar solutions can induce activation of nasal cells, which may at least partly explain rhinitis caused by exercise. (J ALLERGY CLIN IMMUNOL 1995;96:980-5.)

Key words: Osmolarity, exercise-induced rhinitis, nasal polyp, mast cell

During exercise patients with inflammatory rhinitis sometimes have nasal symptoms.¹⁻⁵ The mechanisms underlying this reaction may be at least partly due to the release of inflammatory or vasomotor mediators.¹ Several hypotheses have been proposed to explain the occurrence of exercise-induced asthma including the release of inflammatory mediators, increased osmolarity of the bronchi,⁶⁻⁸ or changes in vasoactivity.^{9, 10} Hyperosmolarity can induce the release of mediators from

Abbreviations used

HETE:	Hydroxyeicosatetraenoic acid
LDH:	Lactate dehydrogenase
LT:	Leukotriene
PG:	Prostaglandin

basophils¹¹⁻¹³ and mast cells in vitro,¹¹⁻¹⁴ and with nasal challenge it can induce the release of histamine, toluenesulfonylarginine methyl ester esterase, and leukotrienes.^{15, 16} Thus hyperosmolarity of the nasal or bronchial milieu may lead to the release of inflammatory mediators, vasomotor mediators, or both, inducing nasal or bronchial symptoms in patients with rhinitis or asthma.

Epithelial cells, metachromatic cells, and macrophages may be activated by hyperosmolar stimulus. Epithelial cells represent the first barrier between the external environment and the nasal or bronchial mucosa, and they are activated in patients with rhinitis and asthma as shown by increased release of mediators and expression of

From ^aClinique des Maladies Respiratoires and CJF INSERM 92/10, Hôpital Arnaud de Villeneuve, Centre Hospitalier Universitaire, Montpellier-Cedex; ^bService d'ORL, Centre Hospitalier Universitaire; and ^cIstituto di Fisiopatologica Respiratoria, CNR Palermo.

Drs. Campbell and Vignola are recipients of an EC Mobility grant.

Received for publication June 8, 1994; revised Feb. 22, 1995; accepted for publication Mar. 16, 1995.

Reprint requests: A. M. Campbell, PhD, INSERM CJF 92-10, Hôpital Arnaud de Villeneuve, Avenue du doyen G Giraud, 34295 Montpellier-Cedex 5, France.

Copyright © 1995 by Mosby-Year Book, Inc.

0091-6749/95 \$5.00 + 0 1/1/64977

surface markers.¹⁷⁻¹⁹ 15-Hydroxyeicosatetraenoic acid (15-HETE),^{18,20} fibronectin,¹⁸ and prostaglandin E₂ (PGE₂)^{18,21} are released by nasal or bronchial epithelial cells spontaneously or after activation. Mast cells and basophils can be activated to release a range of mediators including histamine and eicosanoids. Macrophages release a wide array of mediators including leukotriene B₄ (LTB₄),²² PGE₂,²³ and fibronectin²⁴ spontaneously or after activation.

Cells dispersed from nasal polyps represent an interesting model to study airway inflammatory diseases, because they are derived from an inflamed tissue and are primed or stimulated in comparison with cells obtained from the nasal mucosa in normal subjects. Epithelial cells, macrophages, mast cells, and eosinophils are among the cells recovered from nasal polyps.

A study was carried out with dispersed cells obtained from surgically resected nasal polyps to examine the release of inflammatory (15-HETE, LTB₄, and fibronectin) and vasoactive (PGE₂) mediators after stimulation by solutions covering a wide range of osmolarities.

METHODS

Cell recovery

Nasal polyps were obtained, after polypectomy, from 10 patients (seven male patients) ranging in age from 16 to 60 years who had nonallergic rhinitis. None exhibited an aspirin intolerance. The polyp was dissected with scissors into pieces of approximately 1 mm³; cell disaggregation was achieved in a shaking water bath at 37° C by enzymatic digestion in 4 volumes of RPMI 1640 cultured medium (Gibco, Paisley, Scotland) containing 0.75 mg/ml hyaluronidase (Sigma Co., St. Louis, Mo.), 1.5 mg/ml collagenase (Sigma), and 2 mg/ml protease type XIV (Sigma) for a 2-hour period according to a method previously used for human lung cells.²⁵ Then the cells were filtered sequentially to separate them from any undigested tissue. The cells were resuspended in 15 ml of RPMI and washed three times to remove the enzymes.

