

Evidence for the involvement of free light chain immunoglobulins in allergic and nonallergic rhinitis

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Background: Allergic rhinitis is characterized by mast cell degranulation induced by antigen cross-linking of IgE. It has been proposed that some patients with rhinitis show nasal allergy in the absence of systemic markers of atopy, termed *entopy*. Recent murine studies suggest the existence of an IgE-independent hypersensitivity response involving antigen-induced mast cell activation, mediated by immunoglobulin free light chains (FLCs).

Objectives: To determine whether FLC is associated with mast cell-mediated nasal hypersensitivity and its relationship with eosinophilic activity in allergic and nonatopic rhinitis.

Methods: Patients with allergy and nonallergic rhinitis with eosinophilia syndrome (NARES) had levels of soluble FLC measured in nasal secretions and serum. In addition, levels of the nasal inflammatory mediators mast cell tryptase and eosinophil cationic protein were quantified. Cellular expression of κ and λ FLC was characterized in the nasal mucosa of allergic and nonatopic idiopathic rhinitis and control subjects by using immunohistochemistry. Immunopositive cells were phenotyped by using laser microdissection and PCR.

Results: Free light chain was significantly increased in nasal secretions of subjects with allergy and NARES, and in serum of patients with NARES. Nonatopic patients with allergy showed significantly increased nasal mast cell tryptase and eosinophil cationic protein. FLC-positive cells were significantly increased in allergic and nonatopic mucosa, and were shown to be mast cells and plasma cells.

Conclusion: Nasal FLC is significantly increased in allergic and nonatopic rhinitis nasal mucosa, suggesting a role in nasal hypersensitivity. Further studies are needed to identify which allergens trigger FLC-mediated responses in nonatopic rhinitis. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Rhinitis, allergy, nonallergic, immunoglobulin, free light chains

Allergy commonly manifests in the upper and lower airways as allergic rhinitis and asthma, respectively. Allergic rhinitis¹ and asthma² appear to share a unified disease pathway³ attributed to an inflammatory cell cascade involving T_H2⁴ cells with the production of allergen-specific IgE antibodies from activated B cells (plasma cells). Subsequently, IgE is taken up by receptors on mast cells, and when cross-linked by allergen, degranulation occurs with the release of inflammatory mediators.⁵ Mast cells are abundant in the nasal mucosa of allergic rhinitis mucosa and submucosal lung tissue,⁶ consistent with their role in maintaining inflammation in allergic airway disease. More recently, it has been shown that some patients with nonallergic rhinitis also show increased numbers of mast cells in their nasal mucosa, supporting the proposal that a proportion of nonatopic patients have an inflammatory-type pathology, localized to the nasal mucosa.^{7,8}

A significant amount of IgE is produced locally in the submucosal region of allergic lung and nasal tissue^{9,10} and may account for allergen-specific IgE in subjects with an absence of systemic IgE.^{11,12} By definition, nonallergic rhinitis and nonallergic asthma have a pathophysiology that does not involve IgE,¹³ but reports showing the presence of mucosal inflammatory cells^{7,14-16} and physiological responses¹⁷ compatible with a local allergic-type immune response in some nonatopic subjects confound this definition. Other explanations have been sought for the immediate allergic-type responses found in some patients who apparently have nonatopic rhinitis and asthma in response to allergen challenge. Recently, evidence was provided of a novel and alternative IgE-independent antigen-specific hypersensitivity pathway, mediated by immunoglobulin free light chain (FLC).¹⁸ Just as passive IgE-mediated hypersensitivity can be transferred to nonatopic individuals, a mouse model has demonstrated that FLC-mediated sensitivity can be conferred to nonsensitized mice. Moreover, it is proposed that the FLC pathway might have a role in asthma because antigen-specific induced mast cell activation and bronchoconstriction has been shown to be associated with functional FLC activity in a mouse model.¹⁹ Although it was shown that FLCs bind to cultured murine mast cells, no direct evidence of FLC binding to mast cells in human tissue has yet been provided.

In healthy individuals, polyclonal (κ and λ) FLC are produced in excess, but their precise function remains unknown. In disease states such as myeloma, increased levels of monoclonal FLC are detectable as a consequence of clonal expansion of plasma cells, and furthermore, increased concentration of oligoclonal/polyclonal FLC is seen in a number of autoimmune/inflammatory

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Abbreviations used

ECP:	Eosinophil cationic protein
FLC:	Immunoglobulin free light chain
HDM:	House dust mite
κ:	κ Free light chain
λ:	λ Free light chain
MCT:	Mast cell tryptase
NARES:	Nonallergic rhinitis with eosinophilia syndrome
PAR:	Perennial allergic rhinitis
SPT:	Skin prick test

diseases.²⁰⁻²⁴ Because FLCs are involved in mediating skin and airway hypersensitivity^{18,19} in mice, we hypothesized that they might serve as candidate mediators for the allergic responses seen in some systemically IgE-negative subjects with rhinitis, warranting further investigation.

In this study, levels of secreted FLC in nasal secretions were measured. Furthermore, we investigated the presence and localization of FLC-expressing cells in the nasal mucosa of patients with allergic and a form of nonallergic (nonallergic rhinitis with eosinophilia [NARES]) persistent rhinitis, and sought to identify the cell types binding/producing FLC. Our *in vivo* studies showed increased levels of bound and secreted FLC in the nasal mucosa of individuals with persistent rhinitis, irrespective of their atopic status, compared with healthy controls. These findings suggest a role for FLC in chronic upper airway inflammation.

