

ORIGINAL PAPER

Gene and protein expression of protease-activated receptor 2 in structural and inflammatory cells in the nasal mucosa in seasonal allergic rhinitis

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Clinical and Experimental Allergy

Summary

Background Protease-activated receptor 2 (PAR 2) has been shown to be responsible for trypsin and mast cell tryptase-induced airway inflammation. Here, the present study aimed to explore the expression of PAR 2 in the nasal mucosa of seasonal allergic rhinitis (SAR).

Methods Study subjects were recruited for the study by medical history, physical examination and laboratory screening tests. Using immunohistochemistry, laser-assisted cell picking and subsequently real-time PCR, nasal mucosa biopsies of SAR patients were investigated for PAR 2 gene and protein expression in complex tissues of the nasal mucosa.

Results Gene and protein expression of PAR 2 was firstly detected in nasal mucosa of SAR patients. The relative gene expression level of PAR 2 was significantly increased in complex tissues of the nasal mucosa of SAR (6.21 ± 4.02 vs. controls: 1.38 ± 0.86 , $P = 0.004$).

Moreover, PAR 2 mRNA expression in epithelial cells (SAR: 4.78 ± 4.64 vs. controls: 0.84 ± 0.61 , $P = 0.003$) but not in mucus (SAR: 1.51 ± 1.15 vs. controls: 1.35 ± 1.02 , $P = 0.78$) and endothelial cells (SAR: 1.20 ± 0.57 vs. controls: 1.73 ± 1.30 , $P = 0.5$) was found to be significantly changed in the nasal mucosa in SAR. Using double immunohistochemistry the present study demonstrated that the total numbers of mast cells ($P = 0.0003$) and eosinophils ($P = 0.03$) and the numbers of eosinophils expressing PAR 2 ($P = 0.006$) were significantly elevated in the nasal mucosa of SAR compared with the controls.

Conclusion The abundant presence and distribution of gene and protein expression of PAR 2 in different cell types in the nasal mucosa under normal situation, the increased expression of PAR 2 in epithelial cells and the increased number of eosinophils with PAR 2 suggest that PAR 2 may contribute to the pathogenesis of allergic diseases such as SAR.

Keywords human nasal mucosa, non-allergic rhinitis (NR), perennial allergic rhinitis, protease-activated receptor 2 (PAR 2), seasonal allergic rhinitis (SAR), Taqman

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Introduction

The family of protease-activated receptors (PARs) consists of PAR 1, 2, 3 and 4 which are G-protein-coupled membrane receptors and can be activated by the proteolytic enzymes like thrombin, trypsin and mast cells tryptase [1]. Of these receptors, PAR 2 has been found to

be associated with inflammation in many organ system of animal models such as skin [2, 3], gastro-intestine [4], urinary system [5] and respiratory tracts of human and animal [6]. Activation of PAR 2 has been reported to release pro-inflammatory peptides such tachykinins substance P/neurokinin A and calcitonin gene-related peptides (CGRP) from sensory nerves to mediate neurogenic inflammation [7]. However, the molecular mechanism of PAR 2-mediated inflammation is still poorly understood.

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In recent years, many studies have been designed to examine the presence, localization and distribution of PAR 2 expression in various organs of many species. With respect to the airways, PAR 2 expression was detected in epithelial, airway smooth muscle, endothelial cell and fibroblast of human airways under normal and pathophysiological conditions such as bronchitis [6]. There is conflicting evidence whether PAR 2 activation has a protective or inflammatory role in lower airways [8, 9]. Concerning the upper airways, expression of PAR 2 in the nasal mucosa has been not investigated so far. In this respect, the present study aimed to investigate the presence and localization of gene and protein expression of PAR 2 in the nasal mucosa under normal and pathophysiological situation such as seasonal allergic rhinitis (SAR) by immunohistochemical analysis and laser-assisted microdissection combined with quantitative RT (*real-time*) PCR. The present study demonstrated for the first time that PAR 2 is expressed by structural as well as by inflammatory cells such mast cells and eosinophils that are known to play a central role as important cells in human allergic diseases [10].

Materials and methods

Patients

Nasal biopsies were obtained from 25 patients (15 patients with SAR, 10 non-allergic rhinitis (NR) subjects undergoing surgical therapy for chronic nasal obstruction caused by septum deviation and hyperplasia of the inferior turbinates during the peak of the grass pollen season. The diagnosis for SAR was made on the basis of clinical criteria of nasal (nasal itching, nasal congestion, and rhinorrhea) or ocular (red eyes, watery) symptoms. Additionally, further investigation such as rhinological and allergological history taking of SAR for more than two years, nasal examination and positive prick test were performed. Also, a serum IgE counts by to RAST completed the diagnostic procedures. The positive results of the skin testing and RAST to four common aeroallergens (grass pollen, birch pollen, mites and cat) were compared with questionnaires that have to be completed by each patient who was then classified as having SAR or NR for consistency of the classification.