Characterization and viability of cells

The epithelial origin of cells was characterized on slides prepared by cytocentrifugation with the use of immunohistochemistry with a highly specific monoclonal antibody anticytokeratin (MNF-116, Dako Versailles, France)²⁶ and an alkaline phosphatase, antialkaline phosphatase system²⁷ (Dako). A negative control slide was prepared with an unrelated antibody of the same isotype. Another slide was stained with May Grunwald Giemsa stain to characterize the other cell types present. Viability was assessed before stimulation and at the end of the incubation period by measuring the levels of the

lactate dehydrogenase (LDH) in the supernatant by colorimetric determination with a commercially available kit (Sigma). We have examined LDH release in nasal polyp cells after freezing and thawing to kill them and found that LDH levels ranged from 99 to 122 U/10⁶ cells. In contradistinction, viable cells released between 1.8 to 12 U of LDH per 10⁶ cells.

Stimulation of cells

The cells were then resuspended at a concentration of 1 × 10⁶ cells/ml in RPMI. Osmolarities were selected on the basis of previous studies.¹⁰⁻¹³ In the trachea the osmolarity of secretions has been demonstrated to be 360 mOsm/kg H₂O.¹² Anderson et al.¹⁰ suggested that after exercise bronchial secretions may result in an osmolarity of 900 mOsm/kg H₂O. Cells were stimulated by iso-osmolar (280 mOsm/kg H₂O) and hyperosmolar buffer solutions (360, 500, 600, 750, and 900 mOsm/kg H₂O) obtained by the addition of mannitol (Sigma) (final concentration 0.1 to 0.6 mol/L) in Hanks' balanced salt solution (Gibco). The osmolarity of the solutions was controlled with a cryo-osmometer (Osmomat 030; Gonotec, Berlin, Germany). The cells were incubated at 37° C in 5% CO₂ for 15 minutes. After incubation the cells were centrifuged, and supernatants were frozen at -20° C until measurement of mediators was performed.

Measurement of mediators

15-HETE. 15-HETE was measured by radioimmunoassay with a commercially available kit (Amersham, Buckinghamshire, U. K.). The lower limit of detection of this assay is 16 pg per tube, and at 50% of the maximum binding efficiency the antibody shows a cross-reactivity of 41% with 15-hydroxyperoxy-eicosotetraenoic acid, 2% with 5,15di-HETE, and less than 0.4% with any other eicosanoid tested.

Fibronectin. Fibronectin was measured with an ELISA. Goat anti-human fibronectin antibody, rabbit anti-human fibronectin antibody coupled to peroxidase, and fibronectin standards were obtained from Calbiochem (La Jolla, Calif.). The lower limit of detection was 500 pg/ml.¹⁷

PGE₂. PGE₂ was measured with a commercially available enzyme immunoassay kit (Cayman Chemical Company, Ann Arbor, Mich.). The lower limit of detection of this assay is 7 pg/ml, and at 50% maximum binding the antibody shows a cross-reactivity of 100% with PGE₃, 9.2% with 15-keto PGE₂, and less than 0.02% with any other prostanoid tested.

LTB₄. LTB₄ was measured with a commercially available enzyme immunoassay kit (Cayman Chemical Company). With a sensitive ELISA reader (MR 5000 Dynatech) the lower limit of detection of this assay is 4 pg/ml. At 50% of the maximum binding efficiency the antibody shows a cross-reactivity of 100% with LTB₅, 39% with 6-*trans*-LTB₄, 0.7% with 6-*trans*-12-*epi* LTB₄, 0.5% with 20-hydroxy LTB₄, 0.03% with 5(s)-HETE, and less than 0.01% with other eicosanoids.

