
Reduction of TLR2 gene expression in allergic and nonallergic rhinitis

Lewis J. Vanhinsbergh, BMedSci*; Desmond G. Powe, PhD*; and Nicholas S. Jones, MD†

Background: Immunomodulators, including toll-like receptors (TLRs) and defensins, produced in response to pathogenic stimuli, can direct the developing immune system toward a T_H1 nonallergic phenotype. Increased human β -defensin (HBD) 4 expression is associated with infection.

Objective: To determine whether reduced mucosal levels of TLRs and defensins contribute to the inflammation seen in chronic allergic and nonallergic rhinitis.

Methods: Real-time polymerase chain reaction was used to determine gene expression levels of HBDs 1 through 4 and TLRs 2 and 4. Immunohistochemical analysis was used to study the localization and distribution of protein for α -defensins 1 through 3, HBD2, neutrophil elastase, and TLR2 in sections of nasal turbinate tissue from adults with persistent allergic and idiopathic rhinitis, healthy nasal mucosa, and tonsil tissue.

Results: Allergic mucosa showed a significant ($P = .02$) reduction in TLR2 messenger RNA expression compared with control mucosa and generally reduced expression for TLR4 and HBDs. Although not significant, the nonallergic group also showed reduced expression for TLRs and HBDs. With the exception of HBD4, increased target gene levels were seen in tonsil tissue. Protein expression of HBD2 and TLR2 was localized in lining and submucosal glandular epithelium but insignificant differences were seen for HBDs, TLRs, neutrophils, and α -defensin between the rhinitic and control patient groups.

Conclusions: Subjects with allergic and nonallergic rhinitis show reduced TLR and HBD gene expression. The significant reduction in TLR2 gene expression in allergic adults supports the concept that increased TLR2 protects against the development of allergy. The low levels of HBD4 detected in both rhinitis groups suggest lack of an underlying infection pathophysiological feature.

Ann Allergy Asthma Immunol. 2007;99:509–516.

INTRODUCTION

Allergic rhinitis is an IgE-mediated response indicated by affirmative responses to clinical tests for atopy.¹ On the contrary, persistent idiopathic rhinitis is defined as a noninfectious nonallergic disease² with an ill-defined disease pathomechanism. Recent evidence^{2,3} suggests that some nonallergic subjects have features of a highly localized T_H2, IgE-mediated, cellular immunopathological feature confined to the nasal mucosa. In common with those with allergic rhinitis, this subgroup of patients without allergic rhinitis is characterized by significantly increased numbers of activated mast cells, believed to be important contributors in inflammatory disease.²

Toll-like receptors (TLRs) are an evolutionarily conserved component of the human innate immune system present in inflammatory cells^{4,5} and nasal mucosa.⁶ The role of TLRs is to activate and recruit phagocytes, tissue dendritic cells, mast cells, and naïve CD4⁺ T lymphocytes to respond to microbial

pathogens through the secretion of chemokines and cytokines and to express the costimulatory molecules needed to activate adaptive immunity.^{7,8} Each of the known TLRs recognizes 1 or more pathogen-associated molecular patterns, generally by direct interaction with molecules on the pathogen surface. Recently, there has been interest in a possible role for these immunopeptides in the development of allergy. The hygiene hypothesis proposes that bacterial⁸ and parasitic⁹ infections induce T_H1-biased immunoresponses, protecting the host from developing allergic disease.^{1,10,11} The TLRs and human defensins might possibly facilitate this mechanism by serving as intermediaries between the innate and adaptive immune system. Evidence supporting this is shown in the lower incidence of allergy in farmers' families, who are exposed to increased levels of lipopolysaccharide (LPS) endotoxin¹² and significantly increased levels of blood CD14 and TLR2 compared with control subjects.^{13,14}

Human β -defensins (HBDs) are involved in inflammation and are found in mammalian granulocytes, particularly neutrophils, in the cytoplasm of respiratory epithelial cells, and in the airway surface liquid.¹⁵ The HBDs chemoattract immature dendritic cells and memory T lymphocytes and, similar to TLRs, link the innate and adaptive immune systems.^{15–19} More important, HBD2 can also directly recruit and activate mast cells through an induced G protein phospholipase C signaling pathway,^{15,20} suggesting a possible relevance to rhinitis. Furthermore, TLRs and HBDs are intrinsically asso-

* Division of Pathology, Queen's Medical Centre, Nottingham University Hospitals Trust, Nottingham, England.

† Division of Otorhinolaryngology, Queen's Medical Centre, Nottingham University Hospitals Trust, Nottingham, England.

Authors have nothing to disclose.

This study was supported by the Nasal Research Fund Charity, Nottingham.

Received for publication December 4, 2006.

Received in revised form May 21, 2007.