During their stay in hospital, daily symptom severity of nasal itching, congestion and rhinorrhea and ocular symptoms such as red eyes and watery were recorded. Daily symptom scores for each patient were calculated. Patients with nasal complain caused by septum deviation and negative for skin test were classified as NR. Patients with a history of other airway diseases such as bronchial asthma, chronic obstructive pulmonary diseases (COPD), acute and chronic upper *infectious* airway diseases and positive test to other allergens like *mites and cat* or with

drug treatment with corticosteroids and antihistamines within a previous month were excluded from this study. Informed consent of each individual was obtained. Biopsies of the lower nasal conchae were taken during surgery and immediately washed twice in phosphate-buffered saline (PBS). Then the nasal mucosa biopsies were snap-frozen in liquid nitrogen and stored in cryotubes (Nunc, Wiesbaden, Germany) at -80°C until use.

RNA extraction

Total RNA was extracted from nasal samples by using TRIzol reagent according to the protocol provided by the manufacturer (Molecular Research Center, Cincinnati, USA). Two micrograms of total RNA each probe was reverse transcribed to single-strand cDNA using oligo-(dT) primer and Superscript II (Life Technologies Inc., Grand Island, NY, USA) in a 20 μL reaction volume. The RT reaction was performed at 42°C for 70 min, followed by heating at 70°C for 15 min. For the extraction of RNA from isolated cells, up to 10 isolated cells were scraped off the coverslip individually and were transferred and lysed into 10 μL of a modified first-strand buffer containing 4% RNase inhibitor in 52 mM Tris-HCl (pH 8.3), 78 mM KCl and 3.1 mM MgCl_2 .

Reverse transcription-polymerase chain reaction

PCR was undertaken using primers specific for PAR-2: forward 5'-GGC TGC TGA TTC TGG TC-3' and reverse 5'-GAC ACT TCG GCA AAG GAG AG-3', (PCR product 497 bp) and for GAPDH: forward 5'-CAT CAC CAT CTT CCA GGA GCG A-3' and reverse 5'-GTC TTC TGG GTG GCA GTG ATG G-3', (PCR product 342 bp, picture not shown) (Tib Molbiol, Berlin, Germany). For PCR 2 μL cDNA, 5 μL 10 $\times$ buffer (Perkin-Elmer, Überlingen, Germany), 7.5 μL dNTP-mix (2 mM) (MPI Fermentas, St Leon-Rot, Germany), 2 μL of each primer and 0.5 μL Taq DNA polymerase (5 U/ μL) (Perkin-Elmer) were supplemented with aq. dest. to a final volume of 50 μL . DNA was amplified using the following conditions: 3 min 94°C ; 35 cycles 30 s 94°C , 45 s 55°C and 1 min 72°C ; 1 min 72°C . Amplification products were visualized via gel electrophoresis.

Laser-assisted cell picking

For the single-cell real-time PCR cryosections (6 μm) of the nasal mucosa biopsies were collected on coverslips, which had been placed in acetone overnight, transferred into 100% ethanol and H_2O_2 for 2 h and 30 min, respectively, briefly rinsed in sterile water and stored in 100% ethanol before use. The sections were stained for Mayer's haematoxylin (30 s), briefly rinsed in sterile water and were then stained with eosin (10 s). After the haematoxylin and eosin staining the sections again stored in 100%

ethanol until use. Up to 10 epithelial, endothelial, mucus or inflammatory cells were isolated by laser microdissection (P.A.L.M. robot-micro beam; PALM, Bernried, Germany) [11, 12].

Real-time reverse transcription-polymerase chain reaction

The quantification of PAR 2 transcripts was carried out using the Taqman PCR Reagent Kit and the ABI Prism 7700 Sequence Detection system (Applied Biosystems, Überlingen, Germany) according to the manufacturer's protocol. Following primer pairs PAR 2: forward 5'-ATTCCTGCCCTGAACATCAC-3', reverse 5'-ATAGGCA-GAGGCTGTGAGGA-3', internal probe 5'-CTTC CTCTC-TCTGGCCATTG-3' (PCR product 135 bp) and for GAPDH, forward 5'-GAA GGT GAA GGT CGG AGT C-3', reverse 5'-GAA GAT GGT GAT GGG ATT TC-3' and internal probe 5'-CAA GCT TCC CGT TCT CAG CC-3' (PCR product 225 bp) were used in the present study. The cycling conditions were: 50 °C for 2 min and denaturing at 95 °C for 10 min, 50 cycles at 95 °C for 15 s, 60 °C for 1 min. For quantification of PAR 2 gene expression in relation to an internal control, amplification standard curves of PAR 2 gene and of the reference gene human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were carried out, as previously described [13]. Briefly, the purified PCR content were measured by optical density spectrophotometer and calculated to the corresponding copy number. Serial dilutions (1 : 10) of the purified PCR amplicon (ranging from 10^6 down to 10^1 input cDNA copies) were used as standard curve in each PCR run. To minimize inter-assay variability, all samples analysed from a single experiment were performed with the same dilution series of a standard. Linear regression analysis of all the standard curves showed a high correlation with an average $r^2 \geq 0.982$.