TABLE I. Global results

	No. of experiments	Osmolarity (mOsm/kg H ₂ O)						Friedman test
		Isotonic	360	500	600	750	900	
LDH (U/ml)	9	1.8 0-24	4.0 0-30	6.7 0-37	10.3 0-45	9.7 0-49	9.9 0-54	12.1 $p < 0.04$
Fibronectin (ng/ml)	9	2.3 0.3-5.2	2.4 0.2-8.2	2.0 0.15-8.1	2.1 1.5-13.9	1.0 0.1-5.4	3.6 0.3-8.5	4.0 NS
15-HETE (pg/ml)	8	4.4 0.6-8.4	4.4 0.6-8.2	4.6 0.6-9.6	4.1 0.6-9.2	4.9 0.6-8.4	5.8 0.8-25	14.6 $p < 0.012$
PGE ₂ (pg/ml)	9	62 7-240	67 7-227	81 9-771	97 5-541	112 6-1169	120 23-6200	22.8 $p < 0.004$
LTB ₄ (pg/ml)	9	4.9 3.9-11.5	5.5 3.9-9.6	5.6 3.9-13.5	5.2 3.9-14.8	5.8 3.9-19.1	9.8 3.9-28.2	18.0 $p < 0.003$

Results are expressed as medians (first line) and range (second line).

Influence of the osmolarity on the assay of mediators. The addition of hyperosmolar solutions to the assays did not modify the performance of any test.

Statistical analyses

Statistical analyses were performed with the Friedman test to assess whether osmolarity can affect the release of a marker. When the Friedman test was significant, the Wilcoxon W nonparametric test with Bonferroni's correction was used. Results are given as the median value and the range.

RESULTS

Characteristics of cells

Epithelial cells composed 42% (28% to 61%) (median, range) of the cell population as shown with an anticytokeratin antibody. Mononuclear cells (lymphocytes and macrophages) (47%, 32% to 62%), mastocytes (5%, 0% to 17%), neutrophils (3%, 0% to 6%), and eosinophils (2%, 0.7% to 26%) were the other cell types recovered.

Viability of cells

Hyperosmolar solutions increased the release of LDH (Table I), but no dose-response effect was observed, and no significant increase was seen for a given osmolarity.

Release of mediators

15-HETE. 15-HETE was released from cells at all osmolarities tested, and a significant increase was seen in 15-HETE release for an osmolarity of 900 mOsm/kg H₂O ($p < 0.01$, Wilcoxon W test) (Table I and Fig. 1, A). No correlation was seen between the amount of 15-HETE release and LDH levels (Spearman rank test), indicating that the release of 15-HETE was not related to cell mortality.

Fibronectin. Fibronectin was released from cells at all osmolarities tested, but although a sharp increase of fibronectin levels was seen in four of 10 experiments at an osmolarity of 900 mOsm/kg H₂O, no overall significant increase was seen in fibronectin release (Table I).

PGE₂. PGE₂ was released from cells at all osmolarities tested, and a significant increase was seen in PGE₂ release for an osmolarity of 900 mOsm/kg H₂O ($p < 0.008$, Wilcoxon W test) (Table I and Fig. 1, B).

LTB₄. LTB₄ was released from cells at all osmolarities tested, and a significant increase was seen in LTB₄ release for an osmolarity of 900 mOsm/kg H₂O ($p < 0.007$, Wilcoxon W test) (Table I and Fig. 1, C).

DISCUSSION

This study showed that cells dispersed from nasal polyps can be activated by hyperosmolar solutions to release mediators including 15-HETE, LTB₄, and PGE₂ at a concentration of 900 mOsm/kg H₂O.

We used cells obtained by enzymatic digestion, because work with chopped lung tissue has shown that only qualitative and not quantitative data are obtained with tissue fragments.²⁸ In this study we did not attempt to purify the different cell types found in the polyps, because this purification could mask interactions that would occur in vivo, although in future work it would be interesting to study the cellular provenance of the mediators.