METHODS**Patient selection**

Groups of volunteers were recruited to assess the presence of soluble FLC, mast cell tryptase (MCT), eosinophil cationic protein (ECP), and IgE in nasal secretions.

Patients were recruited in the Department of Otorhinolaryngology, Head and Neck Surgery, Ludwig-Maximilians-University, Munich, Germany. All subjects underwent an examination by a rhinologist, including nasal endoscopy, atopy screening test, and if needed, computed tomography scans of paranasal sinuses. Rhinosinusitis and/or nasal polyposis constituted specific exclusion criteria for this study, as did local or systemic anti-inflammatory medication. The number of patients with comorbidity for asthma was as follows, based on self-reporting complaints: 14 of 90, nonallergic rhinitis; 4 of 90, NARES; and 2 of 90, controls without rhinitis. None of the patients with idiopathic rhinitis had asthma. Patients with persistent allergic rhinitis ($n = 90$) who had a positive skin prick test (SPT) to house dust mite (HDM) allergen and serum specific IgE (mean *Dermatophagoides pteronyssinus*-specific IgE, 16.5 ± 10.7 U/mL) were compared with patients with NARES ($n = 90$) and nonatopic normal controls ($n = 90$). Patients with NARES were defined as nonatopic with nasal secretion ECP >300 ng/mL. Patient profiles for each of the subject groups are shown in Table I.

To represent the diversity of heterogeneity found in nonallergic rhinitis, a separate group of patients with idiopathic rhinitis of unknown etiology was retrospectively identified from tissue archives belonging to patients previously referred to the Division of Otorhinolaryngology, Queen's Medical Center, Nottingham University Hospitals Trust, Nottingham, United Kingdom, for nasal obstruction. Formalin-fixed tissue blocks of inferior nasal turbinate tissue were obtained from patients with idiopathic rhinitis ($n = 10$; 6 men, 4 women; age range, 18-56 years) and compared with groups of patients with persistent allergic rhinitis ($n = 10$; 7 men, 3 women; age range, 18-50 years) and a normal control group ($n = 10$; 5 men, 5 women; age range, 19-48 years). Strict patient selection criteria were applied as previously described.⁷ All patients were free of asthma and had been withdrawn from topical corticosteroids and antihistamines for periods of 28 and 7 days, respectively.

Patients with allergy had a relevant medical history of allergic rhinitis and a positive SPT to HDM (*D pteronyssinus*) allergen indicated by a wheal greater than 3 mm in diameter and HDM specific IgE in the serum (UniCAP; Freiburg, Germany). In contrast, the idiopathic rhinitis group had negative SPT and serum specific IgE tests but displayed persistent (perennial) rhinitis symptoms.

The Ethics Committee at each institution approved the study, and informed consent was given by patients and volunteers who participated in this study.

Measurement of total IgE, FLC, MCT, and ECP

Patients attending the Department of Otorhinolaryngology, Munich, had nasal secretions collected by the absorption method, as previously described and validated in more than 1700 patients²⁵ (see this article's **Methods** in the Online Repository at www.jacionline.org).

Atopic condition was determined by measuring total serum IgE and by using the SX1 atopy screening test (Phadiatop; both UniCAP; Phadia, Freiburg, Germany), according to the supplier's instructions. Atopic individuals underwent further *in vivo* and *in vitro* allergy tests, including SPT, nasal allergen provocation, and allergen-specific IgE determination in serum (UniCAP; Phadia, Freiburg, Germany).

Nasal secretions of all patients were analyzed for MCT as a marker of mast cell degranulation, ECP as a marker of tissue eosinophil activation, and total IgE (all UniCAP; Phadia, Freiburg, Germany) in accord with the manufacturer's instructions as previously described.²⁵ FLC concentrations were measured by using an ELISA technique specific for λ and κ as described.¹⁸

Immunohistochemistry for κ and λ-FLC expressing cell counts

Blocks of nasal mucosa were stored at -80°C or fixed in formol calcium for immunohistochemistry (see additional information in the Online Repository, **Methods**). Sections of nasal mucosa were immunostained for κ and λ by using validated primary mouse mAbs (kindly donated by Dr A. Solomon, Knoxville, Tenn). The antifree κ (Fκ-C8) and antifree λ (Fλ-G9) antibodies have previously been shown to bind specifically only to κ and λ Bence Jones protein fragments, respectively^{26,27} (see the Online Repository, **Methods**, for additional information concerning validation and immunohistochemistry). Cell counts of κ and λ-positive cells were performed by using an eyepiece graticule by counting the number of cells in alternate fields within the epithelium and submucosal regions with an $\times 40$ magnification objective lens. The resulting counts were multiplied by a factor to arrive at cell counts per square millimeter. Frozen sections of nasal mucosa were immunostained for κ and λ for comparison with the paraffin-processed tissue.

Sequential immunostaining

Sequential immunostaining was performed to determine the cell localization of λ stained cells seen in serial paraffin sections of allergic and nonallergic rhinitic mucosa by using the labeled streptavidin biotin immunoperoxidase technique. Alternate sections were immunostained with the λ antibody with intervening sections stained for MCT (DakoCytomation, Cambs, UK), CD68 (macrophages; DakoCytomation), CD138 (plasma cells; DakoCytomation), CD20 (B cells; DakoCytomation), and CD34 (blood vessels; NovaCastra, Newcastle, United Kingdom).

