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A comparison of cytokine release from epithelial cells cultured from nasal biopsy specimens of atopic patients with and without rhinitis and nonatopic subjects without rhinitis

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Abstract

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Background: Recent studies have suggested that airway epithelial cells of atopic and nonatopic

individuals may differ in their ability to produce proinflammatory cytokines.

Methods: We have cultured human nasal epithelial cells (NECs) as confluent explant cultures from nasal biopsy specimens of well-characterized nonatopic normal volunteers without rhinitis ($n = 8$), atopic volunteers without rhinitis ($n = 9$), and atopic patient volunteers with rhinitis ($n = 10$) and measured the amounts of IL-1 β , IL-8, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor- α , and RANTES released spontaneously into the culture medium by these cells in vitro. NECs from patients with allergic rhinitis were cultured from biopsy specimens obtained on two different occasions, during and after the pollen season.

Results: In general, NECs from atopic individuals released significantly greater amounts of IL-1 β , IL-8, granulocyte-macrophage colony-stimulating factor, tumor necrosis factor- α , and RANTES than NECs from nonatopic individuals. IL-8 was released in greatest quantity and IL-1 β in lowest quantity, regardless of whether the NECs were derived from atopic or nonatopic volunteers. Of the atopic individuals, NECs of atopic patients with rhinitis naturally exposed to pollen released greater quantities of all these cytokines, compared with NECs of atopic patients with rhinitis and atopic patients without rhinitis who were not exposed to allergen.

Conclusions: These results suggest that NECs of atopic individuals, who are genetically predisposed to upper airway disease, release increased amounts of proinflammatory cytokines and that natural exposure to allergen enhances the release of these cytokines, exacerbating the symptoms of allergic disease. (J Allergy Clin Immunol 1997;99:65-76.)

AR	Allergic rhinitis
GM-CSF	Granulocyte-macrophage colony-stimulating factor
ICAM-1	Intercellular adhesion molecule-1
mAb	Monoclonal antibody
NECs	Nasal epithelial cells
TNF-α	Tumor necrosis factor- α

(Click on a term to search this journal for other articles containing that term.)

Key Words: [Atopy](#), [allergic rhinitis](#), [nasal epithelial cells](#), [cytokines](#), [eosinophils](#)

The immune response to inhaled allergen is controlled at least in part by the atopic state of the exposed individual, now thought to be regulated by genes present on chromosome 5q31.1.¹ Although there is good evidence of an association between atopy and inflammation of the airways, it is not clear why all atopic individuals do not develop allergic inflammation of the airways, even though they may have increased IgE levels. In addition to atopy, the development of allergic disease is dependent on many other factors including the level of exposure to inhaled allergen and interaction with airway irritants (e.g., cigarette smoke and infection).²⁻⁵

Studies of allergic rhinitis (AR) have demonstrated that there is infiltration of the nasal mucosa by several inflammatory cell types including mast cells, eosinophils, macrophages, and lymphocytes.⁶⁻

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Although these cells have been shown to synthesize a variety of inflammatory mediators and have been associated with the pathogenesis of allergic airway disease, recent evidence suggests that the airway epithelial cell may also contribute to airway inflammation. Both in vivo and in vitro studies have demonstrated that human nasal and bronchial epithelial cells can generate several cytokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF), IL-6, IL-8, tumor necrosis factor- α (TNF- α), and the C-C chemokine RANTES,^{[12](#) [17](#)} which feature prominently in the allergic inflammatory response, influencing the growth, differentiation, proliferation, and activation of the inflammatory cells.

There is some evidence to support the concept that there may be differences in the airway epithelial cell-derived cytokines of atopic and nonatopic individuals, and these differences may play an important role in the genesis and persistence of allergic airway diseases. Sousa et al.^{[18](#) [19](#)} have demonstrated that the epithelium of patients with asthma synthesizes greater quantities of GM-CSF and monocyte chemoattractant protein-1 than that of nonasthmatic subjects. Similarly, studies of nasal epithelial cell (NEC) cultures from nonatopic subjects without rhinitis, patients with AR, and patients with nasal polyps have demonstrated that the epithelial cells from patients with rhinitis and those with nasal polyps synthesize significantly greater quantities of GM-CSF and IL-8 than cells of healthy nonatopic individuals without rhinitis.^{[14](#) [20](#) [21](#)} To date, however, there is no specific information on differences in cytokine levels constitutively generated by NECs from well characterized atopic or nonatopic subjects. Furthermore, there is no information on the effect of natural exposure to allergen on the synthesis of proinflammatory cytokines in patients with AR.

We have cultured human epithelial cells from nasal biopsy tissue of nonatopic normal volunteers without rhinitis, atopic patient volunteers without rhinitis, and atopic patient volunteers with rhinitis and investigated the ability of these cells to release specific proinflammatory cytokines including IL-1 β , IL-8, GM-CSF, TNF- α , and RANTES. We have also investigated the effect of natural exposure to pollen on the production of these cytokines in atopic patients with rhinitis by culturing cells from nasal biopsy specimens collected from patients with AR during and after the pollen season in the United Kingdom.

Methods

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Subjects

Twenty-seven volunteers (18 women and 9 men), with a mean age of 24 years (range, 18 to 38 years), were entered into the study. Of these, eight were nonatopic without rhinitis (normal volunteers), nine were atopic without rhinitis (patients with atopic eczema), and 10 were atopic patients with rhinitis (patients with hay fever). Atopy was defined as the presence of one or more positive skin prick test reactions to either house dust mite (*Dermatophagoides pteronyssinus*), grass pollen, or *Aspergillus fumigatus* (ALK, Allergologist Laboratorium; Horsholm, Denmark). Wheals with a mean diameter of more than 3 mm with associated flare and itching, in the absence of any reaction to the negative control, were considered positive skin prick test reactions. All atopic patients without rhinitis were clinically diagnosed as having atopic eczema. None of the patients had asthma. Characteristics of normal and patient volunteers and treatment requirements at entry into the study are shown in Table I.

