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Expression of cysteinyl leukotriene synthetic and signalling proteins in inflammatory cells in active seasonal allergic rhinitis

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Summary

Background Cysteinyl leukotrienes (CysLTs) are bioactive lipids that have been shown to contribute to allergic and inflammatory diseases. Eosinophils and mast cells have the capacity to produce large amounts of CysLTs after allergic or non-allergic stimulation. Molecular identification of both the synthetic and signalling proteins in the CysLT pathway allows the investigation of expression of the CysLT enzymes and receptors in active allergic rhinitis.

Objective We examined the expression of the proteins involved in the synthesis of CysLTs and the cysteinyl leukotriene-1 ($CysLT_1$) and cysteinyl leukotriene-2 ($CysLT_2$) receptors in inflammatory cells from patients with active seasonal allergic rhinitis.

Methods Nasal lavage samples were obtained from patients during active seasonal allergic rhinitis. Specific cellular immunocytochemical techniques were used to detect the cysteinyl leukotriene synthetic proteins, namely 5-lipoxygenase (5-LO), 5-lipoxygenase-activating protein (FLAP) and leukotriene C_4 synthase (LTC4S). *In situ* hybridization and immunocytochemical techniques were used to identify the mRNA and proteins for the $CysLT_1$ and $CysLT_2$ receptors.

Results 5-LO, FLAP and LTC4S, and the $CysLT_1$ and $CysLT_2$ receptors were expressed in the majority of eosinophils and in subsets of mast cells and mononuclear cells. 5-LO, FLAP and the $CysLT_1$ receptor, but not LTC4S or the $CysLT_2$ receptor, were expressed in a subset of nasal neutrophils.

Conclusions Our study demonstrates the presence of CysLT pathway proteins in key allergic and inflammatory cells from the upper airway of patients with active seasonal allergic rhinitis. Our expression data highlight the potential of CysLT-modifying agents to treat both upper and lower airway symptoms in patients suffering from allergic rhinitis and asthma.

Keywords airway inflammation, allergic rhinitis, immunology, leukotriene receptor biology
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Introduction

The cysteinyl leukotrienes (CysLTs) LTC_4, LTD_4 and LTE_4 are produced in large quantities by activated mast cells and eosinophils and have been proposed to contribute to the pathology of a number of allergic diseases, including asthma and allergic rhinitis [1, 2]. In mast cells and eosinophils, the synthesis of CysLTs is stimulated by antigen-IgE or cytokine activation of calcium release, translocation of the cytosolic 5-lipoxygenase (5-LO) to the inner nuclear membrane, where in the presence of 5-lipoxygenase-activating protein (FLAP), arachidonic acid is converted to LTA_4 [3, 4]. Subsequently, leukotriene C_4 synthase (LTC4S) or microsomal glutathione transferase II (MSGT-II) converts LTA_4 to LTC_4, which is exported to the blood and hydrolysed by peptidases to LTD_4 and then to LTE_4 [3, 4]. The CysLTs activate smooth muscle and inflammatory cells through specific, high-affinity interactions with the $CysLT_1$ and $CysLT_2$ receptors, putative seven transmembrane domain G protein-coupled receptors [5, 6]. The CysLT receptors are coupled to the second-messenger calcium and, depending on the cell type, may result in contraction, cellular migration or inflammatory mediator release [3]. The human $CysLT_1$ receptor is expressed on lung macrophages and smooth muscle cells and in peripheral blood eosinophils and subsets of basophils, monocytes, B cells and $CD34^+$ stem cells [7]. The human $CysLT_2$ receptor has been identified on heart Purkinje conducting fibre cells, adrenal chromaffin cells, in the brain and in subsets of peripheral blood leucocytes, including eosinophils [6, 8].

A wealth of evidence exists for the role of CysLTs in the pathology of both allergic and non-allergic asthma [9]. The expression of the 5-LO pathway enzymes in eosinophils and macrophages within bronchial mucosa has been shown to correlate with the deterioration of lung function during seasonal allergen exposure [10]. Leukotriene synthesis inhibitors or $CysLT_1$ receptor antagonists reduce asthmatic

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symptoms, improve pulmonary function and decrease eosinophilia in the airway and blood [11–14]. $CysLT_1$ antagonists are effective in patients receiving β-agonists alone and in patients receiving inhaled glucocorticoids for chronic asthma [9, 11–17].