The quantities of PAR 2 gene expression and of the reference gene GAPDH were determined by using standard curves. The mRNA copy numbers of these targets were calculated for each sample from the standard curve by measuring the threshold cycle value. The target amount was then divided by the reference gene amount to obtain a normalized target value and presented as relative rates compared with the expression of the reference gene GAPDH.

Sequencing

Sequencing of the PCR products to verify amplification specificity was carried out according to the manufacturer's protocol (ABI Prism sequencing protocol) using the Ampli-Taq[®] FS Big Dye Terminator (Perkin-Elmer).

Immunohistochemistry

The immunohistochemical staining protocol has been previously described [14–18]. Briefly, all sections were

first incubated for 1 h with PBS containing 10% normal swine serum to block non-specific protein binding sites, and 0.5% tween 20 to enhance penetration of immunoreagents. Cryo-sections of nasal mucosa were then incubated with polyclonal rabbit antibody (Santa Cruz Biotechnology Inc., dilution 1 : 100) or mouse monoclonal antibody (Santa Cruz Biotechnology Inc., dilution 1 : 100) that react with PAR 2 of human, mouse and rat. To study the co-localization of PAR 2 expression on mast cells and eosinophils, double immunohistochemistry using polyclonal rabbit PAR 2 antibody in combination with mouse monoclonal anti-human tryptase (Abcam, Cambridge, UK, dilution 1 : 200) for mast cells or with polyclonal goat anti-MBP-antibody (Santa Cruz Biotechnology Inc., dilution 1 : 200) for eosinophils were performed on human biopsy sections. After rinsing in PBS, a biotinylated donkey anti-goat IgG (Amersham, Freiburg, Germany, dilution 1 : 100) was used for 1 h. Following several washing steps, sections were then incubated with the reagents Streptavidin–Texas Red conjugate (Amersham, dilution 1 : 200) and fluorescein (FITC)-conjugated AffiniPure Donkey anti mouse IgG (Dianova, Hamburg, Germany, dilution 1 : 100). Finally, sections were washed again in PBS and mounted in carbonate-buffered glycerol at pH 8.6. Specific immunohistochemical signals also disappeared after pre-incubation of the antibody for PAR 2 peptide (sc-8205 P, Santa Cruz Biotechnology Inc.) or replacement of the primary antisera by PBS.

Immunohistochemical quantification

PAR 2 and/or immunoreactive cell subpopulations were counted at the length of the base membrane at one grid depth (0.45 mm) with an epifluorescence microscope (Olympus BX-60, Olympus, Hamburg, Germany). The results were expressed as the number of immunoreactive cells per square millimetre.

Statistical analysis

Differences between PAR 2 gene expression in epithelial, endothelial and mucus cells of SAR and NR were analysed by the Mann–Whitney *U*-test using SPSS Software (Version 11) for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as median values. The statistical significance between the groups was set at $P < 0.05$.

Results

Study subjects

Patients with SAR were between 15 and 66 years old (mean 28.8) and in the controls between 17 and 46 years old (mean 31.8). All patients with positive allergy history for grass pollen, positive skin test for grass, high total IgE

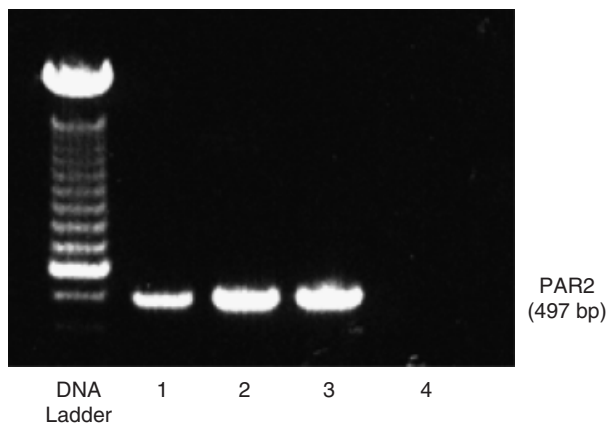


Fig. 1. Expression of protease-activated receptor 2 (PAR 2) mRNA in human nasal mucosa. Expression of PAR 2 mRNA by RT-PCR. Lane 1, expression of PAR 2 mRNA in the nasal mucosa of non-allergic rhinitis; lanes 2 and 3, expression of PAR 2 mRNA in the nasal mucosa of patients with SAR; lane 4, the negative control with aqua dest.

and positive RAST test for grass pollen were recruited for further investigation (data not shown). Six patients were excluded from further study as they showed positive skin testing for grass pollen and also mites indicating of having both SAR and perennial allergic rhinitis or were treated with drugs such as antihistamines within 4 weeks before the study.

Qualitative gene expression of protease-activated receptor 2 in human nasal mucosa

To assess the expression of PAR 2 mRNA in the nasal mucosa, qualitative RT-PCR studies were performed using human-specific primers. PAR 2 mRNA was found in human nasal mucosa of SAR and non-allergic subjects (Fig. 1). The PCR product identities were confirmed by direct sequencing (MWG biotech, Ebersberg, Germany) which revealed identity with the published sequences.