Table I. Characteristics of study volunteers and treatment requirements at time of entry into study

Group	Subject	Age (yr)	Sex	Atopy (SPT)			Treatment		
				HDM	GP	AF	AH	CS	IT
Nonatopic without rhinitis (normal)	CD	22	F	-	-	-	-	-	-
	DM	21	M	-	-	-	-	-	-
	LN	23	F	-	-	-	-	-	-
	HS	22	F	-	-	-	-	-	-
	MD	18	M	-	-	-	-	-	-
	SCh	26	F	-	-	-	-	-	-
	DW	22	M	-	-	-	-	-	-
	HW	21	F	-	-	-	-	-	-
Atopic without rhinitis (eczema)	MS	38	F	-	+	-	-	+	-
	RJ	28	F	-	+	-	-	+	-
	GD	31	M	+	+	-	-	+	-
	AC	23	F	+	-	-	-	+	-
	JB	24	F	+	-	+	+	+	-
	NG	27	M	-	-	+	+	+	-
	AB	22	F	-	-	+	-	+	-
	AG	21	F	+	-	+	+	-	-
	RP	22	M	+	+	-	-	+	-
Atopic with rhinitis (hay fever)	MF	38	M	-	+	-	+	+	-
	SP	32	F	+	+	-	+	-	-
	DT	33	F	-	+	-	+	+	-
	HD	28	F	-	+	-	+	-	-
	JW	37	F	-	+	-	+	-	-
	RT	23	F	-	+	-	+	-	-
	EC	26	F	-	+	-	+	-	-
	TB	25	M	-	+	-	+	-	-
	MH	35	M	-	+	-	+	-	-
	LS	23	F	-	+	-	+	-	-

SPT, Skin prick test; *HDM*, house dust mite; *GP*, grass pollen; *AF*, *Aspergillus fumigatus*; *AH*, antihistamines; *CS*, topical corticosteroids; *IT*, immunotherapy.

Normal and patient volunteers were not studied within 1 month of upper respiratory tract infections.

Smokers and patients with nasal deformity or mechanical obstruction (e.g., caused by polyps, surgery, or trauma) were excluded. Patient volunteers were asked to stop all treatment for rhinitis at least 15 days before the study. Although the patients with atopic eczema were receiving topical corticosteroids, none of them were taking systemic corticosteroids. None of the patients had previously received immunotherapy. Approval was obtained from the Ethics Committee of St. Bartholomew's Hospital, and normal and patient volunteers provided written informed consent to participate in the study.

Study protocol

Nasal biopsy specimens from nonatopic normal volunteers without rhinitis and atopic patients without rhinitis were obtained outside the pollen season (October to November 1992 and February 1993, respectively). Nasal biopsy specimens from patients with AR were obtained both during (July 1994) and after the pollen season (February 1995) in the United Kingdom. To avoid significant differences in the duration and amount of natural exposure to allergen among the patients, especially during the pollen season, biopsy specimens were obtained from all subjects during the same week.

Reagents

All reagents were of tissue culture grade and purchased from Sigma Chemical Company (Dorset, U. K.), unless stated otherwise.

Nasal tissue

Nasal biopsy specimens were obtained from the anterior end of the inferior turbinate, after application of local anesthetic. The local anesthetic was instilled by placing a small pledget of cotton wool soaked with 0.5 to 0.75 ml of 10% cocaine hydrochloride sterile solution between the inferior turbinate and the septum for 10 minutes.²² A biopsy specimen of approximately 2 to 3 mm³ in size was obtained from each subject by using cup biopsy forceps (Gerritsma forceps; Viru Instruments, Oegstgeest, The Netherlands) and immediately placed in chilled culture medium (Medium 199; Northumbria Biologicals Ltd., Northumberland, U.K.) containing antibiotics. Each biopsy specimen was processed for tissue culture within 30 minutes of resection.

Culture of human NECs

NECs were cultured by using the explant cell culture technique developed in our laboratory.²³ Briefly, the epithelium was dissected away from the underlying lamina propria; and after further dissection into smaller sections of approximately 0.5 mm³, the epithelium was washed three times with fresh, sterile Medium 199. Single sections of the epithelium were explanted into 9 mm diameter Falcon cell culture inserts (Becton Dickinson Ltd., Oxford, U.K.); and 100 μ l aliquots of culture medium supplemented with 2.5% fetal bovine serum and a variety of growth factors including human transferrin (2.5 μ g/ml), epidermal growth factor (20 ng/ml), bovine pancreatic insulin (2.5 μ g/ml), hydrocortisone (0.36 μ g/ml), L-glutamine (0.02 mg/ml), and 1.5% (vol/vol) antibiotic and antimycotic solution were added to each insert.

The inserts were placed in 24-well culture plates (Becton Dickinson Ltd.) containing 400 µl of the complete culture medium, as described above, in each well and incubated at 37° C in a humidified 5% CO₂ in air atmosphere. The media in the insert and the well were replaced with culture media containing 2.5% NUSERUM IV Culture Supplement (Universal Biologicals Ltd., London, U.K.) after 3 days, and then every 48 hours. The cultures were observed for epithelial cell outgrowth until the cells had grown to confluence. The explants were removed, and the cultures were incubated further until the area left vacant by the explant was overgrown with epithelial cells.

The identity of the epithelial cells was confirmed in all cultures by light microscopy and in randomly selected cultures by electron microscopy and immunocytochemical staining for cytokeratin with monoclonal antibody (mAb) preparation CAM 5.2 (Becton Dickinson Ltd.). Contaminating cell types were analyzed by staining for T and B lymphocytes with mAbs CD3 and CD37, respectively (Serotec Ltd., Oxford, U.K.); and fibroblasts, mast cells, neutrophils, and macrophages were analyzed by staining with mAbs 5B5, AA1, NP57, and Ber-MAC3, respectively (Dako Ltd., Bucks., U.K.). Eosinophils were analyzed by staining cultures with Kimura's stain.²⁴ The viability of the NECs was determined by trypan blue exclusion, and only cultures found to be greater than 95% viable were used in further investigations.

In order to standardize for both the age and therefore size of cultures (cell numbers), only 2- to 3-week-old confluent cell cultures from each subject were used. This was necessary because these could contribute to gross differences in the amounts, and possibly in the types, of inflammatory cytokines generated by these cells. Before use, all cultures were washed and incubated overnight in SF-1 Supplement medium (Serum free medium; Hyclone Europe Ltd., Northumberland, U.K.). The cultures were then washed three times with fresh SF-1 medium. Five hundred microliters of SF-1 medium was added to each insert, 400 µl of SF-1 medium was added to each well, and the cultures were incubated for 6 hours at 37° C in 5% CO₂ in air atmosphere. All experiments were carried out in SF-1 medium to ensure that the cytokines detected were derived from the cells and not from serum, a potential source of cytokines. At the end of incubation, the medium was collected from each cell culture and stored immediately at -70° C until analysis for cytokines was performed. The insert membrane, containing the attached epithelial cells, was removed and stored by freezing at -20° C in 250 µl of 1 mol/L NaOH until analysis for total cellular protein was done with the method of Lowry et al.²⁵

Estimation of cytokines released

The levels of IL-1β, IL-8, GM-CSF, TNF-α, and RANTES released into the culture medium over a period of 6 hours were measured by using commercially available ELISA kits (R&D Systems Europe Ltd., Oxford, U.K.). These assays were highly specific, with no significant cross-reactivity or interference between any of the cytokines being investigated. These assays were also highly sensitive, as demonstrated by their sensitivities, ranging from 0.3 pg/ml for IL-1β to 4.4 pg/ml for TNF-α. To account for any differences resulting from these parameters, cytokine release was expressed per unit weight of intracellular protein analyzed for each culture; therefore, all results were expressed as picograms of cytokine per microgram of cellular protein.