CysLTs have also been implicated in the pathology of allergic rhinitis [18–21]. Increases in CysLTs in nasal lavage fluids have been measured after specific allergen challenge to seasonal allergic individuals and in perennial rhinitis [18–21]. By contrast, CysLTs did not change in nasal lavage from saline nasal challenge or in non-atopic healthy volunteers with nasal allergen challenge [2]. LTB_4 has also been shown to increase in nasal lavage fluids post-allergen challenge, but these also occur in non-atopic individuals and are suggested to be a result of repeated nasal lavage procedures [2]. CysLT single-dose nasal challenge studies showed that both LTC_4 and LTD_4 induced increases in nasal airway resistance and nasal mucosal blood flow, but did not cause nasal pruritus or sneezing [22, 23]. Clinical studies have confirmed that LT synthesis inhibition or CysLT receptor antagonism is beneficial in allergic rhinitis and may be complementary to the action of antihistamines [24–26].

The study described in this manuscript characterizes the expression of both CysLT synthetic and signalling proteins in patients suffering from seasonal allergic rhinitis with natural exposure to allergen. From previous studies in seasonal allergic asthma, we predict that inflammatory nasal lavage cells from such patients would express the CysLT synthetic proteins [10]. There is no published study on the expression of the CysLT receptors in allergic or non-allergic asthma. One previous study has been published on the localization of the CysLT receptors in human nasal mucosa obtained by turbinectomy from six patients with nasal obstruction [27]. Only three of these six patients had a history of allergies and it is unclear whether these subjects were symptomatic at the time of the turbinectomy. In the latter study, $CysLT_1$ and $CysLT_2$ receptor mRNA was identified by RT-PCR in human nasal mucosa, and the $CysLT_1$ receptor mRNA was shown by *in situ* hybridization in blood vessels and interstitial cells but not in epithelial cells [27]. Using immunocytochemical techniques, the $CysLT_1$ receptor protein was demonstrated in the nasal mucosa in interstitial eosinophils, mast cells, macrophages and neutrophils, but not in epithelial cells [27]. Our study confirms the previous CysLT receptor observations in the nasal mucosa and extends these to include both synthetic and signalling proteins in nasal lavage inflammatory cells from patients with active seasonal allergic rhinitis.

Methods

Patients

Twenty subjects with active seasonal allergic rhinitis were studied. The ages of the subjects ranged from 20 to 57 years. The diagnosis of allergic rhinitis was based on the presence of clinical symptoms and the presence of positive prick skin tests (>5 mm diameter) to three or more components of the Virginia allergen panel. This positive diagnosis for allergic rhinitis is based on a conservative definition of atopy as used to define asthma and allergy phenotypes in genetic studies of asthma. The study was approved by the Human Investigation Committee of the University of Virginia and all subjects gave signed consent. All subjects were symptomatic for at least one week and skin tested positive to a relevant allergen (i.e. tree pollens) at the time of enrolment. Upper respiratory infections were excluded by the presence of symptoms of greater than 1 week duration, the associated presence of allergic conjunctivitis, and the absence of fever and purulent nasal discharge. Subjects were not using topical or systemic anti-inflammatories (nasal/systemic steroids, LT modifiers or cromolyn). Antihistamine medications were not withheld from subjects prior to sampling. No subjects were receiving immunotherapy.

Cell-sampling technique

Representative nasal cells were obtained by nasal lavage with $\sim 5\,cm^3$ of normal saline, which was insufflated into one nare as we have previously described [28]. Saline was collected, filtered and centrifuged. Cell pellets were resuspended in phosphate-buffered saline (PBS), resuspended in OCT compound (Sakura Technologies, Torrance, CA, USA), placed in a cryomold (Miles Inc., Elkhart, IN, USA) and snap-frozen in liquid nitrogen. Limited numbers of cells were obtained from each patient; therefore, each protein could not be studied in each patient. Each data set describes how many patients were used for each determination. In the initial stages of this study, nasal lavage samples were taken from subjects with perennial non-allergic rhinitis in order to have a comparative group for the active seasonal allergic rhinitic group. These perennial non-allergic rhinitic subjects had >5 consecutive days of symptoms of nasal congestion, rhinorrhoea and posterior pharyngeal drainage. However, these nasal lavage samples contained mainly epithelial cells and very limited numbers of inflammatory cells, preventing a comparison of the levels of expression between perennial non-allergic vs. seasonally active allergic rhinitis subjects.