Accepted for publication May 30, 2007.

ciated with each other through their effect on mononuclear phagocytes. The LPS binding to surface TLR4 induces the activation of phagocytes, resulting in the production of interleukin (IL) 1 β and tumor necrosis factor α , which induce HBD2 and HBD3 expression through their action on nuclear factor κ B.²¹

α -Defensins 1 through 4 (also known as human neutrophil peptides [HNPs] 1–4) are stored within phagocytes but are also produced by B and T lymphocytes and have the capacity to chemoattract immature dendritic cells and CD8 T lymphocytes.^{22–24} In addition to recruiting neutrophils through the production of IL-8 in airway epithelium,^{23,26–29} HNPs can also stimulate mast cell degranulation.²⁵ The HNPs 1, 2, and 3 are detected in nasal mucosa, possibly in response to local inflammation.²²

The concept that defensins and TLRs have a role in mediating adaptive immunity has been widely accepted, but their role in rhinitis has not been fully investigated. Our study investigated the gene and protein expression levels of TLRs (TLR2 and TLR4) and defensins (HBD1–HBD4) in allergic and nonallergic rhinitis mucosa to test the hypothesis that these subjects have an underlying deficiency in these immune regulatory peptides consistent with the selection of a T_H2 rather than a T_H1 disease pathway, seen in those with allergic rhinitis.

METHODS

Tissue samples were obtained from patients attending the Queen's Medical Centre, Nottingham University Hospitals Trust, for nasal surgery between 1997 and 2004. Patients gave informed consent, and ethical approval was obtained from the Queen's Medical Centre Research and Development and Ethics Committees. Allergic rhinitis (n = 22) was confirmed in patients by positive skin prick and radioallergosorbent test results and a relevant medical history, whereas those with idiopathic rhinitis (n = 21) were chosen based on persistent rhinitic symptoms with negative systemic atopy (skin prick and radioallergosorbent test results) and otherwise unexplained cause. Healthy control patients (n = 20) required surgery after trauma and had no rhinitis or history of allergy. The perennial allergic and idiopathic patients underwent turbinectomy because of markedly swollen turbinates, resulting in breathing difficulties and rhinitis symptoms. Rhinitis med-

ication was withdrawn from patients for a minimum of 4 weeks for corticosteroids and 5 days for antihistamines. None of the patients had chronic sinusitis, polyposis, or a history of asthma. Patients in all groups had a minimum of 2 of the following symptoms, nasal discharge, sneezing, and nasal obstruction, for an average of at least 1 hour per day for at least 5 days. None of the patients had purulent discharge, asthma, or eczema; none received medication; and none were pregnant. In all patients, the computed tomographic scan results were normal and the blood chemistry results were normal. (Blood chemistry included levels of sodium, potassium, calcium, total protein, albumin, urea, creatinine, bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, γ -glutamyl transpeptidase, and hemoglobin.) Also, all patients had normal levels of angiotensin-converting enzyme and antineutrophil cytoplasmic antibody. Additional patient details are given in Table 1. Inferior nasal turbinate tissue was removed, and separate samples were formalin fixed and embedded in paraffin or frozen in liquid nitrogen and stored at -80°C . Palatine tonsil tissue was obtained from patients undergoing surgery for recurrent tonsillitis because this tissue was previously shown to express TLR and HBD.⁶

Immunohistochemical Analysis for TLR2, HBD2, and α -Defensins

Labeled streptavidin biotin immunohistochemical analysis with microwave antigen retrieval was performed for all listed antigens (Table 2) using a commercially available instrument (Techmate 500; DakoCytomation, Cambridge, England). A universal secondary antibody raised against goat (Vector Labs, Peterborough, England) was used in the detection of the TLR2 goat polyclonal antibody. To validate positive staining, 2 negative controls were included in every experiment, comprising omission of the primary antibody and substitution of the primary antibody for 1 of an equivalent immunoglobulin class and concentration.

Morphometry

Slides stained for HBD2 and TLR2 were graded for staining intensity (perennial allergic rhinitis, 20; idiopathic rhinitis, 18; and healthy patients, 19); otherwise, individual cells were counted for slides stained for α -defensin and neutrophil elas-

Table 1. Inclusion and Exclusion Criteria Used in the Selection of Patients

Variable	Patient group		
	Allergic (n = 22)	Idiopathic NAR (n = 21)	Healthy control (n = 20)
Age range, y	17–63	16–59	21–64
Skin prick test result ^a	Positive	Negative	Negative
RAST result ^b	Positive	Negative	Negative
Duration of symptoms, y	>1	>1	0

Abbreviations: NAR, nonallergic rhinitis; RAST, radioallergosorbent test.

^a Skin prick test allergens used included grass and tree pollens, house dust mite, pet danders, feathers, and *Cladosporium*.

^b The test used was Phadiatop (Phadia AB, Uppsala, Sweden).