Quantitative gene expression of protease-activated receptor 2 in different cell types of the human nasal mucosa by real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction. PAR 2 gene expression level was found to be significantly increased in SAR in comparison to the controls ($P=0.004$). Using laser-assisted cell picking and subsequent real-time PCR, PAR 2 expression level was found to be significantly increased in epithelial ($P=0.003$) but not in endothelial cells ($P=0.50$) and in mucus cells ($P=0.78$) in the nasal mucosa of SAR in comparison the controls. The relative gene expression of PAR 2 in different cell types in the nasal mucosa is presented in (Figs 2 and 3).

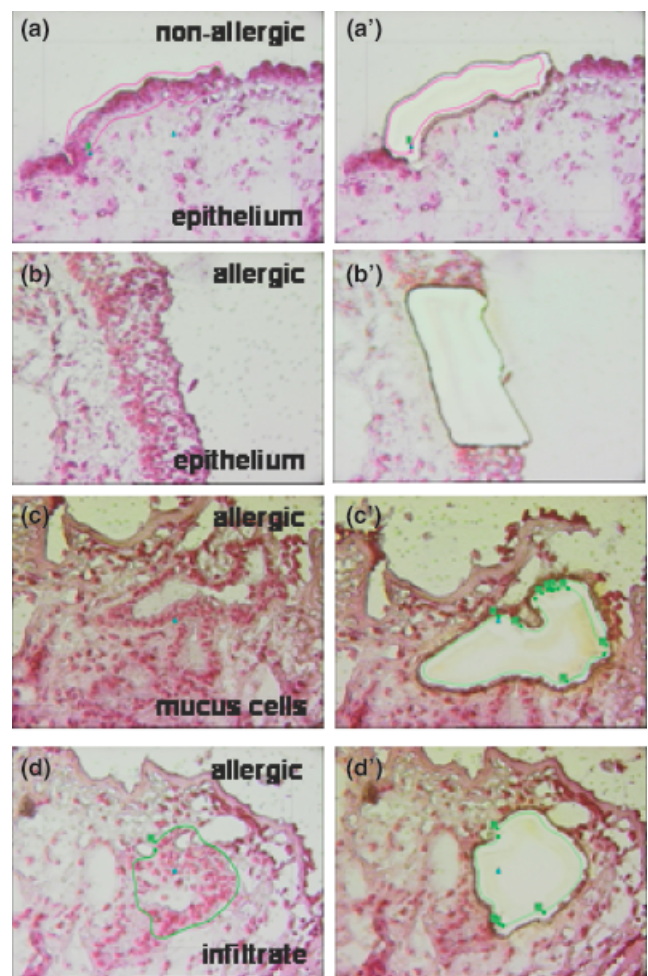


Fig. 2. Nasal mucosa from non-allergic and seasonal allergic rhinitis (SAR) subjects were stained with haematoxylin and eosin (H&E). Expression of PAR 2 gene was investigated in complex tissues of the nasal mucosa. PAR 2 mRNA level was analysed in epithelial cells of SAR (SAR: before, b; after microdissection and cell picking, b') in comparison with the controls (non-allergic: before, a; after microdissection and cell picking, a'); in mucus cells (SAR: before, c; after microdissection and cell picking, c') and infiltrate of SAR subjects, (SAR: before, d; after microdissection and cell picking, d'), magnification; $\times 240$.

Efficiency of picking. The data analysed here are based on 50 samples of epithelial cells (each containing 50–100 picked epithelial cells), 40 samples of endothelial cells (each containing 10–20 picked endothelial cells) and 38 samples of mucus cells (each containing 15–30 picked mucus cells), in 91 samples GAPDH mRNA was detected. Thus, the overall efficiency of RT – and real-time PCR in detecting the housekeeping gene in a limited number of picked cells was 71% (91/128 samples). Negative samples may be mostly caused by methodological imponderables, including degradation of RNA during staining and preparation and failure of the polymerase during PCR cycles.

Immunohistochemistry. The immunohistochemical staining showed that PAR 2 was found in epithelial, mucus and