Cells present in the airways epithelium may play a role in asthma or rhinitis. In this study we used dispersed nasal polyp cells, because polyps represent an inflamed airway tissue that can be recov-

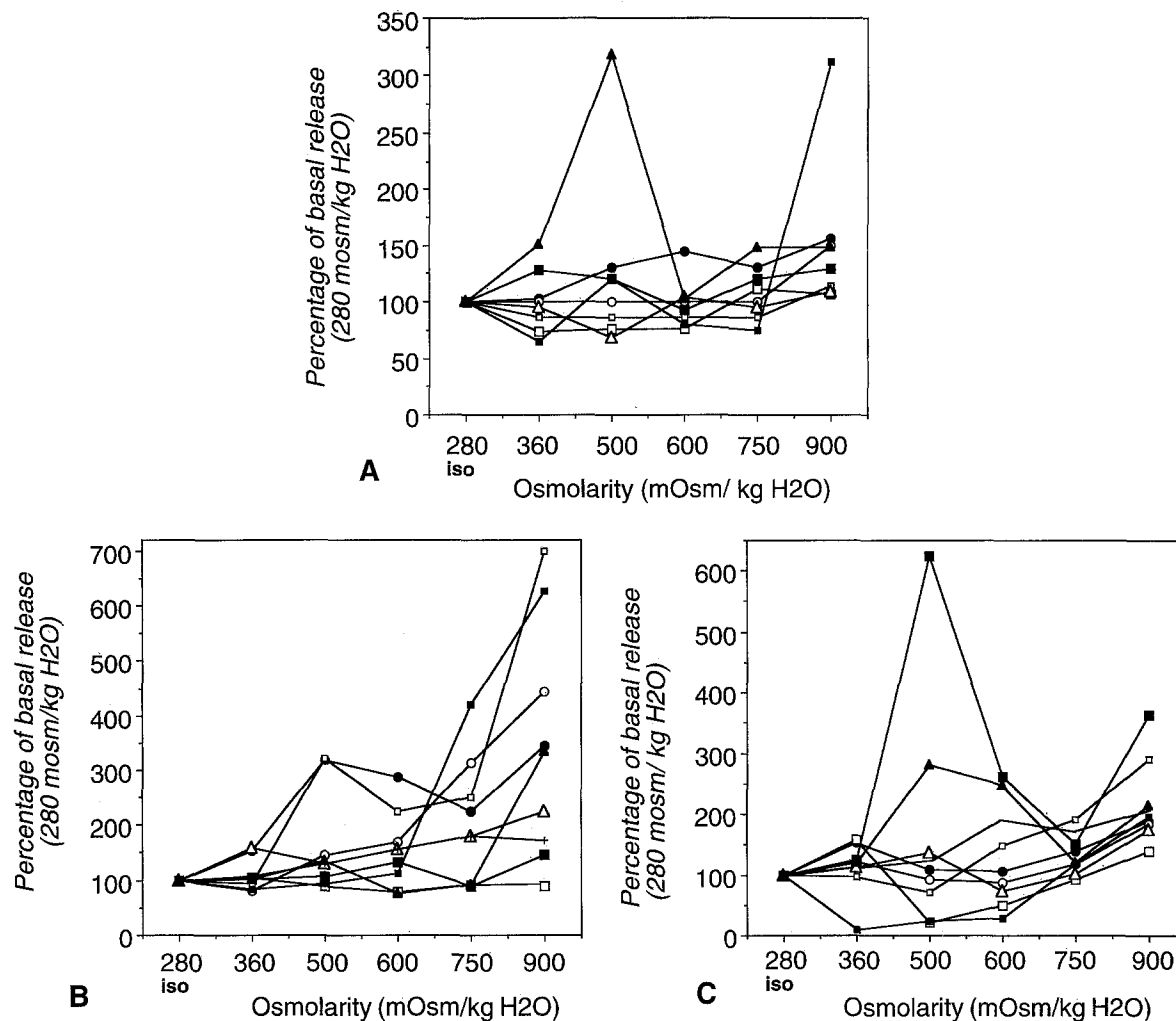


FIG. 1. Release of proinflammatory mediators by dispersed nasal polyp cells stimulated by isotonic and hypertonic solutions. **A**, 15-HETE; **B**, PGE₂; **C**, LTB₄.