Statistical analysis of results

Nonparametric statistical tests were used because of the non-normality of the data. The Kruskal-

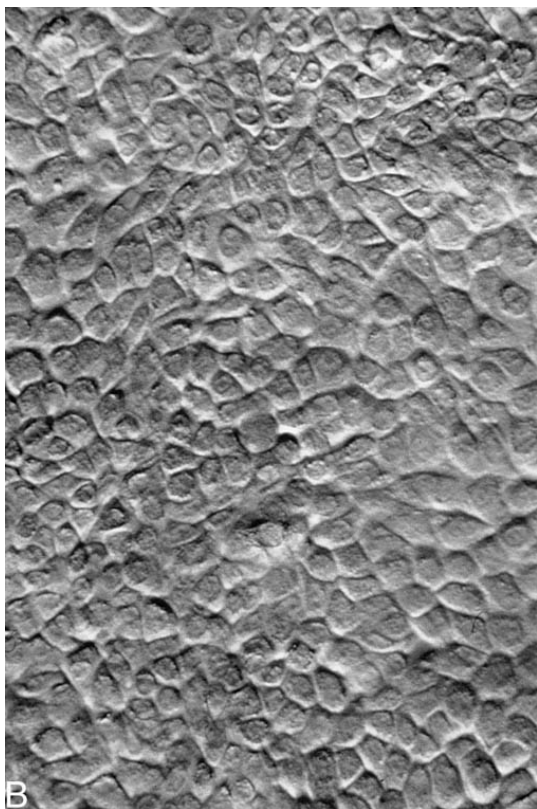
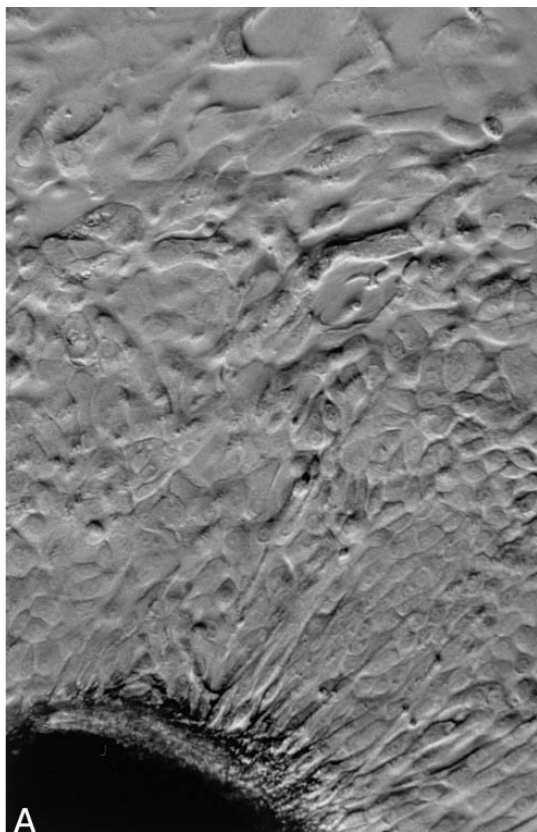
Wallis one-way analysis of variance by ranks was first applied to determine whether there were genuine population differences between cultures from the different groups of subjects. If so, the Mann-Whitney U test was used to compare results for cultures from atopic patients without rhinitis, atopic patients with rhinitis, and nonatopic subjects without rhinitis. Bonferroni's correction was used for multiple comparisons. Wilcoxon's matched-pair, signed-rank test was applied for within-group comparisons of atopic patients with rhinitis during and after the pollen season. The criterion for statistical significance was set at $p \leq 0.05$. All statistical tests were performed with a standard computer package (Minitab Data Analysis Software Release 6.1.1, 1987; Minitab Inc., State College, Pa.).

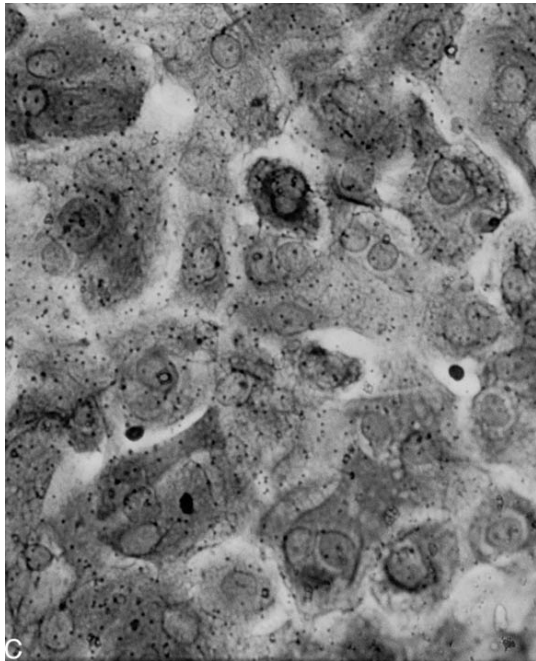
Results

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These studies demonstrated that epithelial cells could be cultured from nasal biopsy tissue of well-characterized nonatopic normal volunteers without rhinitis and atopic patients with and without rhinitis. Fig. 1, A, shows epithelial cell outgrowth from nasal explants after 1 week in culture and demonstrates the presence of a large number of cells with polygonal structure typical of epithelial cells.

Fig. 1. A, Epithelial cell outgrowth from nasal explants after 1 week in culture, demonstrating the presence of a large number of cells with polygonal structure typical of epithelial cells. **B,** A confluent culture of NECs after 2 weeks with the nasal explant removed and the area left vacant by the explant overgrown with epithelial cells (Hoffman Modulation contrast light micrograph, $\times 300$.) Analysis of cultures by immunocytochemistry demonstrated that all these cells stained for cytokeratin (**C**), with no indication of any contaminating cell types including T and B lymphocytes, fibroblasts, mast cells, neutrophils, macrophages, and eosinophils, as determined by immunocytochemical stainings with mAbs specific to these cell types.





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Fig. 1, B, ■ shows a confluent culture of NECs after 2 weeks with the nasal explant removed and the area left vacant by the explant overgrown with epithelial cells. In all cultures the identity of the epithelial cells was confirmed by light microscopy, and in randomly selected cultures, by electron microscopy. The average number of NECs yielded from a confluent culture established on 9 mm diameter cell culture insert was approximately 10.⁶ We have established that regardless of the source of nasal biopsy tissue, the time required for the cultures to grow to confluence did not vary by more than 1 to 2 days.