Nasal cytology evaluation

Immunocytochemistry Lavage samples were rinsed in PBS and fixed in 4% paraformaldehyde (Electron Microscopy Inc., Fort Washington, PA, USA) in PBS (Sigma, St Louis, MO, USA) for 15 min at room temperature, rinsed in PBS and then blocked with 10% normal donkey serum (Jackson Immunoresearch, West Grove PA, USA). Immunocytochemistry was carried out as previously described [7]. Antisera for 5-LO were raised in rabbit against human 5-LO purified to homogeneity from human leucocytes and antisera for FLAP were raised in rabbit against the human FLAP sequence 42–51 [29]. Antisera for LTC4S, from D. Nicholson (Merck Frosst, Montreal, Canada), were raised in rabbit against the human LTC4S sequence 37–51, which has 70% identity to MSGT-II and so may also detect this protein, which is also capable of LTC_4 synthesis [30]. All three antisera were used at a 1 : 200 dilution. Immunocytochemistry for the $CysLT_1$ receptor was carried out using a polyclonal goat anti-full-length recombinant human $CysLT_1$ receptor antisera, as described previously [7], and immunocytochemical detection of the $CysLT_2$ receptor was carried out using 15 μg/mL of

affinity-purified rabbit anti-peptide antisera raised against the first N-terminal 18 amino acids of the human $CysLT_2$ receptor (Cayman, Ann Arbor, MI, USA). Non-immune sera from each animal, used at the same concentration as the specific antisera, were used as a negative control in immunostaining, and either no staining or minimal non-specific staining was observed (data not shown). Following immunohistochemical detection, cells were counterstained with DAPI (Molecular Probes, Eugene, OR, USA) and images were digitally acquired using a Nikon E1000 epifluorescence microscope, Micromax CCD camera (Princeton Instruments, San Diego, CA, USA) and Metamorph Imaging Program (Universal Imagining, West Chester, PA, USA).

In situ *hybridization* Lavage samples were fixed in 4% paraformaldehyde (Electron Microscopy Inc.) in PBS (Sigma) for 20 min at room temperature and digested for 5 min in 10 μg/mL proteinase K (Boehringer Mannheim, Indianapolis, IN, USA) at room temperature. Biotinylated oligonucleotide antisense and sense $CysLT_1$ and $CysLT_2$ receptor probes were prepared and *in situ* hybridization was carried out as described previously [5, 7]. Bound probe was detected using the TSA direct red FISH tyramide amplification kit (NEN Life Sciences, Boston, MA, USA) according to the manufacturer's instructions. Double label cell identification immunocytochemistry was performed as above on specimens demonstrating $CysLT_1$ and $CysLT_2$ receptor mRNA signal. The identification marker antisera used on nasal lavage preparations were Mast Cell Tryptase (DAKO Corporations, Carp, CA, USA) for mast cells and basophils and CD64 (Pharmingen, San Diego, CA, USA) for mononuclear cells. The identification marker for eosinophils was the human CCR3 receptor using a rabbit antiserum from B. Daugherty (Merck Research Laboratories, Rahway, NJ, USA) and a human CCR3 receptor mouse monoclonal antibody (R&D Systems, Minneapolis, MN, USA).

Morphometry and analysis Expression analysis was carried out on images of multiple microscopic fields ($n = 6$), taken at $\times 200$ magnification, for each staining condition. Images were given a uniform threshold prior to analysis. *In situ* mRNA positive cells and cells showing specific immunoreactivity for the protein or cell marker of interest were counted within a 300 μm × 300 μm grid. The expression level of signals was quantified as the ratio of cell identification marker positive cells to total cells counted, which were positive for the mRNA or protein being determined. Data from allergic rhinitis lavage samples from different patients were pooled to ensure that at least 100 cells identified by selective marker or morphology were counted for each CysLT protein determination. Samples from perennial non-allergic rhinitic controls contained too few leucocytes to be included in a comparative analysis (data not shown). A *t*-test was used to determine differences between expression levels for each mRNA or protein in each cell type.