ered in sufficient quantity to allow these experiments to be performed, and it is extremely difficult to obtain adequate quantities of tissue from patients with exercise-induced rhinitis who only rarely present with an indication for nasal surgery. Although nasal polyp cells are closer to cells recovered from the nasal mucosa of patients with rhinitis, they may be similar to bronchial cells of patients with asthma. Moreover, it seems more appropriate to study cells obtained from inflamed tissue than cells from normal tissue, because they are already primed in a manner similar to the inflammation associated with rhinitis and asthma. However, for a more accurate understanding of exercise-induced asthma, cells obtained from bronchial biopsies or brushing will have to be studied. The cells were dispersed according to a method previously used for obtaining cells from lung pa-

renchyma, because cells obtained with enzyme digestion can be used for pharmacologic studies.²⁹ Epithelial cells can be identified by the presence of cytokeratin with a monoclonal antibody,²⁷ and we found that almost half the cells were of epithelial origin. As expected, the cell population also included mononuclear cells, eosinophils, and neutrophils. The release of LDH was measured as an index of cell mortality; this enzyme was released by dead or dying cells. We used LDH measurement rather than assessment of the uptake of a vital stain such as trypan blue, because we (and others) have observed that this stain can be taken up by cells that still have ciliary movement, making assessment of viability difficult and inaccurate.

We challenged cells with hyperosmolar solutions for only 15 minutes to try to mimic the conditions *in vitro* that may occur *in vivo* after exercise in

these patients and to avoid hyperosmolar-induced cytotoxicity, which might have occurred with a longer incubation period. The selection of osmolarities was based on measurement of tracheal secretions¹² and the suggestion that in patients with exercise-induced asthma, osmolarity may reach 900 mOsm/kg H₂O in bronchial secretions.¹⁰

On hyperosmolar stimulation, polyp cells released increased levels of 15-HETE, PGE₂, and LTB₄. The results obtained for fibronectin are less consistent, because a sharp increase occurred in only four experiments. 15-HETE is the major arachidonic acid metabolite released by freshly dispersed epithelial cells³⁰ and is not released in significant quantities by other cells present in the airways (eosinophils and possibly macrophages may also release 15-HETE but to a lesser extent). Cells released significantly greater levels of this mediator after incubation for 15 minutes with 900 mOsm/kg H₂O. Of interest, no significant correlation was found between 15-HETE release and the numbers of eosinophils present, suggesting that the main source of this eicosanoid was the epithelial cell. 15-HETE can be released by dead or dying cells; however, in these experiments no significant increase was seen in the release of LDH (an enzyme released by dead cells) for an osmolarity of 900 mOsm/kg H₂O, and no significant correlation was seen between LDH and 15-HETE levels, indicating that the release of this mediator was not due to cell death. 15-HETE is a biologic mediator capable of stimulating the chemotaxis of inflammatory cells³¹ and inducing the release of mucus glycoprotein from human airways in culture,³² and it can also influence 5-lipoxygenase activity in leukocytes.³³ Lai et al.³⁴ have shown that 15-HETE is capable of enhancing the early bronchoconstrictor response to inhaled allergen in patients with atopic asthma.

Epithelial cells and macrophages in culture produce PGE₂,³⁵ and epithelial cells from patients with asthma can be stimulated to release this mediator, whereas cells obtained from normal subjects cannot.¹⁸ PGE₂ has a proinflammatory function, causing vasodilatation and increasing mucosal edema. However, it has been suggested that PGE₂ may have different effects in the bronchi and in the nasal cavities. Although there is little doubt that PGE₂ generally acts as a vasodilator, reports have been made that this mediator has vasoconstrictor effects in the nasal mucosa, and for this reason it has been tested as a nasal decongestant.³⁶ This finding is important because it has

been shown that a decrease in nasal airway resistance occurs during exercise.^{1, 2, 4}

LTB₄ release was increased at an osmolarity of 900 mOsm/kg H₂O. The major sources of this eicosanoid are monocytes and macrophages, and this study suggests that these cells can be activated by a hyperosmolar stimulus. The major biologic activity of LTB₄ is to act as a potent chemoattractant for inflammatory cells such as neutrophils and eosinophils and to increase vascular permeability.³⁷

In summary, this study shows that cells dispersed from nasal polyps can be activated by hyperosmolar solutions to release a range of mediators. The release of these mediators occurs within 15 minutes, and it is therefore possible that alterations in the osmotic environment may be an important factor in the development of exercise-induced rhinitis and possibly exercise-induced asthma, although studies with bronchial cells are necessary to confirm the latter hypothesis.