Analysis of cultures by immunocytochemistry demonstrated that all these cells stained for cytokeratin (Fig. 1, C ■), with no indication of any contaminating cell types including T and B lymphocytes, fibroblasts, mast cells, neutrophils, macrophages, and eosinophils, as determined by immunocytochemical staining with mAbs specific to these cell types. The viability of the NECs was determined by trypan blue exclusion, and only cultures found to be greater than 95% viable were used in further investigations.

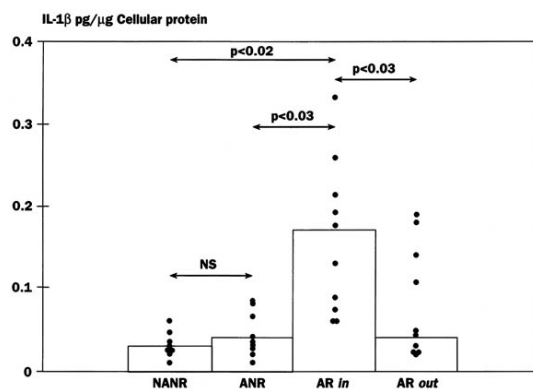
Analysis of the proinflammatory cytokines released by these cells in vitro demonstrated that cultured NECs released IL-1 β , IL-8, GM-CSF, TNF- α , and RANTES into the culture medium. Of the different cytokines investigated in this study, IL-1 β was found to be released in the lowest concentrations and IL-8 in the greatest concentrations, regardless of whether the cells were cultured from nasal tissue of atopic patients with rhinitis (in or outside the pollen season), atopic patients without rhinitis, or nonatopic subjects without rhinitis.

Release of IL-1 β

IL-1 β was released in significantly greater amounts from NEC cultures from atopic patients with rhinitis who underwent biopsy during the pollen season as compared with NEC cultures from normal volunteers without rhinitis, from atopic patients without rhinitis, and from atopic patients with rhinitis who underwent biopsy outside the pollen season. No statistical differences were found between the

amounts of IL-1 β released from NECs of atopic patients with rhinitis outside the pollen season and atopic patients without rhinitis or normal volunteers. No statistical differences were found between the amounts of IL-1 β released from NECs of atopic patients without rhinitis and normal volunteers (Fig. 2, Tables II and III).

Fig. 2. Levels of IL-1 β released by cultured human NECs from nonatopic subjects without rhinitis (NANR, $n = 8$), atopic patients without rhinitis (ANR, $n = 9$), and atopic patients with rhinitis (AR, $n = 10$). Nasal biopsy specimens from the AR group were taken during (AR in) and outside (AR out) the pollen season. Columns represent median values. Average amounts of total cellular protein were 75.0, 75.0, 84.4, and 77.4 $\mu\text{g/ml}$ for NANR, ANR, AR in, and AR out, respectively.



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Table II. Median (range) values of all the cytokines investigated in all groups

Group	IL-1 β	IL-8	GM-CSF	TNF- α	RANTES
NANR	0.03 (0.01-0.06)	1.13 (0.20-2.47)	0.18 (0.09-0.30)	0.34 (0.15-0.71)	0.20 (0.09-0.41)
ANR	0.04 (0.01-0.08)	4.7 (0.67-9.73)	0.48 (0.16-0.82)	0.90 (0.40-1.50)	0.21 (0.10-0.44)
AR in	0.17 (0.06-0.33)	8.18 (2.75-26.66)	0.78 (0.41-1.40)	1.97 (0.97-2.64)	1.19 (0.70-1.65)
AR out	0.04 (0.02-0.19)	6.87 (1.44-14.89)	0.30 (0.08-1.19)	0.11 (0.09-0.4)	0.75 (0.23-1.40)

All values expressed as picograms of cytokine per microgram of cellular protein.
NANR, Nonatopic without rhinitis; *ANR*, atopic without rhinitis; *AR in*, atopic rhinitis in pollen season; *AR out*, atopic rhinitis outside pollen season.

Table III. Bonferroni-corrected *p* values for all comparisons

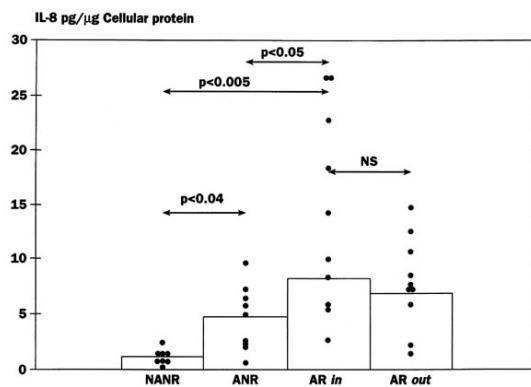
	IL-1β	IL-8	GM-CSF	TNF-α	RANTES
					0.81
NANR vs ANR	0.57 (NS)	< 0.04	< 0.04	< 0.03	(NS)
NANR vs AR in	< 0.02	< 0.005	< 0.006	< 0.006	< 0.02
NANR vs AR out	0.15 (NS)	< 0.01	0.32 (NS)	< 0.03	< 0.03
ANR vs AR in	< 0.03	< 0.05	< 0.02	< 0.02	< 0.02
ANR vs AR out	0.47 (NS)	0.09 (NS)	1.0 (NS)	< 0.002	< 0.03
AR in vs AR out	< 0.03	0.35 (NS)	< 0.03	< 0.009	< 0.04

NANR, Nonatopic without rhinitis; *ANR*, atopic without rhinitis; *AR in*, atopic rhinitis in pollen season; *AR out*, atopic rhinitis outside pollen season; *NS*, not significant.

Release of IL-8

IL-8 was released in significantly greater amounts from NEC cultures from atopic patients with rhinitis who underwent biopsy during the pollen season as compared with NEC cultures from normal volunteers and atopic patients without rhinitis. IL-8 was released in greater amounts by NEC cultures from volunteers who underwent biopsy during the pollen season than NEC cultures from the same volunteers who underwent biopsy outside the pollen season; however, the difference did not achieve statistical significance. NEC cultures from atopic patients with rhinitis who underwent biopsy outside the pollen season released significantly greater amounts of IL-8 than cell cultures from normal volunteers and atopic patients without rhinitis. NEC cultures from atopic patients without rhinitis released significantly more IL-8 than cell cultures from normal volunteers (Fig. 3, Tables II ■ and III ■).