Laser microdissection and PCR to investigate the identity of λ-positive cells

To investigate the candidacy of cells positively stained with λ a rapid immunohistochemistry technique was performed on frozen sections from a single subject with nonallergic nasal mucosa. A PALM Microbeam laser microdissector (Zeiss, Bernreid Germany) was used to obtain 500 single cells stained for λ. Total RNA was extracted and converted to cDNA for PCR by using exon-spanning PCR primers for MCT (mast cell-specific), CD138 (plasma cell-specific), and 18s ribosomal RNA. PCR products from the

TABLE I. Patient profiles for the allergic and nonallergic (NARES) rhinitis groups and normal control group

Group	Sex profile	Age (y), range (mean)	Mean nasal symptom score (range, 0-3)	Serum specific IgE
Normal controls (n = 90)	36 M	11-79 (42.6)	Obstruction 1.19 (SD 1.1)	None
	54 F		Secretion 1.16 (SD 0.9)	
			Sneezing 1.17 (SD 1.0)	
			Itching 0.84 (SD 0.9)	
Allergic rhinitis (n = 90)	57 M	10-68 (27.1)	Obstruction 2.18 (SD 0.9)	HDM only, 14/90
	33 F		Secretion 1.74 (SD 0.9)	HDM and S, 24/90
			Sneezing 1.70 (SD 1.1)	HDM and P, 13/90
			Itching 1.29 (SD 1.0)	HDM and S/P, 39/90
NARES (n = 90)	63 M	8-92 (36.2)	Obstruction 1.95 (SD 1.2)	None
	27 F		Secretion 1.52 (SD 1.0)	
			Sneezing 1.20 (SD 1.1)	
			Itching 0.89 (SD 1.0)	

F, Female; M, male.

Sensitivity to allergens was assessed by detection of serum specific IgE, and patients were categorized into groups showing sensitivity to HDM, seasonal (S; timothy grass pollen, birch pollen, herbs), and perennial (P; storage mites, pet danders, molds).

MCT and plasma cell DNA samples were sequenced by using an Applied Biosystems (ABI) 3130 instrument (PerkinElmer, Waltham, Mass; see the Online Repository, Methods, for additional information).

Statistics

The FLCs in nasal lavage secretions and serum were compared in a Mann-Whitney *U* test by using an SPSS program (version 15; SPSS Inc, Chicago, Ill). Correlations between FLC and MCT, ECP, and IgE in nasal secretions were investigated by using the Spearman test. Numbers of κ and λ -stained cells were compared in the 3 Nottingham patient groups by using a 2-tailed ANOVA. Subsequently, a follow-on Mann-Whitney *U* test was performed on pairs of the patient groups. A *P* value equal to or less than .05 was considered significant.

RESULTS

IgE measurements

Total IgE levels were measured in serum and nasal secretions. Significantly increased IgE was found in serum from the allergic group compared with healthy controls ($P < .0001$), but not in patients with NARES ($P = .7411$). Similarly, levels of nasal IgE were significantly increased in the allergic group ($P < .0001$) but not in the NARES group ($P = .3213$; Fig 1, A).

FLC, MCT, and ECP measurements

Levels of κ and λ were measured in nasal secretions of patients with perennial allergic rhinitis (PAR) and NARES and in control normal counterparts. κ was significantly increased in nasal secretions of subjects with allergy ($P = .019$) and NARES ($P < .0001$). A similar increase was also seen for λ in PAR ($P = .0007$) and NARES ($P < .0001$) samples (Fig 1, B). Levels of serum κ and λ were significantly increased in patients with NARES ($P < .05$) but not in the allergic group.

The inflammatory mediators MCT and ECP were measured in nasal secretions to assess degranulation of mast cells and eosinophils, respectively, as markers of mucosal inflammation. MCT and ECP levels were significantly increased in allergic and nonallergic secretions ($P < .0001$; Fig 1, C).

Significant associations were seen between secreted κ and λ and secreted MCT (κ , $r = 0.2599$; λ , $r = 0.2549$) and ECP (κ , $r = 0.3535$; λ , $r = 0.3706$) for the allergic group ($P < .05$). In addition,

a significant association was found between κ and λ and nasal IgE (κ , $r = 0.2759$; λ , $r = 0.3755$) in the PAR group ($P < .05$). No such associations were detected in NARES samples.

Immunohistochemistry for κ and λ FLC

The pattern of κ and λ immunostaining was similar in frozen and paraffin-embedded allergic and nonallergic perennial rhinitis tissues (Fig 2) with cells localized to the lamina propria. More cells stained for λ and the staining was more intense than that obtained with the anti- κ antibody. A significant increase in numbers of κ -stained cells was detected in allergic ($P = .005$) and idiopathic rhinitis ($P = .035$) mucosa compared with controls. Numbers of λ -stained cells were also increased in allergic ($P = .06$) and idiopathic rhinitis ($P = .0001$) mucosa (Fig 3). In rhinitic mucosa, increased numbers of FLC stained cells were localized in stroma between the submucosal glands, where plasma cells predominate. In the more superficial lamina propria, FLC staining was seen in granulated cells with a morphology resembling mast cells or CD68-positive macrophages. Serial sections immunostained with a panel of antibodies indicated localization of FLC to mucosal areas containing plasma cells and mast cells. In addition, a band of FLC staining was seen associated with the epithelial basement membrane and the adjacent basal epithelium, particularly in frozen sections, but the staining did not appear localized to single cells. Some CD34-positive capillaries and small vessels also showed localization of FLC.