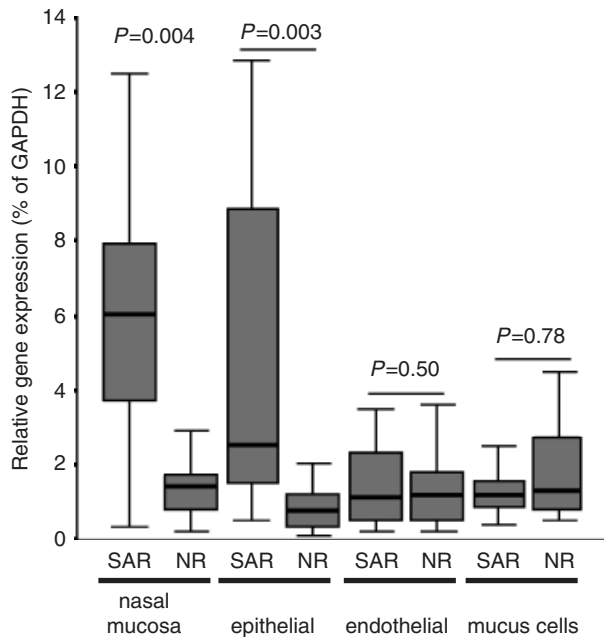


Fig. 3. Protease-activated receptor 2 (PAR 2) gene expression in various nasal mucosa cell types of seasonal allergic rhinitis (SAR) and non-allergic rhinitis (NR). The first grey bar shows PAR 2 gene expression in the nasal mucosa of SAR (grey bar 1; relative PAR 2 gene expression in the nasal mucosa of SAR) in comparison with the NR controls (grey bar 2; relative PAR 2 gene expression in the nasal mucosa of NR), grey bar 3; relative PAR 2 gene expression in epithelial cells of SAR, grey bar 4; relative PAR 2 gene expression in epithelial cells of NR, grey bar 5; relative PAR 2 gene expression in endothelial cells of SAR, grey bar 6; relative PAR 2 gene expression in endothelial cells of NR, grey bar 7; relative PAR 2 gene expression in mucus cells of SAR, grey bar 8; relative PAR 2 gene expression in mucus cells of NR. The boxes represent the first (25%) and the third (75%) quartile of the distribution, the cross-line denotes the median, the low whisker the 5th and the upper whisker 95th percentile.

endothelial cells of human nasal mucosa. The present study demonstrated, PAR 2 is strongly expressed and localized predominantly on the epitheliums of the nasal mucosa. PAR 2 was also found in mucus cells and endothelial cells. Interestingly, PAR 2 was also found to be strongly expressed on inflammatory cells of SAR.

Further investigation using double immunohistochemistry with antibody against PAR 2 and human tryptase or antibody raised against MBP (eosinophils marker) showed that PAR is expressed on mast cells as well as on eosinophils in the nasal mucosa. Moreover, quantitative immunohistochemistry showed that there was a significant increase in the number of both mast cells (162.10 ± 91.08 vs. 51.15 ± 20.26 , $P = 0.0003$) and eosinophils (39.30 ± 45.07 vs. 10.07 ± 5.72 , $P = 0.03$) in the nasal mucosa of SAR in comparison with the controls. Mast cells and eosinophils with non-immunoreactivities for PAR 2 were also found in the nasal mucosa of SAR and NR. The number of PAR 2-immunoreactive mast cells was not significantly changed between the two groups

(58.70 ± 28.74 vs. 44.15 ± 23.08 , $P = 0.19$). In contrast to the PAR 2-immunoreactive mast cells, the number of eosinophils expressing PAR 2 (29.66 ± 19.60 vs. 10.40 ± 5.96 , $P = 0.006$) in the nasal mucosa was significantly elevated in the nasal mucosa of SAR if compared with the controls (Figs 4–6). For the negative controls, the same slides showed immunoreactivity for major basic proteins, but not for PAR 2 when the primary antibody raised for PAR 2 was replaced by PBS and strong immunoreactivity for PAR 2 was detected, but there was no staining signal for major basic proteins when the primary antibody raised for MBP was removed (Fig. 5).

Discussion

The PAR family is known to be present in the lower airways of different species including human. With respect to the important role of PARs in the airways and lack of information on the expression of PARs in the upper airways, therefore the present study was designed to explore the gene and protein expression of this receptor on human nasal mucosa and demonstrated for the first time that PAR 2 is present in the nasal mucosa under normal situation and pathophysiological conditions such as SAR.

In the present study, further investigations using laser-assisted cell picking in combination with subsequently *real-time* PCR were able to show the distribution and localization of gene expression of PAR 2 in different cells of the human nasal mucosa. Supporting our findings, PAR 2 is known to be widely distributed on the cells of different human tissues including the human airways under normal [19, 20] and chronic obstructive pulmonary diseases (COPD) [21, 22]. In this respect, PAR 2 has been implicated to contribute to the pathogenesis of airway inflammation as stimulation of PAR 2 in epithelial cells has been found to open tight junctions, cause desquamation, produce cytokines and chemokines, and growth factors [10]. The high basal expression of PAR 2 in epithelial cells in the nasal mucosa of normal subjects and its increased level of expression under pathological conditions such as SAR suggest a physiological and/or pathophysiological role of PAR 2 in SAR. In asthmatic airways, the expression of PAR 2 has been described to be increased [23]. In human nasal mucosa the expression of PAR 2 has not been reported so far. The reasons for the increased expression of PAR 2 in the nasal mucosa of SAR patients are unclear but may reflect the presence of inflammation in the nasal mucosa *per se*.