Fig. 3. Levels of IL-8 released by cultured human NECs from nonatopic subjects without rhinitis (NANR, $n = 8$), atopic subjects without rhinitis (ANR, $n = 9$), and atopic patients with rhinitis (AR, $n = 10$). Nasal biopsy specimens from the AR group were taken during (AR *in*) and outside (AR *out*) the pollen season. Columns represent median values. Average amounts of total cellular protein were 93.8, 87.5, 99.7, and 81.3 $\mu\text{g/ml}$ for NANR, ANR, AR *in*, and AR *out*, respectively.

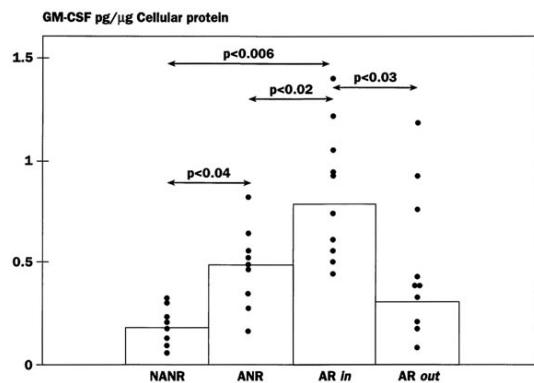


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Release of GM-CSF

GM-CSF was released in significantly greater amounts by NEC cultures from atopic patients with rhinitis naturally exposed to pollen as compared with NEC cultures from atopic patients without rhinitis and normal volunteers. Cell cultures from atopic patients with rhinitis naturally exposed to grass pollen released significantly greater amounts of this cytokine when compared with those cell cultures from specimens obtained outside the pollen season. No statistical differences were found when amounts of GM-CSF obtained from NECs cultured from atopic patients with rhinitis who underwent biopsy outside the pollen season were compared with the amounts of GM-CSF released by normal volunteers and atopic patients without rhinitis. NECs from atopic patients without rhinitis released significantly greater amounts of GM-CSF than NECs from normal volunteers (Fig. 4, Tables II ■ and III ■).

Fig. 4. Levels of GM-CSF released by cultured human NECs from nonatopic subjects without rhinitis (NANR, $n = 8$), atopic subjects without rhinitis (ANR, $n = 9$), and atopic patients with rhinitis (AR, $n = 10$). Nasal biopsy specimens from the AR group were taken during (AR in) and outside (AR out) the pollen season. Columns represent median values. Average amounts of total cellular protein were 75.0, 31.3, 43.8, and 69.5 $\mu\text{g}/\text{ml}$ for NANR, ANR, AR in, and AR out, respectively.

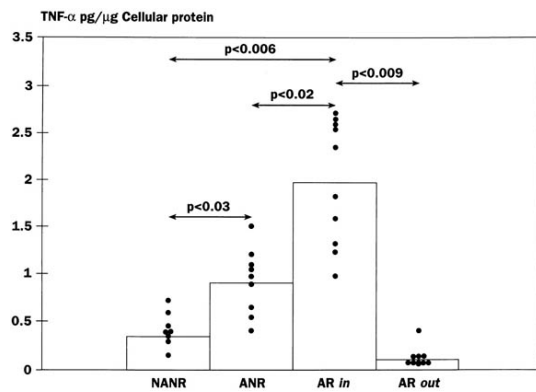


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Release of TNF- α

NEC cultures from the atopic group with rhinitis who underwent biopsy during the pollen season released significantly greater amounts of TNF- α as compared with NEC cultures from the atopic patients without rhinitis and normal volunteers. Cell cultures from atopic patients with rhinitis naturally exposed to grass pollen released greater amounts of this cytokine when compared with those cell cultures from specimens obtained outside the pollen season. This result was highly significant. The amounts of TNF- α released by NEC cultures from atopic patients without rhinitis and normal subjects were significantly greater than the amounts released by NEC cultures from atopic patients with rhinitis obtained outside the pollen season. NEC cultures from atopic patients without rhinitis released significantly more TNF- α than cell cultures from normal volunteers (Fig. 5, Tables II ■ and III ■).

Fig. 5. Levels of TNF- α released by cultured human NECs from nonatopic subjects without rhinitis (NANR, $n = 8$), atopic patients without rhinitis (ANR, $n = 9$), and atopic patients with rhinitis (AR, $n = 10$). Nasal biopsy specimens from the AR group were taken during (AR *in*) and outside (AR *out*) the pollen season. Columns represent median values. Average amounts of total cellular protein were 31.9, 77.4, 54.1, and 46.6 $\mu\text{g}/\text{ml}$ for NANR, ANR, AR *in*, and AR *out*, respectively.

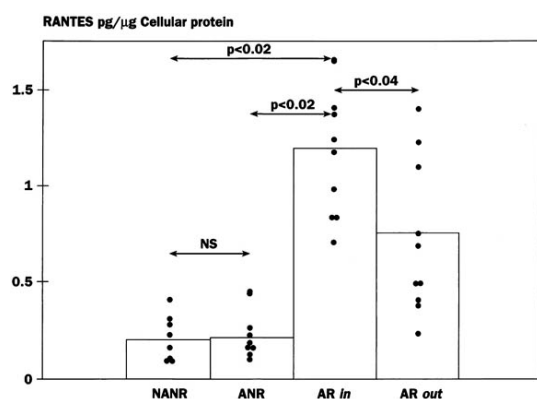


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Release of RANTES

RANTES was produced in significantly greater amounts by NEC cultures from patients with AR naturally exposed to pollen as compared with cell cultures from the atopic patients without rhinitis and normal volunteers. Cell cultures from atopic patients with rhinitis naturally exposed to grass pollen released significantly greater amounts of this cytokine when compared with those NEC cultures obtained outside the pollen season. NEC cultures from atopic patients with rhinitis who underwent biopsy outside the pollen season released significantly greater amounts of RANTES than cell cultures from atopic patients without rhinitis and normal volunteers. No differences were found between the amounts of RANTES released by NEC cultures from atopic patients without rhinitis and normal volunteers (Fig. 6, Tables II ■ and III ■).

Fig. 6. Levels of RANTES released by cultured human NECs from nonatopic subjects without rhinitis (NANR, $n = 8$), atopic patients without rhinitis (ANR, $n = 9$), and atopic patients with rhinitis (AR, $n = 10$). Nasal biopsy specimens from the AR group were taken during (AR *in*) and outside (AR *out*) the pollen season. Columns represent median values. Average amounts of total cellular protein were 103.1, 95.3, 79.7, and 81.3 $\mu\text{g}/\text{ml}$ for NANR, ANR, AR *in*, and AR *out*, respectively.