REFERENCES

1. Silvers WS. Exercise-induced allergies: the role of histamine release. *Ann Allergy* 1992;68:58-63.
2. Stanford CF, Stanford RL. Exercise induced rhinorrhoea (athlete's nose). *BMJ* 1988;297:660.
3. Forsyth RD, Cole P, Shephard RJ. Exercise and nasal patency. *J Appl Physiol* 1983;55:860-5.
4. Hasegawa M, Kabasawa Y, Ohki M, Watanabe I. Exercise-induced change of nasal resistance in asthmatic children. *Otolaryngol Head Neck Surg* 1985;93:772-6.
5. Syabbalo NC, Bundgaard A, Widdicombe JG. Effects of exercise on nasal airflow resistance in healthy subjects and in patients with asthma and rhinitis. *Bull Eur Physiopathol Respir* 1985;21:507-13.
6. Smith CM, Anderson SD. A comparison between the airway response to isocapnic hyperventilation and hypertonic saline in subjects with asthma. *Eur Respir J* 1989;2:36-43.
7. Anderson SD. Is there a unifying hypothesis for exercise-induced asthma? *J Allergy Clin Immunol* 1984;73:660-5.
8. Finney MJ, Anderson SD, Black JL. Terfenadine modifies airway narrowing induced by the inhalation of nonisotonic aerosols in subjects with asthma. *Am Rev Respir Dis* 1990;141:1151-7.
9. McFadden E Jr. Hypothesis: exercise-induced asthma as a vascular phenomenon. *Lancet* 1990;335:880-3.
10. Anderson SD, Daviskas E. The airway microvasculature and exercise induced asthma. *Thorax* 1992;47:748-52.
11. Findlay SR, Dvorak AM, Kagey-Sobotka A, Lichtenstein LM. Hyperosmolar triggering of histamine release from human basophils. *J Clin Invest* 1981;67:1604-13.
12. Eggleston PA, Kagey-Sobotka A, Schleimer RP, Lichtenstein LM. Interaction between hyperosmolar and IgE-mediated histamine release from basophils and mast cells. *Am Rev Respir Dis* 1984;130:86-91.
13. Eggleston PA, Kagey-Sobotka A, Lichtenstein LM. A comparison of the osmotic activation of basophils and human lung mast cells. *Am Rev Respir Dis* 1987;135:1043-8.
14. Makhadmeh A, Pearce F. Hyperosmolar induced histamine