In this study epithelial, endothelial and mucus cells were cut, isolated and collected by single cell using laser pressure catapulting technology (LPC), unlike previous study where the epithelial, endothelial and mucus cells were cut and collected as a whole tissues [24]. However, the fact that PAR 2 mRNA in inflammatory cells may

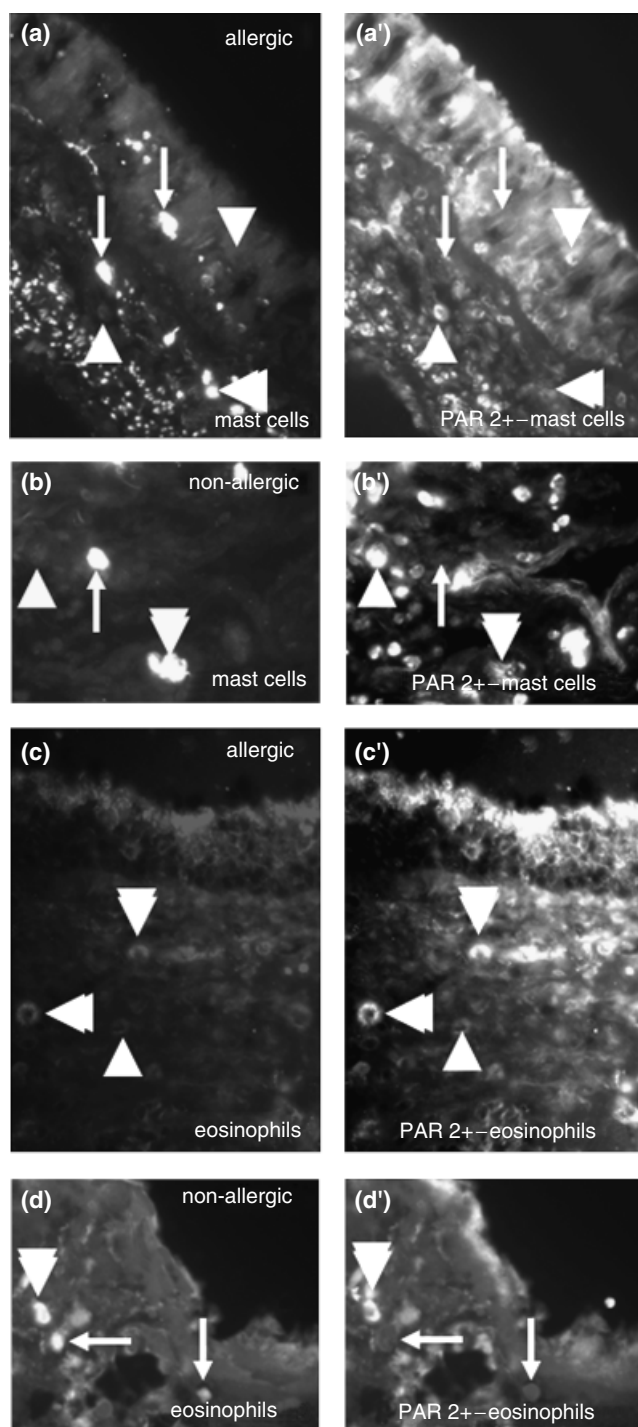


Fig. 4. Expression of Protease-activated receptor 2 (PAR 2) in nasal mucosa of non-allergic and seasonal allergic rhinitis. PAR 2-immunoreactivity (IR) was found in epithelial cells in the nasal mucosa of SAR and the controls (a', c' and d'). A large number of inflammatory cells expressed PAR 2-immunoreactivities. Most of them did not express tryptase (arrowheads in a–b') or major basic proteins (arrowheads in c–c') indicating they were not mast cells or eosinophils. A substantial population of mast cells (immunoreactivity for tryptase) (arrows in a–b') and eosinophilic population (positive immunoreactivity for major basic proteins) was found to show negative PAR 2 immunoreactivities (arrows in a–d') (magnification $\times 400$).

contribute to the PAR 2 mRNA levels of epithelial, endothelial and mucus cells could not be totally excluded in the present study.

Concerning allergic airway inflammation, PAR 2 activation of epithelial cells has been proposed to be involved in electrolyte transport in mouse and human airways [25–27] and reduce epithelial response to lipopolysaccharide in guinea-pig airways [28]. Activation of PAR 2 has been also reported to induce morphologic change and cytokine production in epithelial cells by fungal allergens [29]. *In vivo* studies using PAR 2 $-/-$ mice demonstrated that ear edema and infiltration of inflammatory cells were reduced in PAR 2 $-/-$ mice after 24 h topical application of picryl chloride and oxazolone compared with the wild type indicating an important role of PAR 2 in allergic dermatitis [30]. For the mouse lower airways, eosinophils infiltration, airway hyper-reactivity and IgE production were significantly reduced in PAR 2 $-/-$ mice and significantly increased in transgenic mice over-expressing PAR 2 after allergen challenge if compared with the wild-type animals [8]. Activation of PAR 2 can cause multiple motor effects such as *in vivo* bronchoconstriction in guinea-pig airways [31].