Results

Expression of 5-LO, FLAP and LTC_4 synthase proteins in nasal cells from patients with active seasonal allergic rhinitis

We investigated the expression of CysLT synthetic proteins in specific inflammatory cells, namely eosinophils, mononuclear cells, mast cells and neutrophils identified by cell-selective

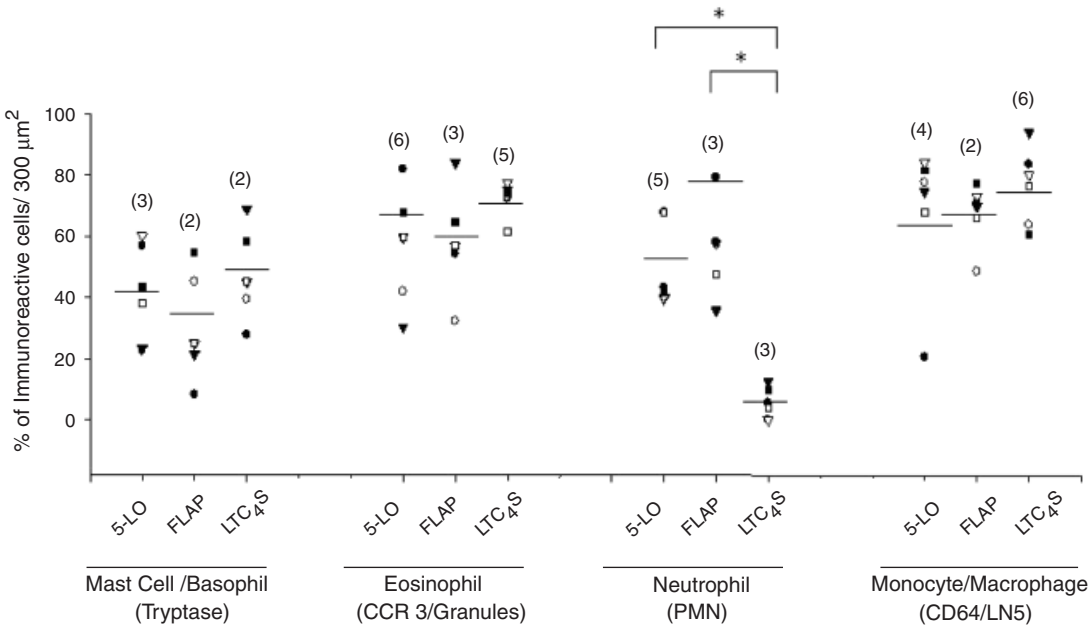

Fig. 1. Immunohistochemical expression of the cysteinyl leukotriene synthetic pathway proteins on inflammatory cells from allergic nasal secretions. The graph shows percent values for each leukotriene-synthesizing protein within each cell population. Each point represents percent derived for each 300 μm^2 microscopic grid analysed for each condition. Horizontal lines depict the mean percentage value within each group. The figure in parentheses represents the number of different allergic rhinitis patients used to perform analysis, ensuring that more than 100 cells for each type were analysed. LTC4 synthase (and/or MSGT-II) show a significantly lower expression on neutrophils than both 5-LO and FLAP (*P*-values < 0.05).

markers and morphology, in active seasonal allergic rhinitic nasal lavage samples (Fig. 1). The majority of eosinophils and mononuclear cells in these samples were positive for 5-LO, FLAP and LTC4S (Fig. 1). Only about 30% of cells co-staining with tryptase (i.e. mast cells or basophils) expressed the CysLT pathway proteins (Fig. 1). Neutrophils showed a strong expression of 5LO and FLAP, but less than 5% were positive for LTC4S (Fig. 1). Examples of nasal lavage eosinophils, positive for 5-LO (Fig. 2a) or FLAP (Fig. 2b), identified both by bi-lobed nuclei morphology and by co-localization with the eotaxin receptor CCR3 are shown in Fig. 2. Figure 2 illustrates examples of mast cells in nasal lavage samples that were positive for 5-LO (Fig. 2c) or FLAP (Fig. 2d). A subset of mast cells was also positive for LTC4S (Fig. 1, Fig. 2g). Neutrophils in nasal lavage samples were identified by poly-lobed nuclei and were positive for both 5-LO (Fig. 2e) and FLAP (Fig. 2f), but expressed minimal LTC4S (Fig. 1 and data not shown). Greater than 50% of $CD64^+$ mononuclear cells expressed all the proteins in the CysLT synthetic pathway, including LTC4S (Fig. 2h).