Table 2. Primary Antibody Details Used in the Immunohistochemistry Technique

Antibody	Dilution	Details/supplier
TLR2	1:1,200	Goat polyclonal to TLR2 (ab1655)/Abcam, Cambridge, England
HBD2	1:500	Rabbit anti-HBD2 (14338-v)/Peptide Institute, Louisville, Kentucky
HNP	1:1,500	Mouse monoclonal (DEF3) to α -defensin 1 plus α -defensin 2 plus α -defensin 3 (ab24247)/Abcam
NE	1:200	Mouse monoclonal antihuman neutrophil elastase (M0752)/DakoCytomation, Cambridge

Abbreviations: HBD, human β -defensin; HNP, human neutrophil peptide; NE, neutrophil elastase; TLR, toll-like receptor.

tase (perennial allergic rhinitis, 13; idiopathic rhinitis, 14; and healthy patients, 15). Slides were scored in a blind manner without knowledge of their disease categorization. Staining intensity was graded using a scale of 0 to 3, where 3 was strong staining and 0 was negative staining. Cell counts were made using a 10 $\times$ 10-mm graticule in a light microscope with a $\times$ 25 objective lens. Counts of positive cells in epithelium and superficial and deep submucosa of each graticule field were made and converted to cell counts per millimeter squared.

Quantitative Real-time Polymerase Chain Reaction

Cryostat sections (10 μ m) of nasal turbinate tissue were homogenized in RLT buffer and 1% β -mercaptoethanol (Qiagen, Crawley, England) using 1-mm glass beads and a minibead beater (Biospec Products Inc, Bartlesville, Oklahoma), followed by centrifugation in tubes (Qiashredder; Qiagen). RNA was extracted using a commercially available kit (RNeasy Micro kit; Qiagen), incorporating a DNase digest according to the supplier's instructions. The extracted total RNA was quantified using a spectrophotometer (NanoDrop, Wilmington) and used to generate complementary DNA. Reactions contained 1 μ L of 10-mmol/L deoxyribonucleotide triphosphate (ABGene, Epsom, England), 0.5 μ L (500 μ g/mL) of random hexamer primers (Promega, Southampton, England), 500 ng of RNA, and molecular grade water to 13 μ L. After heating to 65°C for 5 minutes, 1 μ L (200 U/ μ L) of reverse transcriptase (Superscript III), 1 μ L of RNase inhibitor (Ambion, Huntingdon, England), 1 μ L of 0.1M dithio-

threitol, and 4 μ L of 5 $\times$ first strand buffer measures were added to each tube. The mixtures were incubated at 50°C for 1 hour and then heated to 70°C for 15 minutes.

Complementary DNA was used in the quantitative real-time polymerase chain reactions (PCRs) comprising 0.5 μ L (10-mmol/L) of forward/reverse primers and 11 μ L of 2 $\times$ a commercially available kit (Fastart DNA Master SYBR Green; Roche Diagnostics, Mannheim, Germany). The primer sequences used in the quantitative real-time PCRs are described in Table 3. Quantitative real-time PCR was performed using a commercially available instrument (Stratagene MX4000; Stratagene, La Jolla, California) with the following cycle conditions: 1 cycle at 95°C for 15 minutes; 40 cycles of 94°C for 30 seconds, 54°C (53°C for HBD3 and 59°C for glyceraldehyde-3-phosphate dehydrogenase [GAPDH]) for 60 seconds, and 72°C for 30 seconds; and 1 cycle of 72°C for 10 minutes. DNA standards were incorporated for each target gene to allow quantitation. The 18S ribosomal RNA and GAPDH housekeeping genes were included for cell normalization. Initially, samples (perennial allergic rhinitis, 13; idiopathic rhinitis, 10; and healthy patients, 11) of messenger RNA (mRNA) were analyzed for each of the primers used, but a few samples were unsuitable for PCR, reflected by the numbers of results given.

Statistical Analysis

Cell counts and staining intensity obtained from immunostained sections were compared between the diseased and healthy patient groups using the Mann-Whitney test (GraphPad Prism, San Diego, California). The Kolmogorov-Smir-

Table 3. Oligonucleotide Primer Sequences Used in Quantitative Real-time PCR

Primer	Sequence		Annealing temperature, °C	Product size, bp
	Forward	Reverse		
HBD				
1	CGCCATGAGAACTTCCTACC	CCACTGCTGACGCAATTGTA	54	126
2	GACTCAGCTCCTGGTGAAGC	GGATCGCCTATACCAACAAA	54	118
3	TCCTGTTTTTGGTGCCTGTT	CGATCTGTTCTCCTTTTGA	53	123
4	ATGCAGAGACTTGTGCTGCT	CTGTATTCTTGGCTGCGACA	54	140
TLR				
2	TGTAGGTTGAAGCACTGGACA	TACCATTGCGGTCAACAAGAC	54	124
4	CTCCTGCGTGAGACCAGAA	GGTTGAGAAGGGGAGGTTGT	54	115
18S	^a	^a	54	112
GAPDH	GGTGAAGGTCGGAGTCAACGGA	GAGGGATCTCGTCTCTGGAA GA	59	240

Abbreviations: bp, base pair; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HBD, human β -defensin; PCR, polymerase chain reaction; TLR, toll-like receptor.

^a Available from the authors on request.

nov test was performed on normalized gene expression levels in the allergic, idiopathic, and healthy control groups to determine normality and then 1-way analysis of variance was used to evaluate differences in gene expression levels between the different groups. $P \leq .05$ was regarded as statistically significant.