Laser microdissection and PCR

Frozen sections of allergic rhinitis mucosa were immunostained for λ to facilitate single cell laser microdissection (see this article's Fig E1 in the Online Repository at www.jacionline.org). Extracted RNA subjected to PCR for MCT, CD138 (plasma cells), and 18S ribosomal RNA resulted in products of expected size (see this article's Fig E2 in the Online Repository at www.jacionline.org). Subsequent sequencing of the putative MCT and CD138 (plasma cell) amplicons confirmed fidelity.

DISCUSSION

In this study, we showed that patients with allergic and nonallergic rhinitis differ significantly from normal healthy

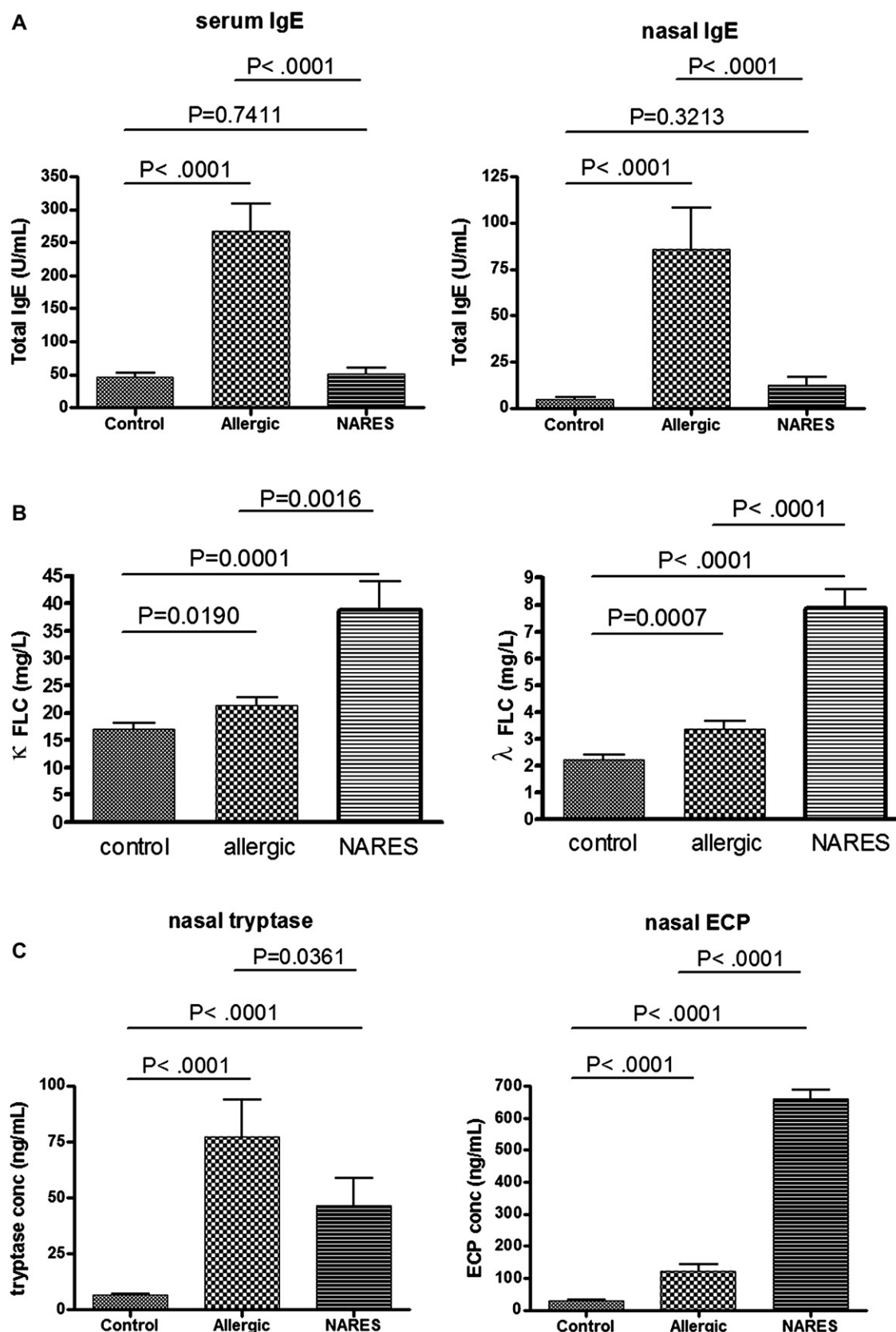


FIG 1. A, Increased serum ($P < .0001$) and nasal ($P < .0001$) IgE was found in the allergic but not in the NARES or control group. B, Levels of nasal κ and λ were significantly increased in patients with allergy and NARES compared with healthy controls. C, The allergic and NARES groups showed increased tryptase and ECP levels compared with control subjects.