- release from mast cells: a mechanism for the pathogenesis of exercise-induced asthma? *Agents Actions* 1993;38: C192-3.
15. Togias AG, Naclerio RM, Proud D, et al. Nasal challenge with cold, dry air results in release of inflammatory mediators: possible mast cell involvement. *J Clin Invest* 1985;76: 1375-81.
16. Silber G, Proud D, Warner J, et al. In vivo release of inflammatory mediators by hyperosmolar solutions. *Am Rev Respir Dis* 1988;137:606-12.
17. Ramis I, Catafau JR, Serra J, Bulbena O, Picado C, Gelpi E. In vivo release of 15-HETE and other arachidonic acid metabolites in nasal secretions during early allergic reactions. *Prostaglandins* 1991;42:411-20.
18. Campbell AM, Chanez P, Vignola AM, et al. Functional characteristics of bronchial epithelium obtained by brushing from asthmatic and normal subjects. *Am Rev Respir Dis* 1993;147:529-34.
19. Vignola AM, Campbell AM, Chanez P, et al. HLA-DR and ICAM-1 expression on bronchial epithelial cells in asthma and chronic bronchitis. *Am Rev Respir Dis* 1993;148:689-94.
20. Kumlin M, Hamberg M, Granstrom E, et al. 15(S)-hydroxyeicosatetraenoic acid is the major arachidonic acid metabolite in human bronchi: association with airway epithelium. *Arch Biochem Biophys* 1990;282:254-62.
21. McKinnon KP, Madden MC, Noah TL, Devlin RB. In vitro ozone exposure increases release of arachidonic acid products from a human bronchial epithelial cell line. *Toxicol Appl Pharmacol* 1993;118:215-23.
22. Radeau T, Godard P, Chavis C, Michel FB, Descomps B, Damon M. Effect of nedocromil sodium on sulfidopeptide leukotrienes-stimulated human alveolar macrophages in asthma. *Pulm Pharmacol* 1993;6:27-31.
23. Pueringer RJ, Hunninghake GW. Lipopolysaccharide stimulates de novo synthesis of PGH synthase in human alveolar macrophages. *Am J Physiol* 1992;262:L78-85.
24. Hershkovich R, Alon R, Gilat D, Lider O. Activated T lymphocytes and macrophages secrete fibronectin which strongly supports cell adhesion. *Cell Immunol* 1992;141: 352-61.
25. Campbell AM, Chanez P, Marty-Ane C, et al. Modulation of eicosanoid and histamine release from human dispersed lung cells by terfenadine. *Allergy* 1993;48:125-9.
26. Moll R, Franke WW, Schiller DL, Geiger B, Krepler R. The catalog of human cytokeratins: patterns of expression in normal epithelia, tumors and cultured cells. *Cell* 1982; 31:11-24.
27. Cordell JL, Falini B, Erber WN, et al. Immunoenzymatic labeling of monoclonal antibodies using immune complexes of alkaline phosphatase and monoclonal anti-alkaline phosphatase (APAAP complexes). *J Histochem Cytochem* 1984; 32:219-29.
28. Church MK, Young KD. The characteristics of inhibition of histamine release from human lung fragments by sodium cromoglycate, salbutamol and chlorpromazine. *Br J Pharmacol* 1983;78:671-9.
29. Holgate S, Burns G, Robinson C, Church M. Anaphylactic and calcium-dependent generation of prostaglandin D₂ (PGD₂), thromboxane B₂ and other cyclooxygenase products of arachidonic acid by dispersed lung cells and relationship to histamine release. *J Immunol* 1984;133:2138-44.
30. Hunter JA, Finkbeiner WE, Nadel JA, Goetzl EJ, Holtzman MJ. Predominant generation of 15-lipoxygenase metabolites of arachidonic acid by epithelial cells from human trachea. *Proc Natl Acad Sci U S A* 1985;82:4633-7.
31. Nagy L, Lee T, Goetzl E, et al. Complement receptor enhancement and chemotaxis of human neutrophils and eosinophils by leukotrienes and other lipoxygenase products. *Clin Exp Immunol* 1986;47:541-7.
32. Marom Z, Shelhamer JH, Kaliner M. Effects of arachidonic acid, monohydroxyeicosatetraenoic acid and prostaglandins on the release of mucous glycoproteins from human airways in vitro. *J Clin Invest* 1981;67:1695-702.
33. Goetzl EJ. Leukocyte recognition and metabolism of leukotrienes. *Fed Proc* 1983;42:3128-31.
34. Lai CK, Polosa R, Holgate ST. Effect of 15-(s)-hydroxyeicosatetraenoic acid on allergen-induced asthmatic responses. *Am Rev Respir Dis* 1990;141:1423-7.
35. Devalia J, Spsford R, Cundell D, Rusnak C, Campbell A, Davies R. Human bronchial epithelial cell dysfunction following in vitro exposure to nitrogen dioxide. *Eur Resp J* 1993;6:1308-16.
36. Jackson RT, Birnbaum JE. Nasal vasoconstrictor activity of a novel PGE₂ analog. *Prostaglandins* 1981;21:1015-24.
37. Musser JH, Kreft AF. 5-lipoxygenase: properties, pharmacology, and the quinolinyl(bridged)aryl class of inhibitors. *J Med Chem* 1992;35:2501-24.