RESULTS

Immunohistochemical Analysis for HBD2 and TLR2

Strong immunostaining for HBD2 and TLR2 was localized to surface and glandular epithelium in nasal mucosa in all patient groups, including healthy mucosa (Fig 1A). Variation in staining intensity was seen in some cases for TLR2 and HBD2 staining, especially at the air-surface epithelium, where staining was most intense on the apical aspect. A statistical intergroup comparison revealed insignificant TLR2 ($P = .34$) and HBD2 ($P = .77$) differences in the intensity of staining between the patient groups. The submucosa appeared predominantly negative, although individual cells (possibly macrophages) stained for TLR2, with insignificant differences seen between patient groups (analysis of variance, $P = .77$).

Human Neutrophil Peptides 1 to 3

α -Defensin immunoreactivity was seen in individual neutrophils and macrophages within the airway epithelium and submucosal layer (Fig 1B). Large clusters of cells were often localized in the lumen of superficial blood vessels but did not appear in the submucosa. Differences in cell counts between patient groups were statistically insignificant ($P = .98$).

Quantified PCR Gene Expression

Low-level expression of all genes was detected in all the tissues investigated (Fig 2A-F), with the lowest expression for HBD2 and TLR4. Median gene expression was generally

lowest in the allergic rhinitic mucosa and intermediate in the nonallergic group compared with healthy mucosa. The level of TLR2 was significantly reduced in allergic compared with control mucosa ($P = .02$), with the nonallergic group also showing a reduced expression level ($P = .03$). Regarding the other genes, P values for differences between patient group mucosal expression, using 1-way analysis of variance, are as follows: TLR4, $P = .94$; HBD1, $P = .31$; HBD2, $P = .66$; HBD3, $P = .07$; and HBD4, $P = .12$. Excluding HBD4, expression levels of mucosal HBD and TLR were reduced compared with that seen in tonsil tissue. The TLR and HBD gene expression data were consistently similar irrespective of whether normalization was performed using GAPDH or 18S housekeeping genes.

DISCUSSION

In this study, we tested the hypothesis that adult patients with persistent allergic and idiopathic nonallergic rhinitis have a deficiency in the T_H1 -promoting immunomodulator TLRs and defensins (HBDs and HNPs) located in their nasal mucosa, contributing to the development of a T_H2 -type allergic disease pathway. We found that gene expression levels for TLR and HBD were generally lower in allergic rhinitic mucosa, with a significant reduction seen for TLR2 compared with healthy mucosa. Our immunohistochemical study suggests that TLRs and HBD proteins are produced in nasal mucosal tissue at low constitutive levels, and they function in protecting against microbial assault as part of the innate immune response. Consistent with the concept that increased TLR2 expression is protective against the development of a T_H2 pathway,^{13,14} our study suggests that adults with allergic rhinitis show a deficiency in TLR and HBD immunopeptides, possibly associated with a propensity to develop a T_H2 disease pathway in response to allergen provocation. Gene ex-

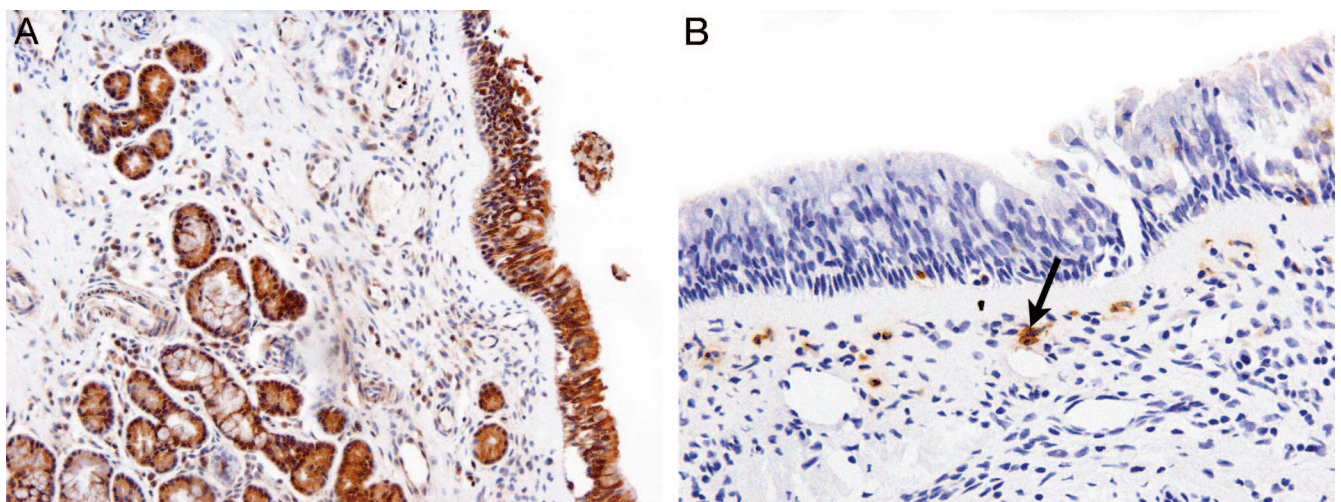


Figure 1. A section of idiopathic rhinitic nasal mucosa immunostained with toll-like receptor 2 (brown staining is localized to airway and glandular epithelium, with isolated cells localized in the submucosal stroma) (A), and α -defensin (arrow) staining localized to individual cells in this allergic rhinitic nasal mucosa (hematoxylin counterstain used) (B).