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Discussion

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These studies have demonstrated that human NECs cultured from biopsy tissue can be grown to confluence on microporous membranes and thus provide a suitable model for the investigation of their role in the development of upper airway disease. This technique for culture of epithelial cells from biopsy tissue was based on the technique for culture of bronchial and NECs from surgical tissue, which we have developed and used extensively in our studies.²³ Although others have cultured and used NECs *in vitro*,¹⁶ a major difference in our technique is that we have obtained explant cultures instead of cultures obtained from tissue after protease treatment. Using this model, we have investigated the capacity of NECs from well-characterized groups of nonatopic subjects without rhinitis (normal volunteers), atopic patients without rhinitis (patients with atopic eczema), and atopic patients with rhinitis (patients with hay fever, studied both during and outside the pollen season), to release several proinflammatory cytokines, which may play a prominent role in the pathogenesis of allergic nasal disease. A time point of 6 hours was chosen for these studies on the basis of our findings in preliminary studies of bronchial epithelial cells cultured from surgical tissues, which allowed time- and dose-response studies to be performed for the release of these cytokines. In this study it was unfortunately not possible to perform such time-course studies because of the constraints imposed by the comparatively much smaller numbers of cultures that could be obtained from the relatively small biopsy specimens.

Our studies have demonstrated that although NECs from each group of individuals studied were

capable of constitutively releasing IL-1 β , IL-8, GM-CSF, TNF- α , and RANTES, the cells from both atopic patients with rhinitis and atopic patients without rhinitis generally released significantly greater amounts of IL-8, GM-CSF, and TNF- α than the cells from nonatopic normal volunteers.

A comparison between epithelial cells cultured from the atopic group without rhinitis and the atopic group with rhinitis studied outside of the pollen season demonstrated that although the cells of the atopic patients without rhinitis released significantly greater amounts of GM-CSF and TNF- α , the cells of the atopic patients with rhinitis released significantly greater amounts of RANTES, with no differences in release of IL-1 β and IL-8.

A comparison of the cells cultured from the atopic group without rhinitis and the atopic group with rhinitis studied during the pollen season, however, demonstrated that the levels of cytokines released from these cells were altered and that the cells of the patients with rhinitis released significantly greater amounts of all cytokines investigated including IL-1 β , IL-8, GM-CSF, TNF- α , and RANTES.

These results suggest that the genetic predisposition of atopic individuals to greater susceptibility to inflammatory processes may, at least in part, be a consequence of the ability of their airway epithelial cells to release greater amounts of proinflammatory cytokines. Furthermore, the observation that nasal cells from atopic patients with rhinitis can release significantly greater amounts of proinflammatory cytokines during the pollen season, as compared with outside the pollen season, suggests that the manifestation of symptoms of AR may be a consequence, at least in part, of the activation of NECs by external factors, such as natural allergen exposure. Although the precise mechanisms underlying natural allergen-induced release of inflammatory cytokines from the NECs are not clear, it is possible that these may be both direct and indirect. Direct effects may result from activation of specific epithelial cell surface receptors, which trigger specific signal transduction cascades essential for the regulation of genes coding for the specific cytokines. Alternatively, it is possible that pollen allergens that have enzymatic potencies, as is the case for house dust mite (*D. pteronyssinus*) allergens, may lead to epithelial cell damage, which itself may stimulate the synthesis and release of inflammatory cytokines.

Our findings that IL-1 β was released in lowest quantity and IL-8 in greatest quantity by NECs are in accordance with the findings of others. Kenney et al.¹⁵ have investigated the synthesis of several cytokines from NECs cultured from surgical nasal turbinate tissue and demonstrated that amounts of cytokines released were in the order IL-8 > IL-6 > IL-1 α > IL-1 β . Bachert et al.²⁶ have investigated the cytokine levels in nasal lavage fluids of allergic and nonallergic individuals and demonstrated that the quantities of cytokines detected were in the order IL-8 > IL-6 > TNF- α > IL-1 β . Our findings for GM-CSF are also in agreement with the findings of Ohtoshi et al.,^{20, 21} who demonstrated that NECs from patients with AR released significantly greater amounts of this cytokine than cells of normal healthy volunteers.

The observation that the release of all the cytokines investigated in this study is significantly enhanced after natural exposure to grass pollen allergen is of particular relevance to the pathogenesis of AR, because it has been demonstrated that each of these cytokines either directly or indirectly influences the activity of inflammatory cells, particularly eosinophils. It is thought that eosinophils are the chief effector cells in the inflammation of allergic diseases, which are characterized by peripheral blood eosinophilia and the presence of activated eosinophils in inflamed

tissues.²⁷

Studies of IL-1 β and TNF- α have demonstrated that these cytokines are potent inducers of endothelial cell adhesion receptors, such as intercellular adhesion molecule-1 (ICAM-1), E-selectin, and vascular cell adhesion molecule-1, which facilitate the adherence and transendothelial migration of leucocytes.^{10 26 28 29} Indeed, recent studies from our laboratory have demonstrated that culture medium collected from confluent bronchial epithelial cell cultures incubated for 24 hours (conditioned medium) significantly increased the percent of eosinophils and neutrophils adhering to endothelial cells in vitro and that anti-human IL-1 β and anti-human TNF- α neutralizing antibodies significantly attenuated this effect.³⁰ In addition, these studies have demonstrated that treatment of endothelial cells, for 1 hour before coculture with eosinophils and neutrophils, with neutralizing antibodies against ICAM-1, E-selectin, and vascular cell adhesion molecule-1 also attenuated the adherence of eosinophils and neutrophils to the endothelial cells.³⁰

Other studies have suggested that TNF- α may be a particularly important cytokine in the pathogenesis of allergic airway disease because: (1) it is a chemoattractant for neutrophils and monocytes; (2) it increases microvascular permeability; and (3) it activates T cells, mast cells, and eosinophils.^{31 33} Nasal biopsy studies of atopic individuals have demonstrated that the number of cells expressing messenger RNA for TNF- α is significantly increased 24 hours after allergen challenge and suggest that this cytokine is likely to play a major role in the allergic reaction.³⁴

Similarly, studies of GM-CSF, IL-8, and RANTES have shown that these cytokines are also likely to play key roles in the pathogenesis of AR, because they are potent chemoattractants and activators of eosinophils and neutrophils.^{35 39} Studies by Roth-en-berg et al.⁴⁰ and Owen et al.⁴¹ have demonstrated that GM-CSF promotes the survival of eosinophils and have suggested that this cytokine plays a role in the maintenance of airway inflammation.