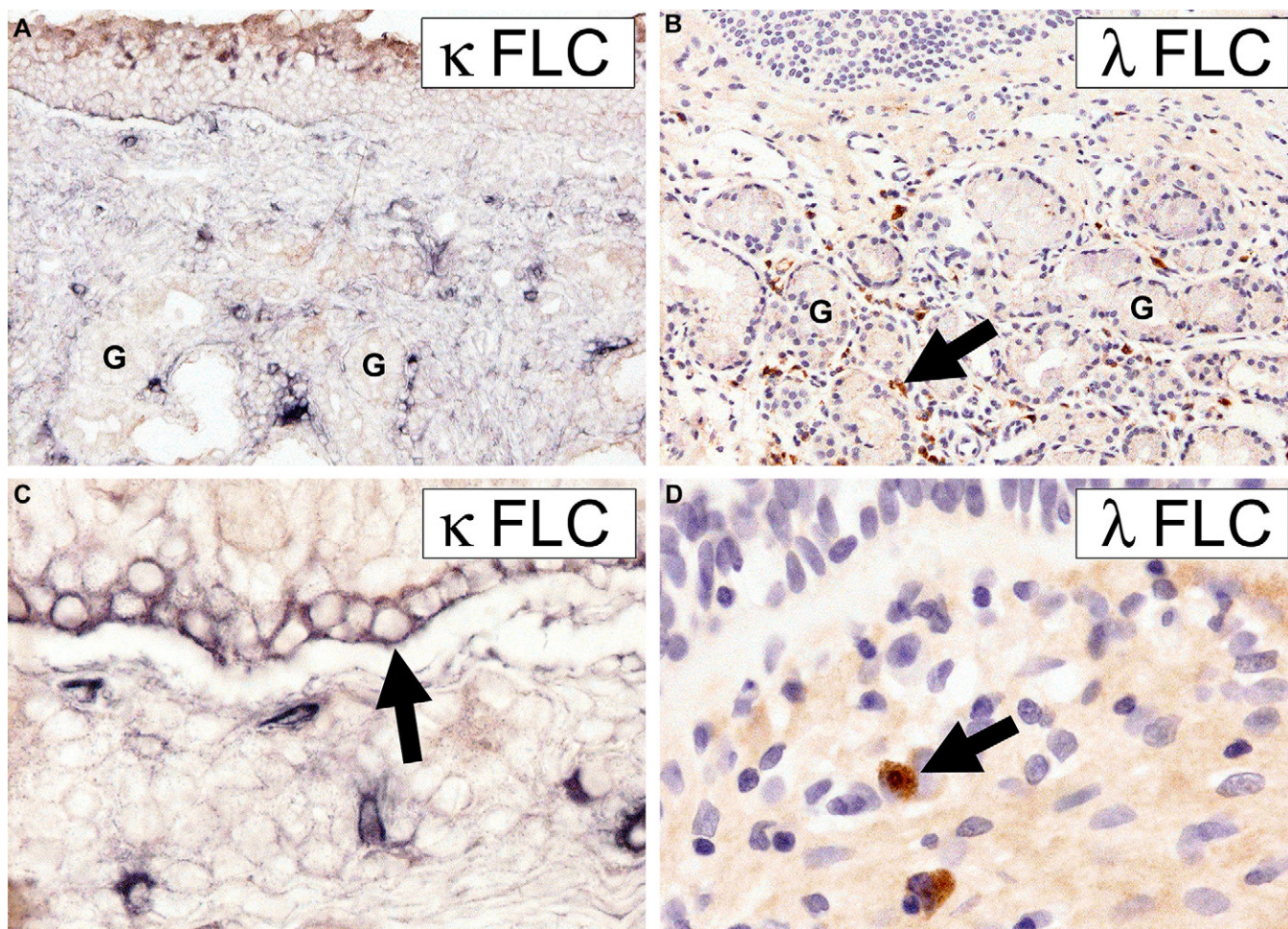


FIG 2. Immunohistochemistry showed FLC localized to stromal cells adjacent to submucosal glands (G) in (A) allergic and (B) idiopathic nasal mucosa, compatible with localization to plasma cells (arrow). FLC staining was seen (C) in the epithelium (arrow) and (D) in granulated cells with morphologic features of mast cells (arrow) in the superficial lamina proprium.

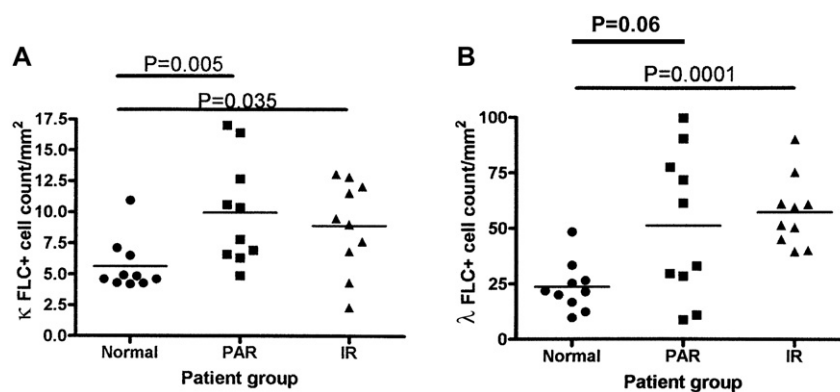


FIG 3. Increased numbers of (A) κ and (B) λ -stained cells appeared in the lamina proprium of nasal mucosa of PAR and idiopathic rhinitis (IR) mucosa compared with normal control subjects.

controls in having increased numbers of FLC-expressing cells in their nasal mucosa, accompanied by increased levels of secreted FLC. Furthermore, we found FLC is associated with mast cells and plasma cells, supportive of a possible local immune hypersensitivity function.