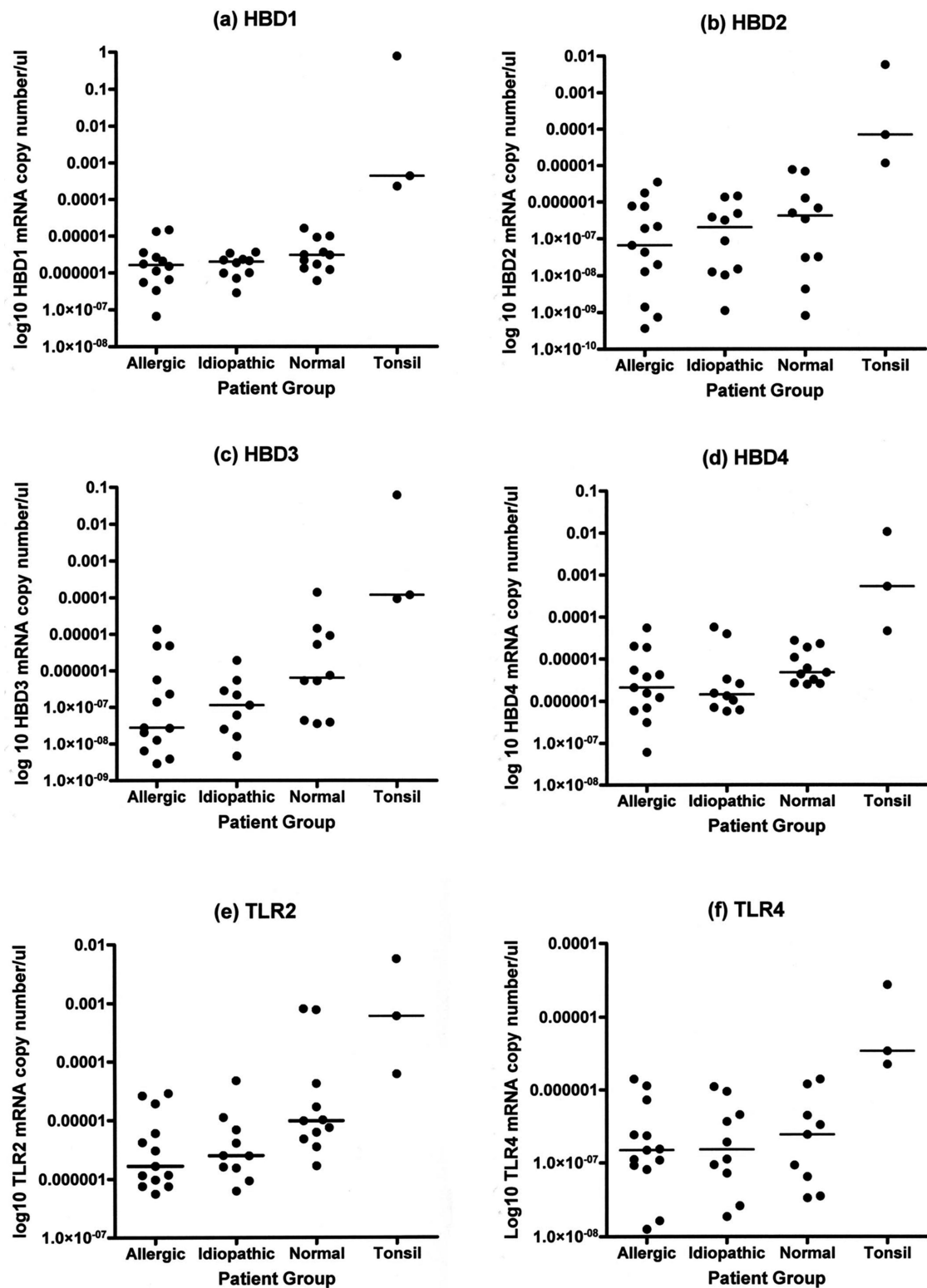


Figure 2. Median normalized gene expression (messenger RNA [mRNA] copies) in nasal mucosa from the 3 patient groups: human β -defensin (HBD) 1 (A), HBD2 (B), HBD3 (C), HBD4 (D), toll-like receptor (TLR) 2 (E), and TLR4 (F). Healthy tonsil tissue is included as a reference.

pression levels of TLR and HBD were generally reduced in the idiopathic patient group compared with healthy mucosa but did not reach the level of significance seen for the allergic group. This observation might be explained by the presence of a proportion of nonallergic subjects possessing local mucosal allergy, entropy, with a similar disease pathway to that occurring in the allergic group.³