Although it is clear that IL-1 β , IL-8, GM-CSF, TNF- α , and RANTES are all likely to play a part in the pathogenesis of AR, of particular interest in this study are the significantly greater amounts of IL-1 β , TNF- α , and RANTES released by NEC cultures from atopic patients with rhinitis naturally exposed to allergen in comparison with NEC cultures from atopic patients without rhinitis and normal nonatopic volunteers. These differences suggest that these cytokines may play a particularly important role in the pathogenesis of the allergic reaction in atopy because of their potential to influence the expression of endothelial cell adhesion receptors and influence the migration of eosinophils. We have previously investigated the nasal epithelial expression of cellular adhesion molecules in biopsy tissue from normal nonatopic volunteers and patients with atopic perennial rhinitis and have demonstrated that ICAM-1 was expressed only in tissue of patients with atopic perennial rhinitis as indicated by positive staining for ICAM-1 with a specific mAb.⁴²

The finding that RANTES is released in significantly greater amounts from NEC cultures from only atopic patients with rhinitis, and not from atopic patients without this disease, suggests that the release of this cytokine from epithelial cells may be central to the development of symptomatic disease in atopic patients. Our findings for syn-thesis of RANTES by NECs are similar to those of Beck et al.,⁴³ who have demonstrated that RANTES is present in the epithelium of nasal polyps.

In summary, our studies have demonstrated that NECs of atopic patients with rhinitis release significantly greater quantities of specific proinflammatory cytokines than NECs of either atopic patients without rhinitis or nonatopic subjects without rhinitis and that natural exposure to allergen may accentuate both the amounts and types of cytokines released from the cells of the patients with rhinitis, possibly leading to exacerbation of their disease.

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References

TOP

1. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Friedhoff LR, Ehrlich-Kautzky E, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-3.

[MEDLINE](#)

2. Duff AL, Pomeranz ES, Gelber LE, Price GW, Farris H, Hayden FG, et al. Risk factors for acute wheezing in infants and children: viruses, passive smoke, and IgE antibodies to inhalant allergens. *Pediatrics* 1993;93:535-40.

3. Sherrill DL, Halonen M, Burrows B. Relationships between total serum IgE, atopy, and smoking: a twenty-year follow-up analysis. *J Allergy Clin Immunol* 1994;94:954-62.

[MEDLINE](#)

[ABSTRACT](#)

[FULL TEXT](#)

4. Bjerke T, Hedegaard M, Henriksen TB, Nielsen BW, Schiotz PO. Several genetic and environmental factors influence cord blood IgE concentration. *Pediatr Allergy Immunol* 1994;5:88-94.

[MEDLINE](#)

5. Byron KA, Varigos GA, Wootton AM. IL-4 production is increased in cigarette smokers. *Clin Exp Immunol* 1994;95:333-6.

[MEDLINE](#)

6. Calderón MA, Lozewicz S, Prior A, Jordan S, Trigg CJ, Davies RJ. Lymphocyte infiltration and thickness of the nasal mucous membrane in perennial and seasonal allergic rhinitis. *J Allergy Clin Immunol* 1994;93:635-43.

[MEDLINE](#)

[ABSTRACT](#)

[FULL TEXT](#)

7. Lozewicz S, Gomez E, Chilstrey S, Gatland D, Davies RJ. Time course of cellular infiltration in the nasal mucosa during the immediate allergic reaction. *Int Arch Allergy Appl Immunol* 1991;95:273-7.

[MEDLINE](#)

8. Lozewicz S, Gomez E, Clague J, Gatland D, Davies RJ. Allergen-induced changes in the nasal mucous membrane in seasonal allergic rhinitis: effect of nedocromil sodium. *J Allergy Clin Immunol* 1990;85:125-31.

[MEDLINE](#)

9. Lozewicz S, Davies RJ. Inflammatory cells in allergic rhinitis [editorial]. *Respir Med* 1991;85:259-61.

[MEDLINE](#)

10. Howarth PH. The cellular basis for allergic rhinitis. *Allergy* 1995;50(suppl 23):6-10.

[MEDLINE](#)

11. Durham SR. Allergic inflammation. *Pediatr Allergy Immunol* 1993;4(suppl 4):7-12.

[MEDLINE](#)

12. Davies RJ, Devalia JL. Epithelial cells. In: Barnes PJ, Rodger IW, editors. *Asthma: basic mechanisms and clinical management*. 2nd ed. London: Academic Press Ltd, 1992:143-56.

13. Devalia JL, Davies RJ. Airway epithelial cells and mediators of inflammation [editorial]. *Respir Med* 1993;87:405-8.

[MEDLINE](#)

14. Mullol J, Xaubet A, Gaya A, et al. Cytokine gene expression and release from epithelial cells. A comparison study between healthy nasal mucosa and nasal polyps. *Clin Exp Allergy* 1995;25:607-15.

[MEDLINE](#)

15. Kenney JS, Baker C, Welch MR, Altman LC. Synthesis of interleukin-1 α , interleukin-6, and interleukin-8 by cultured human nasal epithelial cells. *J Allergy Clin Immunol* 1994;93:1060-7.

[MEDLINE](#)

[ABSTRACT](#)

[FULL TEXT](#)

16. Marini M, Vittori E, Hollomberg T, Mattoli S. Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol* 1992;89:1001-9.

[MEDLINE](#)

17. Devalia JL, Campbell AM, Sapsford RJ, et al. Effect of nitrogen dioxide on synthesis of inflammatory cytokines expressed by human bronchial epithelial cells in vitro. *Am J Respir Cell Mol Biol* 1993;9:271-8.

18. Sousa AR, Poston RN, Lane SJ, Nakhosteen JA, Lee TH. Detection of GM-CSF in asthmatic bronchial epithelium and decrease by inhaled corticosteroids. *Am Rev Respir Dis*

1993;147:1557-61.

MEDLINE

19. Sousa AR, Lane SJ, Nakhosteen JA, Yoshimura T, Lee TH, Poston RN. Increased expression of the monocyte chemoattractant protein-1 in bronchial tissue from asthmatic subjects. *Am J Respir Cell Mol Biol* 1994;10:142-7.

20. Ohtoshi T, Tsuda T, Vancheri C, et al. Human upper airway epithelial cell-derived granulocyte-macrophage colony-stimulating factor induces histamine-containing cell differentiation of human progenitor cells. *Int Arch Allergy Appl Immunol* 1991;95:376-84.

MEDLINE

21. Ohtoshi T, Vancheri C, Cox G, et al. Monocyte-macro-phage differentiation induced by human upper airway epithelial cells. *Am J Respir Cell Mol Biol* 1991;4:255-63.

22. Prior AJ, Calder\$\$\$Aaon MA, Lavelle RJ, RJ Davies. Nasal biopsy: indications, techniques and complications. *Respir Med* 1995;89:161-9.