Allergic rhinitis is defined by IgE-mediated mast cell degranulation, contributing to the hallmark inflammatory symptoms seen in rhinitis. More recently, compelling evidence has shown the importance of locally produced IgE within the nasal mucosa of atopic subjects.²⁸⁻³¹ Interestingly, some apparently nonatopic

subjects with rhinitis share similar histologic mucosal features characterized by increased numbers of mast cells and eosinophils.⁷ Included in this group are patients with NARES³² and idiopathic rhinitis; both are characterized by a pathophysiology involving mast cells and eosinophils.^{15,33} Previously, we showed that some idiopathic subjects have the ability to bind specific allergen to mast cells in their nasal mucosa despite having negative systemic atopic tests for the same allergen. Subsequently, we introduced the term *entopy* to define the presence of a localized immune response in the nasal mucosa of patients with nonatopic rhinitis who tested negative with SPTs and RAST.¹⁴ The precise immune pathway responsible for this observation is not known, but our findings are consistent with studies that have shown anomalous IgE expression in nonatopic patient nasal secretions. Specific IgE has been identified in nasal secretions from RAST-negative patients with nonallergic rhinitis,^{11,12,34,35} and symptoms accompanying physiological airway changes have been documented in similar patient groups.¹⁷

An alternative explanation for the immune type responses seen in some subjects with nonatopic rhinitis might be found in non-IgE-mediated pathways, possibly involving IgG, because it was previously reported that in chronic sinusitis, increased levels of antifungal IgG antibodies are detectable, resulting in an inflammatory response.³⁶ More recently, attention has focused on the role of FLC in chronic inflammatory disease. Using a murine model, we previously showed that FLCs appear to function in a manner that is analogous to IgE and have a pivotal role in T-cell-mediated immediate and delayed hypersensitivity immune responses.³⁷ Moreover, there is a suggestion that local FLC production may account for the pathology seen in contact hypersensitivity, multiple sclerosis, and rheumatoid arthritis because of the formation of germinal center-like structures reported in some chronic inflammatory diseases,³⁸ including rheumatoid arthritis.³⁹ Importantly, there is increasing evidence linking FLC with the sensitization of mast cells and a proposed role in asthma.^{18,19} In the case of some nonatopic forms of rhinitis, mast cells have been proposed to have surrogate T-cell functions in promoting immunoglobulin production in B cells.⁴⁰

Ours is the first study to investigate the localization of FLC in allergic and nonallergic rhinitic tissue by using immunohistochemical and ELISA techniques with monoclonal anti-FLC antibodies of high specificity and sensitivity.^{26,27} From our animal experiments, we know that lymph nodes are certainly producers of FLC in mice,¹⁸ and although likely, it is not known with certainty whether this is the case in human beings. It is known that B lymphocytes localized in nasal mucosa and lymph nodes contribute to systemic and local mucosal IgE levels, respectively, and it is conceivable that FLCs detected in the serum and nasal secretions of our patient cohorts also derive from these cellular sources. Moreover, we showed the apparent localization of FLC to mast cells in idiopathic rhinitic mucosa, leading us to suggest that FLC may function in promoting and supplementing the inflammation seen in atopic and nonatopic rhinitis forms. Our findings of increased levels of secreted FLC in NARES and FLC-positive cells in patients with idiopathic rhinitis, more so than those identified in allergic rhinitis, are in agreement with our previous observation of increased mucosal mast cells in idiopathic rhinitis.⁷ These data suggest that mast cell activation and degranulation could be induced by a FLC-mediated mechanism, but although it is tempting to suggest that FLCs are involved in mediating allergen-induced responses, further studies are

required to investigate this possible function. In the perennial allergic group, levels of secreted FLC predictably showed significant association with the granulocyte mediators MCT and ECP, supporting a possible role for their involvement in the allergic inflammatory cascade. In this study, the increased FLC production is most likely a reflection of polyclonal stimulation of B cells. Increases in κ correlated well with increases in λ in the patients with allergy and NARES. No unidirectional (monoclonal) increase in κ or λ was observed. We hypothesize that increased FLC seen in rhinitis may be a result of increased polyclonal synthesis of antigen-specific FLC (similar to increased synthesis of specific IgE in patients with allergy), but further studies are needed to test this.

In conclusion, we provide evidence of significantly increased FLC-expressing cells in the mucosa of patients with persistent allergic and idiopathic rhinitis, localized to mast cells and plasma cells. In addition, serum FLC levels are increased in subjects with NARES, and further studies are required to explain the significance of this observation. On this evidence, we propose that FLC may provide an additional non-IgE immune pathway to augment or replace IgE-mediated hypersensitivity in chronic mucosal inflammatory disease. In accord with the recommendations of the European Academy of Allergy and Clinical Immunology (EACCI) task force,¹³ the presence of an FLC immune disease pathway in some subjects with idiopathic and NARES rhinitis may require their reclassification as having non-IgE mediated allergic rhinitis. Additional studies are needed to understand better the pathophysiology of nonallergic rhinitis.⁴¹ Among these, further studies are needed to identify which allergens serve as triggers in FLC-mediated mucosal immune responses and could involve measurement of FLC and secretory IgE (sIgE) in nasal secretions from subjects with atopic and nonatopic rhinitis after nasal allergen provocation. If proven, FLC blockade might provide a fresh approach in the treatment of non-IgE-mediated rhinitis, because such antagonists have recently shown promising results in animal models.^{18,19,42,43}

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Clinical implications: Novel treatment strategies involving blocking FLC might be effective in the treatment of allergic and nonallergic rhinitis.