We found no significant differences in the distribution or localization of TLR2 or HBD2 protein localized to airway epithelial cells between the different patient groups. On the contrary, we identified significantly reduced gene expression for TLR2 in allergic mucosa. The disparity seen between gene and protein expression might be because of gene regulatory mechanisms responsible for protein production^{30,31}; the quantity of protein produced cannot easily be predicted from the level of gene expression. In our study, the reduced levels of TLR2 gene expression could represent a legacy of early immunological development and/or might result from the presence of the defining signature cytokine IL-4, a feature of allergic mucosa, reported to cause down-regulation of TLR2 and TLR4.^{32,33} Our findings contrast with those found in the study by Fransson et al,³⁴ who found no significant difference in gene expression for TLR2 and TLR4 between subjects with seasonal allergic rhinitis and control subjects. Fransson et al obtained biopsy specimens for mRNA gene analysis from 2 separate patient groups, one sampled before and the other at 3 to 10 days into the pollen season (M. Fransson, MD, written communication, July 2006). The same researchers reported increased TLR3 gene expression and generally increased protein levels in allergic mucosa after allergen challenge; TLR3 gene expression was not measured in our study. Up-regulation of TLR2 and TLR4 is seen in response to infection, as witnessed in those with chronic rhinosinusitis,³⁵ and in the tonsil control tissue used in our study; in contrast, Claeys et al⁶ found no significant difference in TLRs and defensins between mucosa obtained from patients with chronic sinusitis and nasal polyps compared with healthy controls. Reduced TLR expression and susceptibility of developing chronic rhinosinusitis might be expected, but this was not observed in our study. Our finding of reduced TLR expression in the absence of mucosal infection in the allergic group suggests that additional immunological mechanisms are involved in preventing infection in nasal mucosa, principally secretory IgA.³⁶ A possible explanation for these results might be the use of adult patients in most studies, including our own. The nasal mucosal immune response to allergen may change with age, reflecting a possible decrease in immunoreactivity to allergen with increasing age.³⁷ In addition to the significant reduction in TLR2 seen in allergic mucosa, we noted a trend toward reduced TLR and HBD expression levels in both rhinitic groups, and it is uncertain if similar results would be obtained in the developing nasal mucosa of young children. A long-term study following the mucosal response of infants and adults would address this issue. Anecdotal evidence suggests that exposure to an LPS-rich environment is associated with low levels of allergy.¹²⁻¹⁴

Recently, a nasal explant model was used to investigate the mechanism of LPS-mediated immune modulation. Tulic et al⁵ detected LPS-binding TLR4 localized on CD3 T lymphocytes that, in the presence of LPS, induce IL-10 production, resulting in a T_H1 response in allergic individuals. Crucially, the adaptability of this pathway seems to be lost in adulthood, lessening the potential success for therapy targeted at TLR4.

Several studies report up-regulation of HBD2 mRNA expression in airway epithelial cells after exposure to infection or other inflammatory stimuli³⁸⁻⁴² mediated by IL-1 β .⁴⁰ We detected low levels of HBD2 gene expression in allergic and nonallergic rhinitic and healthy nasal mucosa, consistent with the findings of Kalfa et al⁴³ for those with allergic rhinitis and recurrent sinusitis and healthy controls. But unlike Claeys et al,⁶ we were able to detect immunostaining of the protein for HBD2 in nasal turbinate mucosa and tonsil that would corroborate previous findings of HBD2 staining localized in upper airway epithelial cell cytoplasm.¹⁶ Previous reports^{6,22} suggest that differential levels of HBD2 occur in different regions of airway tissue. Our findings corroborate this because we saw increased HBD2 and HBD3 gene expression in lymphoid tonsil control tissue, in accord with the findings of others.^{6,22} Generally, we detected low levels of expression for HBD3 in rhinitic mucosa, similar to the findings of Claeys et al for chronic sinusitis and nasal polyposis tissues. Our detection of low HBD4 gene expression in the allergic and nonallergic rhinitic groups supports the clinical judgment used in the selection criteria of these patients (ie, patients were free of an underlying infection underpinning their rhinitis).

There are several possible reasons for the observed regional differences of antimicrobial peptide gene expression between tonsil and nasal mucosa. It is tempting to speculate that the intensive mechanical mucociliary clearance occurring in nasal mucosa rapidly transports HBDs and TLRs, limiting contact time with immunostimulatory compounds. In contrast, the deep folds and crypts of the tonsil are not subject to this clearance mechanism, giving prolonged exposure time between pathogens and receptors. A further explanation might be because of an innate deficiency in the induction of HBD production in allergic nasal mucosa. Not only is TLR needed for HBD production, but second response signals, such as IL-1 β , have been shown to induce HBD2 expression in airway epithelia.⁴⁰ There is evidence of increased IL-1 production^{44,45} and frequent IL-1 polymorphisms in allergic mucosa,⁴⁶ but other mediators might also be required. Infection induces HBD4,⁴⁷ and the low levels seen in our study suggest that infection is not responsible for the persistent inflammation seen in our allergic and nonallergic rhinitic mucosa.

In summary, our findings suggest that, compared with healthy nasal mucosa, allergic and some nonallergic rhinitic patients have a tendency for reduced production of TLRs and HBDs; levels of the latter might result from the former. It is still unclear whether the deficiency of TLR and HBD gene expression seen in rhinitic tissue is causal or a consequence of

the T_H2 pathway seen in allergic mucosa. In vitro mucosal explant models donated from subjects with allergic and non-allergic rhinitis would provide a means for further investigation of the role of TLR and HBD in skewing the development of the allergic immune response.