Activation of PAR 2 in endothelial cells can induce vasorelaxation, plasma protein extravasation and endothelial cell proliferation leading to vascular remodeling [19, 32, 33] by calcium mobilization and cell proliferations [34]. In this respect, the present findings of high basal gene expression of PAR 2 in endothelial cells indicate a physiological role of PAR 2 in the nasal mucosa under normal conditions. Contrary our hypothesis that activation of PAR 2 in endothelial cells can cause vasodilatation leading to nasal congestion that presents one of the major symptoms of AR. In contrast to our expectation and previous findings [35, 36], the gene level of PAR 2 in endothelial cells was not increased under SAR. However, the basic expression of PAR 2 is already high. So, a functional role of PAR 2 on these cells cannot be excluded in the present study.

As PAR 2 has been shown to be involved in mucus secretion of gastric mucosa [37–39], the present study addressed to explore PAR 2 gene expression in mucus cells of SAR and found that PAR 2 gene expression was not changed in SAR in comparison with the controls. These findings indicate that PAR 2 may not be involved in mucus hypersecretion in SAR as previously reported for the mucin gene expression in the lower airways [40]. Functional studies remain to be carried out to explore the role of PAR 2 in mucus cells in the nasal mucosa of SAR.

Airway smooth muscle cells, fibroblast and also sensory neurons are known to express PAR 2 [6, 7]. The extend co-localization of PAR 2 and other pro-inflammatory neuropeptides like tachykinins and CGRP in sensory neurons indicate a role of PAR 2 in neurogenic airway inflammation [41]. Moreover, PAR 2 has been shown to express on

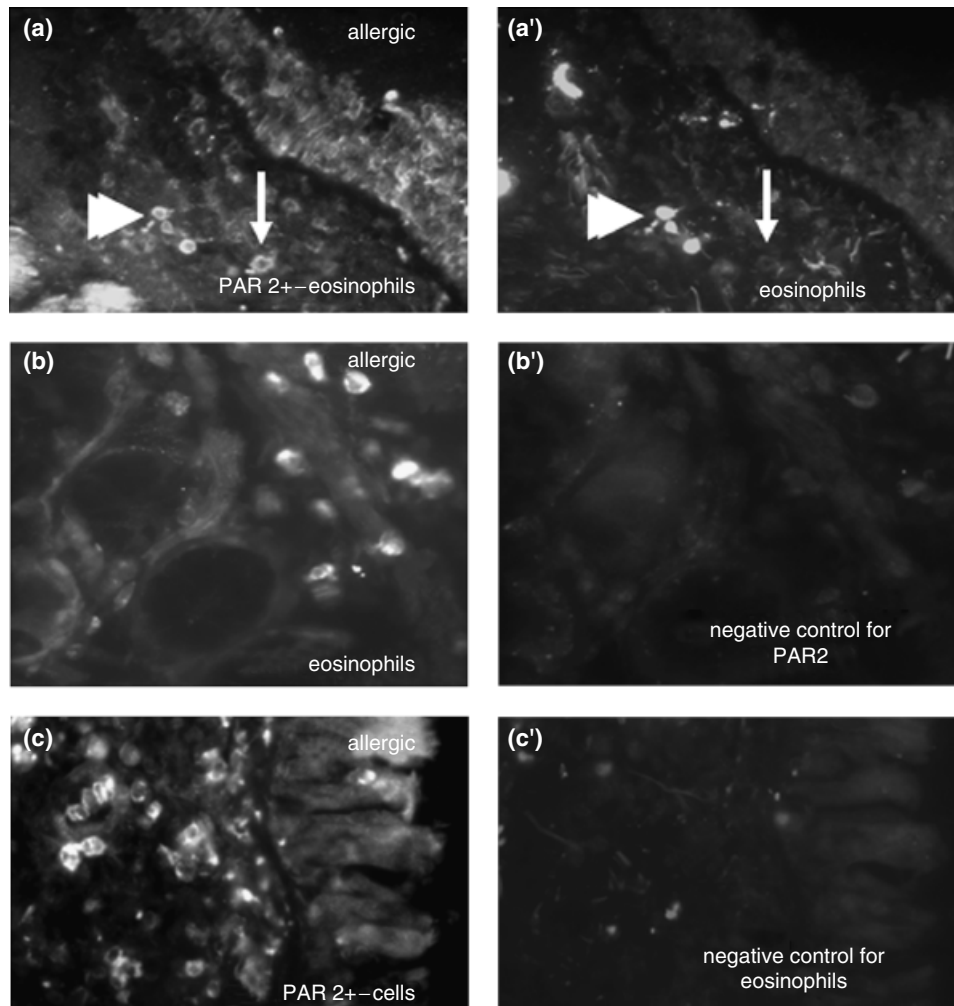


Fig. 5. Expression of Protease-activated receptor 2 (PAR 2) in nasal mucosa of seasonal allergic rhinitis. PAR 2-immunoreactivity (IR) was found in eosinophils of the nasal mucosa of SAR (double arrowheads in a and a'). PAR 2 was also found on other cells (arrows in a and a'). The same slides shows immunoreactivity for major basic proteins, but not for PAR 2 (negative control for PAR 2, primary antibody raised for PAR 2 was replaced by PBS) in b and b'. Strong immunoreactivity for PAR 2 in c, but no staining signal for major basic proteins (MBP) (negative control for MBP, marker for eosinophils, primary antibody raised for MBP was replaced by PBS) in c'. (Magnification $\times 400$).

sensory neurons [42]. Activation of PAR 2 on sensory nerves was able to mediate neurogenic inflammation via tachykininergic pathway [7].