MEDLINE

23. Devalia JL, Sapsford RJ, Wells CW, Richman P, Davies RJ. Culture and comparison of human bronchial and nasal epithelial cells in vitro. *Respir Med* 1990;84:303-12.

MEDLINE

24. Kimura I, Moritani Y, Tanizaki Y. Basophils in bronchial asthma with reference to reagin-type allergy. *Clin Allergy* 1973;3:195-202.

MEDLINE

25. Lowry ON, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951;193:265-75.

26. Bachert C, Hauser U, Prem B, Rudack C, Ganzer U. Proinflammatory cytokines in allergic rhinitis. *Eur Arch Otorhinolaryngol* 1995;252(suppl 1):S44-S49.

27. Frew AJ, Kay AB. Eosinophils and T-lymphocytes in late-phase allergic reactions. *J Allergy Clin Immunol* 1990;85:533-9.

MEDLINE

28. Montefort S, Holgate ST, Howarth PH. Leucocyte-endothelial adhesion molecules and their role in bronchial asthma and allergic rhinitis. *Eur Respir J* 1993;6:1044-54.

MEDLINE

29. Canonica GW, Ciprandi G, Pronzato C, Paolieri F, Scordamaglia A, Bagnasco M. Role of adhesion molecules in allergic rhinitis. In: Basomba A, Sastre J, editors. *Proceedings XVI European Congress of Allergology and Clinical Immunology*. Bologna, Italy: Menduzzi Editore SpD., 1995:137-42.

30. Abdelaziz MM, Devalia JL, Khair OA, et al. The effect of conditioned medium from

cultured human bronchial epithelial cells on eosinophil and neutrophil chemotaxis and adherence in vitro. *Am J Respir Cell Mol Biol* 1995;13:728-37.

31. Bradding P, Mediawake R, Feather IH, et al. TNF α is localized to nasal mucosal mast cells and is released in acute allergic rhinitis. *Clin Exp Allergy* 1995;25:406-15.

MEDLINE

32. Van Overveld FJ, Jorens PG, Rampart M, De Backer W, Vermeire PA. Tumour necrosis factor stimulates human skin mast cells to release histamine and tryptase. *Clin Exp Allergy* 1991;21:711-4.

MEDLINE

33. Baroody FM, Lee BJ, Lim MC, Bochner BS. Implicating adhesion molecules in nasal allergic inflammation. *Eur Arch Otorhinolaryngol* 1995;252(suppl 1):S50-S58.

34. Ying S, Robinson DS, Varney V, et al. TNF α mRNA expression in allergic inflammation. *Clin Exp Allergy* 1991;21:745-50.

MEDLINE

35. Shute J. Interleukin-8 is a potent eosinophil chemoattractant [editorial]. *Clin Exp Allergy* 1994;24:203-6.

MEDLINE

36. Alam R, Stafford S, Forsythe P, et al. RANTES is a chemotactic and activating factor for human eosinophils. *J Immunol* 1993;150:3442-4347.

37. Resnick MB, Weller PF. Mechanisms of eosinophil recruitment. *Am J Respir Cell Mol Biol* 1993;8:349-55.

38. Schweizer RC, Welmers BAC, Raaijmakers JAM, et al. RANTES- and interleukin-8-induced responses in normal human eosinophils: effects of priming with interleukin-5. *Blood* 1994;83:3697-704.

MEDLINE

39. Zhang L, Redington AE, Holgate ST. RANTES: A novel mediator of allergic inflammation? *Clin Exp Allergy* 1994;24:899-904.

MEDLINE

40. Rothenberg ME, Owen WF, Silberstein DS, et al. Eosinophils cocultured with endothelial cells have increased survival and functional properties. *Science* 1987;237:645-7.

MEDLINE

41. Owen WF, Rothenberg ME, Silberstein DS, et al. Regulation of human eosinophil viability, density, and function by granulocyte/macrophage colony-stimulating factor in the presence of 3T3 fibroblasts. *J Exp Med* 1987;166:129-41.

MEDLINE

42. Devalia JL, Calder\$\$\$Aaon MA, Sapsford RJ, McAulay AE, d'Ardenne J, Davies RJ. Cell adhesion molecules expressed by human nasal epithelial cells [abstract]. Clin Exp Allergy 1992;22:120.

43. Beck LA, Schall TJ, Beall LD, Leopold D, Bickel C, Baroody F, et al. Detection of the chemokine RANTES and activation of vascular endothelium in nasal polyps [abstract]. J Allergy Clin Immunol 1994;93:234.

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Articles with References to this Article

TOP

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ABSTRACT

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Induction of the epidermal growth factor receptor and its ligands in nasal epithelium by ozone

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Riccardo Polosa, MD^{a,b}, Raymond J. Sapsford, BSc^c, Dejan Dokic, MD^a, Rossella R. Cacciola, PhD^b, Gaetano Prosperini, MD^b, Jagdish L. Devalia, PhD^c, Stephen T. Holgate, FRCP^a, Peter H. Howarth, FRCP^a, Donna E. Davies, PhD^{a*}

ABSTRACT

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Jean Bousquet, MD, PhD, Paul van Cauwenberge, MD, PhD, Nikolai Khaltsev, MDIn collaboration with the World Health Organization

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Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients in vitro

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Hasan Bayram, MD, PhD^a, Raymond J. Sapsford, BA^b, Muntasir M. Abdelaziz, PhD^c, Omar A. Khair, PhD^d

[ABSTRACT](#)

[FULL TEXT](#)

Comparison of ciliary activity and inflammatory mediator release from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients and the effect of diesel exhaust particles in vitro

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Hasan Bayram, MD^a, Jagdish L. Devalia, PhD^a, Omar A. Khair, MB^a, Muntasir M. Abdelaziz, MB^a, Raymond J. Sapsford, MIST^a, Masaru Sagai, PhD^b, Robert J. Davies, MD^a

[ABSTRACT](#)

[FULL TEXT](#)

Stem cell factor mRNA expression and production in human nasal epithelial cells: Contribution to the accumulation of mast cells in the nasal epithelium of allergy

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Hirokuni Otsuka, MD, PhD^a, Taeko Kusumi, MD, PhD^a, Shozo Kanai, PhD^b, Mamoru Koyama, MD^a, Yoko Kuno, MD^a, Ryuta Takizawa, MD, PhD^a

[ABSTRACT](#)

[FULL TEXT](#)

Effect of fexofenadine on eosinophil-induced changes in epithelial permeability and cytokine release from nasal epithelial cells of patients with seasonal allergic rhinitis

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Muntasir M. Abdelaziz, MRCP, Jagdish L. Devalia, PhD, Omer A. Khair, MRCP, Hasan Bayram, MD, Andrew J. Prior, FRCS, Robert J. Davies, FRCP
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