ACKNOWLEDGMENTS

We thank Janet Williams, Howard Coleman, and Neil Hand, MSc, for their technical assistance and Anne Kane for her assistance with photography.

REFERENCES

- Durham SR. Mechanisms and treatments of allergic rhinitis. In: *Scott Brown's Otolaryngology*. 6th ed. London, England: Butterworth-Heinemann; 1997:1–16.
- Powe DG, Huskisson RS, Carney AS, Jenkins D, Jones NS. Evidence for an inflammatory pathophysiology in idiopathic rhinitis. *Clin Exp Allergy*. 2001;31:864–872.
- Powe DG, Jagger C, Kleinjan A, Carney AS, Jenkins D, Jones NS. "Entopy": localized mucosal allergic disease in the absence of systemic responses for atopy. *Clin Exp Allergy*. 2003;33:1374–1379.
- Kulka M, Alexopoulou L, Flavell RA, Metcalfe DD. Activation of mast cells by double-stranded RNA: evidence for activation through toll-like receptor 3. *J Allergy Clin Immunol*. 2004;114:174–182.
- Tulic MK, Fiset PO, Manoukian JJ, et al. Role of toll-like receptor 4 in protection by bacterial lipopolysaccharide in the nasal mucosa of atopic children but not adults. *Lancet*. 2004;363:1689–1697.
- Claeys S, de Belder T, Holtappels G, et al. Human β -defensins and toll-like receptors in the upper airway. *Allergy*. 2003;58:748–753.
- Bellou A, Schaub B, Ting L, Finn PW. Toll receptors modulate allergic responses: interaction with dendritic cells, T cells and mast cells. *Curr Opin Allergy Clin Immunol*. 2003;3:487–494.
- Sabroe I, Parker LC, Wilson AG, Whyte MK, Dower SK. Toll-like receptors: their role in allergy and non-allergic inflammatory disease. *Clin Exp Allergy*. 2002;32:984–989.
- Pritchard DI. The pro-allergic influences of helminth parasites. *Mem Inst Oswaldo Cruz*. 1997;92(suppl 2):15–18.
- Gangloff SC, Guenounou M. Toll-like receptors and immune response in allergic disease. *Clin Rev Allergy Immunol*. 2004;26:115–125.
- Romagnani S. The increased prevalence of allergy and the hygiene hypothesis: missing immune deviation, reduced immune suppression, or both? *Immunology*. 2004;112:352–363.
- Lazarczyk M, Grzela K, Grzela T. Immunostimulatory oligonucleotides in therapy of allergic diseases. *Expert Opin Biol Ther*. 2005;5:525–536.
- Eder W, Klimecki W, Yu L, et al. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol*. 2004;113:482–488.
- Lauener RP, Birchler T, Adamski J, et al. Expression of CD14 and toll-like receptor 2 in farmers' and non-farmers' children. *Lancet*. 2002;360:465–466.
- Schutte BC, McCray PB Jr. [β]-Defensins in lung host defense. *Annu Rev Physiol*. 2002;64:709–748.
- Carothers DG, Graham SM, Jia HP, Ackermann MR, Tack BF, McCray PB Jr. Production of β -defensin antimicrobial peptides by maxillary sinus mucosa. *Am J Rhinol*. 2001;15:175–179.
- Mahida YR, Cunliffe RN. Defensins and mucosal protection. *Novartis Found Symp*. 2004;263:71–84, 211–218.
- Yang D, Chertov O, Bykovskaia N, et al. β -Defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. *Science*. 1999;286:525–528.
- Yang D, Chertov O, Rosenberg HF, et al. Mobilization of various types of leukocytes by human granulocyte-derived antimicrobial proteins: identification of novel activities and/or receptors for defensins, cathelicidin, and eosinophil-derived neurotoxin (EDN). *J Leukoc Biol Suppl*. 2000;20(No. 63).
- Niyonsaba F, Iwabuchi K, Matsuda H, Ogawa H, Nagaoka I. Epithelial cell-derived human β -defensin-2 acts as a chemotaxin for mast cells through a pertussis toxin-sensitive and phospholipase C-dependent pathway. *Int Immunol*. 2002;14:421–426.
- Tsutsumi-Ishii Y, Nagaoka I. Modulation of human β -defensin-2 transcription in pulmonary epithelial cells by lipopolysaccharide-stimulated mononuclear phagocytes via proinflammatory cytokine production. *J Immunol*. 2003;170:4226–4236.
- Lee SH, Kim JE, Lim HH, Lee HM, Choi JO. Antimicrobial defensin peptides of the human nasal mucosa. *Ann Otol Rhinol Laryngol*. 2002;111:135–141.
- Chertov O, Michiel DF, Xu L, et al. Identification of defensin-1, defensin-2, and CAP37/azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils. *J Biol Chem*. 1996;271:2935–2940.
- Yang D, Chen Q, Chertov O, Oppenheim JJ. Human neutrophil defensins selectively chemoattract naive T and immature dendritic cells. *J Leukoc Biol*. 2000;68:9–14.
- Befus AD, Mowat C, Gilchrist M, Hu J, Solomon S, Bateman A. Neutrophil defensins induce histamine secretion from mast cells: mechanisms of action. *J Immunol*. 1999;163:947–953.
- Baggiolini M. Neutrophil activation and the role of interleukin-8 and related cytokines. *Int Arch Allergy Immunol*. 1992;99:196–199.
- Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines: Cxc and Cc chemokines. *Adv Immunol*. 1994;55:97–179.
- Van Wetering S, Mannesse-Lazeroms SP, Van Sterkenburg MA, Daha MR, Dijkman JH, Hiemstra PS. Effect of defensins on interleukin-8 synthesis in airway epithelial cells. *Am J Physiol*. 1997;272:L888–L896.
- Yang D, Chertov O, Oppenheim JJ. The role of mammalian antimicrobial peptides and proteins in awakening of innate host defenses and adaptive immunity. *Cell Mol Life Sci*. 2001;58:978–989.
- Glanemann C, Loos A, Gorret N, et al. Disparity between changes in mRNA abundance and enzyme activity in *Corynebacterium glutamicum*: implications for DNA microarray analysis. *Appl Microbiol Biotechnol*. 2003;61:61–68.
- Tanaka E, Shimokawa H, Kamiuneten H, et al. Disparity of MCP-1 mRNA and protein expressions between the carotid artery and the aorta in WHHL rabbits: one aspect involved in the regional difference in atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2003;23:244–250.
- Beutler E, Gelbart T, West C. Synergy between TLR2 and TLR4: a safety mechanism. *Blood Cells Mol Dis*. 2001;27:728–730.
- Broide DH. Molecular and cellular mechanisms of allergic disease. *J Allergy Clin Immunol*. 2001;108(suppl 2):S65–S71.
- Fransson M, Adner M, Erjefalt J, Jansson L, Uddman R, Cardell