Expression of $CysLT_1$ and $CysLT_2$ receptor mRNA and protein in nasal cells from patients with active seasonal allergic rhinitis

Both $CysLT_1$ and $CysLT_2$ receptor mRNA and proteins were identified on 70–90% of nasal lavage eosinophils, 50% of mononuclear cells and 30% of mast cells (Fig. 3). A subset of 40% of neutrophils expressed both the $CysLT_1$ receptor mRNA and protein, but not the $CysLT_2$ receptor mRNA and protein (Fig. 3). An eosinophil expressing the $CysLT_1$ receptor is shown in Fig. 4a and co-expression of the $CysLT_1$ receptor with LTC4S on this eosinophil is shown in Fig. 4c. Epithelial cells were found in nasal lavage samples from all patients, and these cells expressed LTC4S but not 5-LO, FLAP or CysLT receptors (Fig. 4a–c and data not shown). An example of a nasal lavage eosinophil expressing the $CysLT_2$ receptor protein is shown in Fig. 4d. A single eosinophil is shown in Fig. 4e–g, expressing the $CysLT_2$ receptor protein (Fig. 4e), $CysLT_1$ receptor protein (Fig. 4f) and co-localization of both receptors (Fig. 4g). Mast cells expressing the $CysLT_1$ receptor are shown in Fig. 5a identified by co-localization in cells expressing tryptase (Figs 5b and c). An example of a mast cell expressing the $CysLT_2$ receptor protein is shown in Fig. 5d. A polymorphonuclear neutrophil expressing the $CysLT_1$ receptor protein is illustrated in Fig. 5e, but very little $CysLT_2$ receptor mRNA or protein was observed in neutrophils (Fig. 5f). A subset of monocytes/macrophages expressed both $CysLT_1$ and $CysLT_2$ receptors (Figs 3 and 5g and h). A nasal lavage monocyte shows co-localization of the $CysLT_1$ receptor protein with the monocytic CD64 marker (Fig. 5g). Localization of the $CysLT_2$ receptor protein to a $CD64^+$ nasal lavage mononuclear cell is shown in Fig. 5h.