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METHODS

Measurement of total IgE, FLC, MCT, and ECP

Patients had nasal secretions collected by the absorption method. Briefly, small cone-shaped cotton wool pieces with a length of about 4 cm and a diameter of about 6 mm were placed into the middle meatus of the nose under anterior rhinoscopic view and were left for 10 minutes, followed by centrifugation of the cotton wool strips at 2000g (8°C) for 10 minutes.

Immunohistochemistry for κ and λ -expressing cell counts

Blocks of full-thickness nasal mucosa were fixed in 10% formal calcium and processed in paraffin wax. Separate pieces of nasal mucosa from the same block were stored at -80°C . Sections of transversely orientated nasal mucosa were immunohistochemically stained for κ and λ . For immunohistochemistry, 4- μm -thick wax sections were dewaxed and subjected to antigen retrieval by using microwave-heated 0.01 mol/L sodium citrate buffer, pH 6.0. Sections were stained using a labeled streptavidin biotin technique on a TechMate 500 instrument (DakoCytomation, Cambs, United Kingdom [UK]) according to the manufacturer's instructions. The antifree κ (F κ -C8) and antifree λ (F λ -G9) antibodies have previously been shown to bind specifically only to κ and λ Bence Jones protein fragments, respectively.^{E1,E2} ELISA assay confirmed an absence of reactivity with total IgG, IgA, and IgM proteins containing the heavy and light immunoglobulin chains.^{E1} In addition, size exclusion chromatography was used to separate serum proteins, and the FLC antibodies specifically bound only to proteins that represented monomeric and dimeric forms of FLC (22-45 kd).

Frozen sections of nasal mucosa were immunostained for κ and λ for comparison with the paraffin-processed tissue. Briefly, cryosections were fixed in acetone and incubated in a 1:5000 dilution of each FLC antibody for 1 hour. An alkaline phosphatase antialkaline phosphatase technique (DakoCytomation) was used with visualization in nitroblue tetrazolium (Sigma, Poole, Dorset, UK). Negative control sections had the primary antibody either omitted or replaced by an inappropriate mouse mAb of the same immunoglobulin class and concentration.

Laser microdissection and PCR to investigate the identity of λ -positive cells

To investigate the candidacy of cells positively stained with λ , a rapid immunohistochemical technique was performed on frozen sections of a single allergic and a nonallergic nasal mucosa sample before laser microdissection. Sections were mounted on PALM PEN-membrane slides (PALM Microlaser

Technologies, Bernried, Germany), incubated in chilled 70% ethanol for 5 minutes, and then rapidly immunostained by using an Envision peroxidase technique (DakoCytomation) according to the supplier's instructions. RNase-free conditions were maintained by adding 40 U/mL RNase inhibitor (Ambion, Warrington, UK) to the immunochemical reagents and by the use of RNase-free wash buffers.

A PALM Microbeam laser microdissector (Zeiss, Bernried, Germany) was used to obtain single cells stained for λ in rhinitic nasal mucosa. Approximately 500 single cells were collected in an adhesive collection cap (PALM), and total RNA was extracted by using a Qiagen RNeasy Micro kit (Qiagen, Crawley, UK). cDNA was generated by using a random hexamer primer mix (Promega, Southampton, UK) and SuperScript III RNase H-reverse transcriptase (Invitrogen, Paisley, Scotland) reaction in accordance with the supplier's recommendations. PCR was performed on the cDNA samples by using exon-spanning PCR primers for MCT (mast cell-specific), CD138 (plasma cell-specific), and 18s ribosomal RNA. PCR reaction mixes (20 μL) contained 1 μL cDNA, 0.5 μL (10 $\mu\text{mol/L}$) forward and reverse primer, respectively, 2.5 μL 10x PCR buffer (Qiagen), 0.5 μL (10 mmol/L) deoxynucleotide triphosphate mix (ABGene, UK), and 0.12 μL Taq polymerase enzyme (Qiagen). The tubes were initially heated to 95°C for 15 minutes, followed by 40 cycles of 94°C for 30 seconds, 56°C for 60 seconds, and then 72°C for 90 seconds before finally being heated to 72°C for 10 minutes in the Techne TC-312 thermocycler (Eurofins MWG Operon, Ebersberg, Germany). The exon-specific primer sequences used were as follows: MCT (forward, cgggagcagcacctctacta; reverse, gagatgttcacgggctcc; amplicons size, 131 bp), CD138 (forward, ttagtgaggagctgggtgctg; reverse, gtattctccccgaggttcc; amplicons size, 122 bp), and 18s ribosomal RNA (forward, aaacggctaccacatccaag; reverse, caattacagggcctcgaaag; amplicons size, 112 bp). A negative control omitting cDNA was included. PCR products were assessed by agarose gel electrophoresis and the amplicon bands excised and purified by using a QiaQuick kit (Qiagen); 2 μL of the mast cell tryptase and plasma cell DNA sample was sequenced by using an Applied Biosystems (ABI) 3130 instrument (PerkinElmer, Waltham, Mass) with an ABI BigDye 3 RRX kit according to the manufacturer's instructions.