-
- LO. Up-regulation of toll-like receptors 2, 3 and 4 in allergic rhinitis. *Respir Res.* 2005;6:100.
35. Dong Z, Yang ZQ, Wang CS. Expression of TLR2 and TLR4 messenger RNA in the epithelial cells of the nasal airway. *Am J Rhinol.* 2005;19:236–239.
36. Brandtzaeg P. Immunology of tonsils and adenoids: everything the ENT surgeon needs to know. *Int J Pediatr Otorhinolaryngol.* 2003;67(suppl 1):S69–S76.
37. Simola M, Böss I, Holopainen E, Malmberg H. Long-term clinical course of hypersensitive rhinitis. *Rhinology.* 1991;29:301–306.
38. Proud D, Sanders SP, Wiehler S. Human rhinovirus infection induces airway epithelial cell production of human β -defensin 2 both in vitro and in vivo. *J Immunol.* 2004;172:4637–4645.
39. Schibli DJ, Hunter HN, Aseyev V, et al. The solution structures of the human β -defensins lead to a better understanding of the potent bactericidal activity of HBD3 against *Staphylococcus aureus*. *J Biol Chem.* 2002;277:8279–8289.
40. Singh PK, Jia HP, Wiles K, et al. Production of β -defensins by human airway epithelia. *Proc Natl Acad Sci U S A.* 1998;95:14961–14966.
41. Harder J, Meyer-Hoffert U, Teran LM, et al. Mucoid *Pseudomonas aeruginosa*, TNF- α , and IL-1 β , but not IL-6, induce human β -defensin-2 in respiratory epithelia. *Am J Respir Cell Mol Biol.* 2000;22:714–721.
42. Hiratsuka T, Nakazato M, Date Y, et al. Identification of human β -defensin-2 in respiratory tract and plasma and its increase in bacterial pneumonia. *Biochem Biophys Res Commun.* 1998;249:943–947.
43. Kalfa VC, Spector SL, Ganz T, Cole AM. Lysozyme levels in the nasal secretions of patients with perennial allergic rhinitis and recurrent sinusitis. *Ann Allergy Asthma Immunol.* 2004;93:288–292.
44. Verhaeghe B, Gevaert P, Holtappels G, et al. Up-regulation of IL-18 in allergic rhinitis. *Allergy.* 2002;57:825–830.
45. Benson M, Wennergren G, Fransson M, Cardell LO. Altered levels of the soluble IL-1, IL-4 and TNF receptors, as well as the IL-1 receptor antagonist, in intermittent allergic rhinitis. *Int Arch Allergy Immunol.* 2004;134:227–232.
46. Joki-Erkkila VP, Karjalainen J, Hulkkonen J, et al. Allergic rhinitis and polymorphisms of the interleukin 1 gene complex. *Ann Allergy Asthma Immunol.* 2003;91:275–279.
47. Garcia JR, Krause A, Schulz S, et al. Human β -defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity. *FASEB J.* 2001;15:1819–1821.
- Requests for reprints should be addressed to:
Desmond G. Powe, PhD
Queen's Medical Centre
Nottingham University Hospitals Trust
Nottingham NG7 2UH, England
E-mail: des.powe@nottingham.ac.uk
-