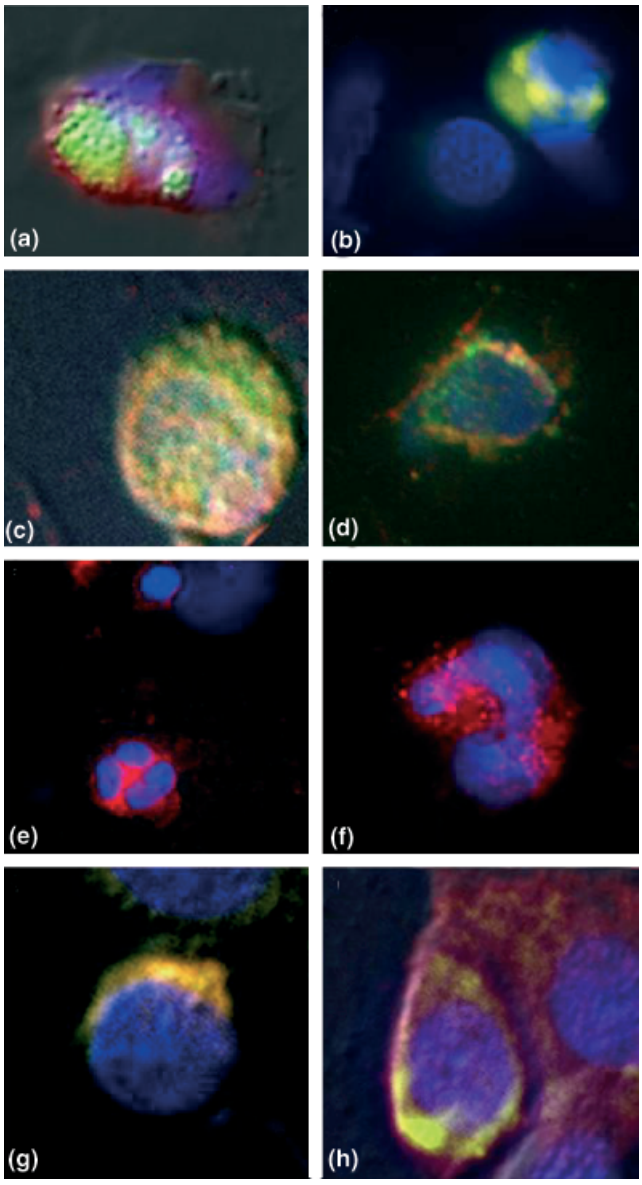

Fig. 2. Cysteinyl leukotriene-synthesizing proteins: expression on eosinophils, mast cells, neutrophils and mononuclear cells. (a) Combined immunohistochemical detection of 5-LO with Texas red and the CCR3 receptor with FITC (green) reveal co-expression of the two proteins (yellow) on an eosinophil. Cell nuclei are detected with DAPI (blue). (b) FLAP protein, detected via IHC with Texas red, is also co-expressed with the CCR3 protein, detected with FITC; co-expression is seen as yellow. (c) Immunohistochemical identification of 5-LO detected with Texas red is seen weakly expressed on a mast cell detected with tryptase FITC (green). Co-expression of the proteins is indicated by yellow. (d) FLAP IHC signal detected with Texas red is co-expressed (seen as yellow) on a tryptase immunoreactive (green) mast cell. (e) 5-LO protein visualization with Texas red; note the typical 'polymorphonuclear' neutrophil morphology. (f) FLAP detected with Texas red is similarly seen expressed by a polymorphonuclear neutrophil. (g) Combined detection of LTC4 synthase (and/or MSGT-II) with Texas red and tryptase visualized with FITC (green) is seen as yellow. (h) LTC4 synthase (and/or MSGT-II) on a $CD64^+$ monocyte. LTC4 synthase is detected with Texas red and CD64 with FITC and seen as green. Nuclei are counterstained with DAPI and seen as blue.

Discussion

Active seasonal allergic rhinitis is characterized by the symptoms of nasal obstruction, rhinorrhoea, nasal itch and sneezing subsequent to the local release of inflammatory mediators from cells within the nasal mucosa [2]. Upon allergen exposure, mast cells release preformed mediators, such as histamine and tryptase, and also rapidly synthesize and release cytokines, PGD_2 and CysLTs. CysLTs remain elevated for 30 min, during which time it is likely that the