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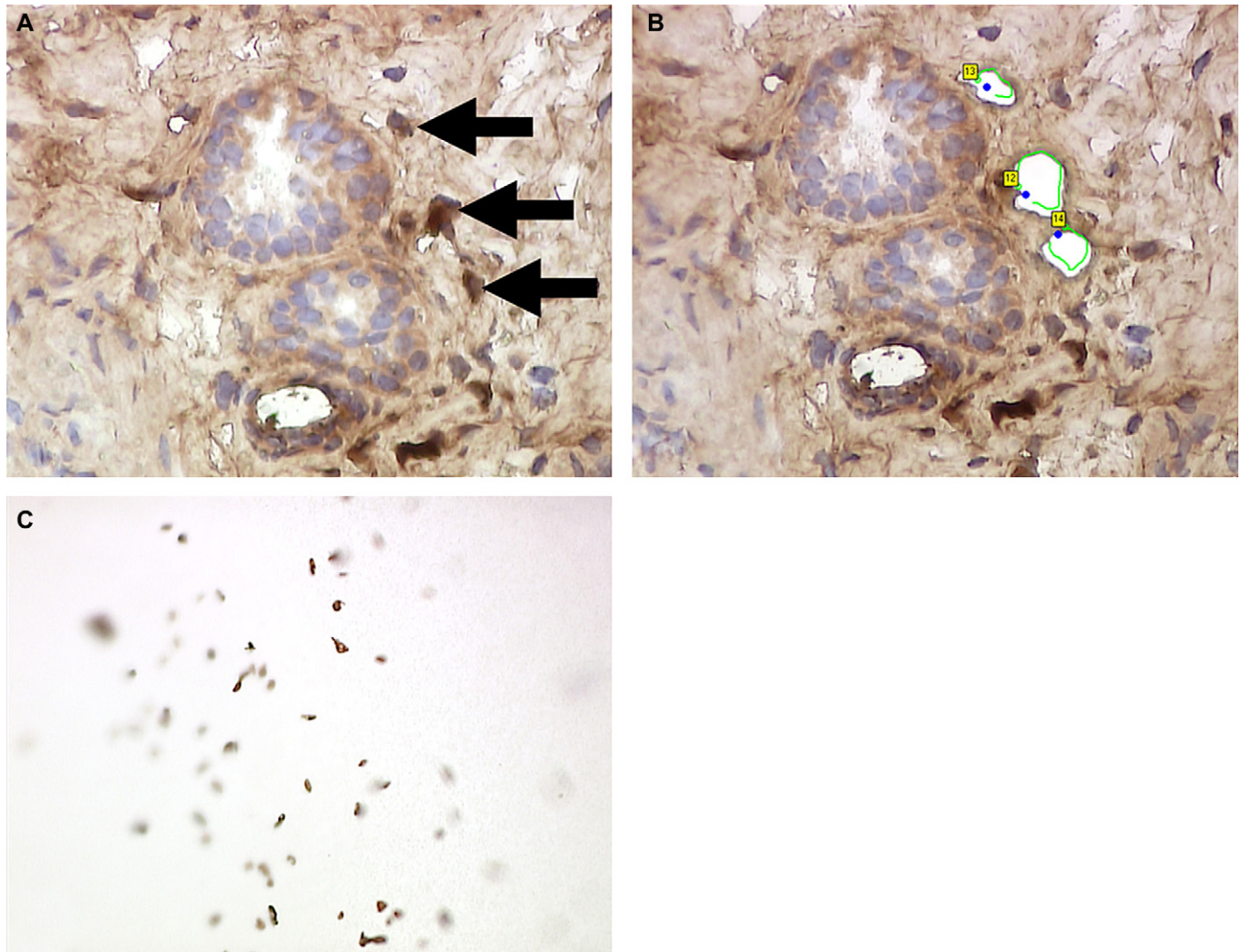


FIG E1. Laser microdissection (LMD) was performed on frozen sections of allergic mucosa after staining for λ -positive cells. **A**, Pre-LMD showing immunostained cells (*arrows*). **B**, Post-LMD. **C**, View of isolated laser-captured cells.

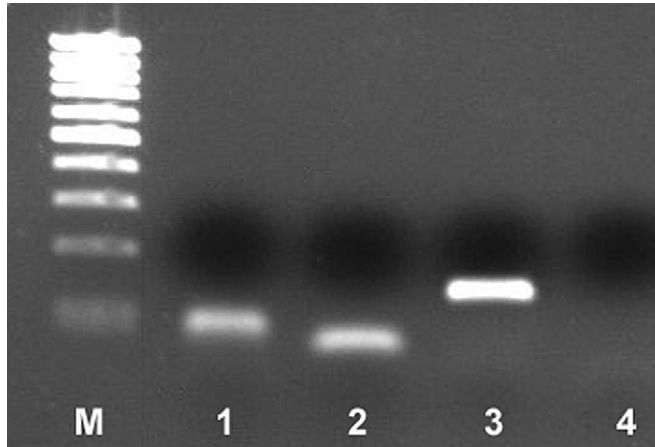


FIG E2. Genotyping of λ -positive cells was performed by using PCR and gel electrophoresis. Primer sequences specific for the detection of plasma cells (*lane 1*; 122 bp), the housekeeping gene 18s rRNA (*lane 2*; 112 bp), and mast cell tryptase (*lane 3*; 131 bp) showed bands of the predicted size compared to DNA (bp) size markers (*M*). A negative control PCR with cDNA template omitted is shown in *lane 4*.