Further investigation of PAR 2 expression on immune cells in the nasal mucosa demonstrated not only an abundant expression of PAR 2 in mast cells and eosinophils but also an increase of eosinophils expressing PAR 2 under SAR condition. Mast cells and eosinophils are known to play a central role in allergic diseases [21, 43, 44]. The fact that the number of mast cells expressing PAR 2 was not significantly increased in SAR did not exclude the possibilities of an important role of PAR 2 in mast cells in SAR as there has been shown that activation of PAR 2 is important for mast cell function such as degranulation of inflammatory cocktails [45, 46], the release granule-associated enzymes from eosinophils [47] and for neutrophil adherence [48]. Contrary the unchanged number of

PAR 2-immunoreactive mast cell subpopulation in SAR, the number of eosinophils expressing PAR 2 was found to be significantly increased in the nasal mucosa of SAR. Under SAR, activation of PAR 2 localized on eosinophils may lead to an influx of eosinophils expressing PAR 2 to the nasal mucosa to mediate allergic airway inflammation. Supporting this hypothesis, PAR 2 has been found to be expressed on human eosinophils with the capacity of modulating eosinophil function [49]. Activation of PAR 2 has been shown to be responsible for the infiltration of eosinophils in the lower airways of mice [8]. In this context, using PAR 2 $-/-$ mouse in a model of allergic airway inflammation, specific PAR 2 agonists have been found to be accounted for the infiltration of eosinophils and macrophages into the airways [8, 50]. However, there are also conflicting reports that shows that PAR 2 agonist has a suppressive effect on the allergic airway

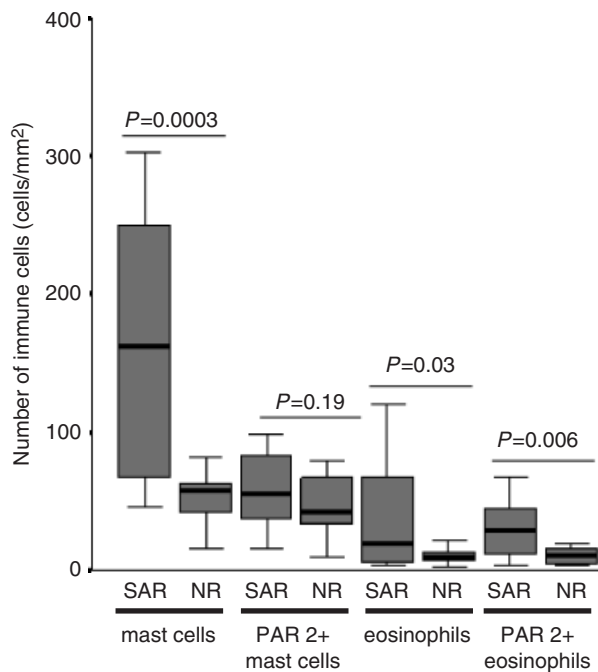


Fig. 6. Quantitative immunohistochemistry of mast cells and eosinophils in the nasal mucosa of SAR and non-allergic rhinitis. There is a significant increase of the number of mast cells (grey bar 1; mast cells in the nasal mucosa of SAR; grey bar 2, mast cells in the nasal mucosa of NR, $P=0.0003$) and eosinophils (grey bar 5, eosinophils in the nasal mucosa of SAR, grey bar 6, eosinophils in the nasal mucosa of NR, $P=0.03$) in the nasal mucosa of SAR in comparison with the controls. The number of eosinophils expressing PAR 2 (grey bar 7, PAR 2-immunoreactive eosinophils in the nasal mucosa of SAR) was found to be significantly higher than PAR 2-immunoreactive eosinophils in the nasal mucosa of the controls (grey bar 8, PAR 2-immunoreactive eosinophils in the nasal mucosa of NR, $P=0.006$) whereas the number of PAR 2-immunoreactive mast cells between the two groups was not significantly changed (grey bar 3, PAR 2-immunoreactive mast cells in the nasal mucosa of SAR and grey bar 4, PAR 2-immunoreactive mast cells in the nasal mucosa of NR).

inflammation [6, 9]. In this respect, the functional role of PAR 2 remains to be determined in future studies. Here, we found that PAR 2 is strongly expressed by mast cells and eosinophils under normal and diseases conditions like SAR. Therefore, the present findings indicate that inflammatory cells expressing PAR 2 may provide new targets for treatment of allergic diseases such as AR [22, 51].

Conclusion

The abundant expression of PAR 2 in epithelial cells and the increased number of eosinophils expressing PAR 2 in the nasal mucosa under diseases conditions such as SAR indicate that PAR 2 might contribute to the pathogenesis of allergic airway inflammation such as SAR.

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