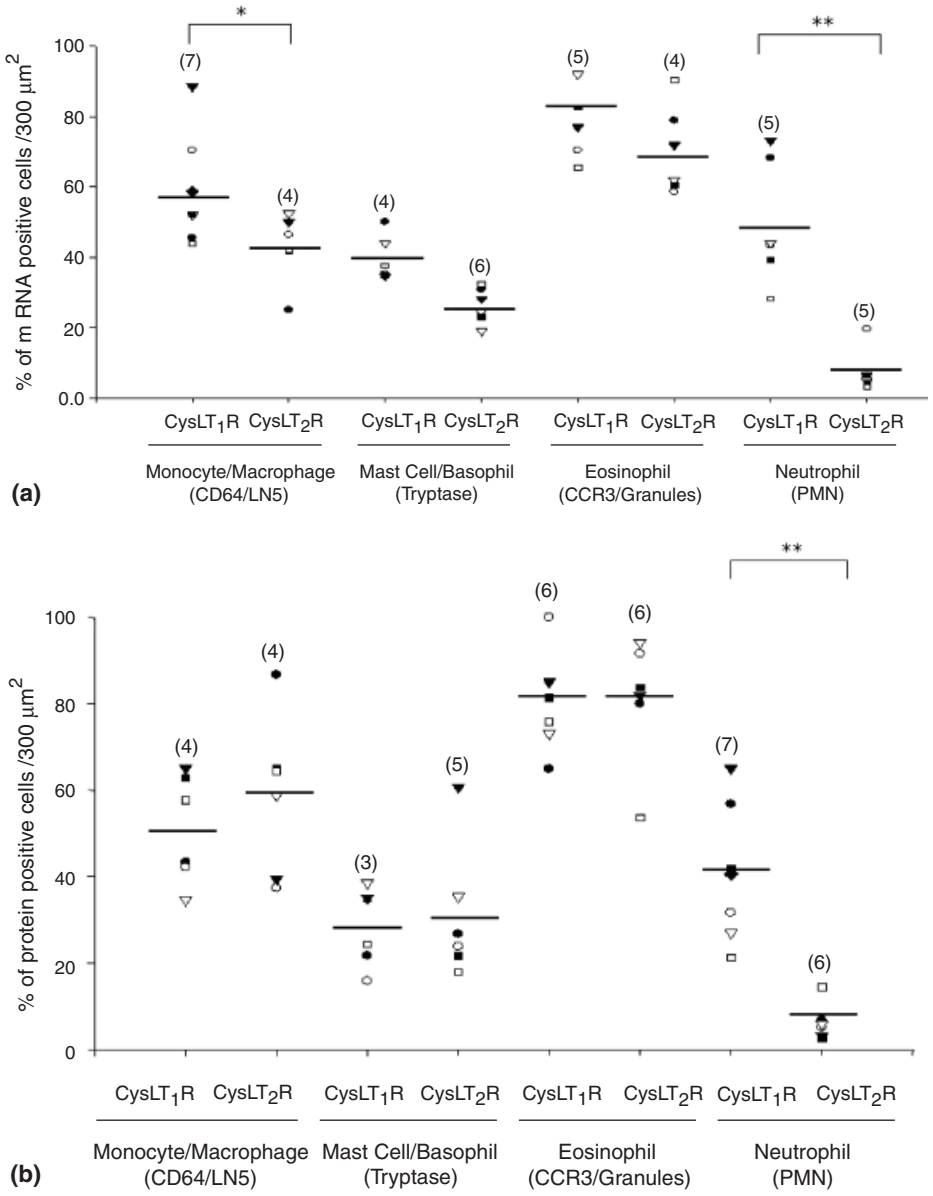

Fig. 3. *In situ* hybridization and immunohistochemistry of the cysteinyl leukotriene receptors in allergic rhinitis expressed as a percentage positive for each cell type. (a) $CysLT_1$ and $CysLT_2$ receptor mRNA-positive cells (>100) for each cell type. Values are expressed as a percent for each $300\,\mu m^2$ microscopic field analysed. The figure in parentheses represents the number of different allergic rhinitis patient samples incorporated into the analysis. Horizontal lines represent the mean value for each condition. The monocyte/macrophage population of cells shows a slightly higher level of CysLT1R mRNA than CysLT2R mRNA ($P<.05$ 0.). Neutrophils show a significantly higher level of CysLT1R mRNA than CysLT2R mRNA ($P<0.01$). (b) Expression of $CysLT_1R$ and $CysLT_2R$ immunoreactive cells within each nasal leucocyte population. Each point represents a $300\,\mu m^2$ microscopic field that was analysed for that staining condition. Horizontal lines represent the mean percent for each group. The figure in parentheses represents the number of different allergic rhinitis patient samples used to complete the analysis. Protein results show no significant difference from mRNA results. Neutrophils show almost no $CysLT_2R$ protein as compared with $CysLT_1R$ ($P< 0.01$).

CysLTs attract inflammatory cells into the nasal mucosa [2]. The late-phase response to allergen occurs 4–5 h post-allergen challenge with nasal obstruction, with some rhinorrhoea and sneezing, and is characterized by a massive influx of eosinophils, neutrophils and mononuclear cells [31]. Our study describes the expression of CysLT synthetic proteins and both CysLT receptors in subsets of nasal mast cells from patients with active allergic rhinitis (Figs 1–3 and 5). These patients are being constantly challenged by the presence of the airborne allergen, so the inflammatory cell content reflects both early- and late-phase cell types as described previously [31–33].

The severity of the nasal blockage in allergic rhinitis has been shown to be proportional to the numbers of eosinophils present [31]. Our results demonstrate expression of the complete CysLT synthetic pathway and both CysLT receptors in eosinophils. Eosinophils preferentially generate CysLTs rather than LTB_4, and CysLT production occurs in these cells on perinuclear membranes and in intracellular lipid bodies [34, 35]. LTD_4 has been shown to promote chemotaxis of eosinophils with the rank order of potency being $LTD_4 > LTC_4 > LTE_4$, the same order of potency of these CysLTs on the $CysLT_1$ receptor [36, 37]. In addition, LTD_4 enhances the release of eosinophil-derived neurotoxin induced by IL-5 and this enhancement is blocked by pranlukast, a $CysLT_1$ receptor-selective antagonist [37]. It is intriguing that while both the $CysLT_1$ and $CysLT_2$ receptors are expressed on the majority of eosinophils in allergic rhinitis, most of the functional activation of eosinophils can be blocked by $CysLT_1$ receptor-specific antagonists [36, 38, 39]. It is not clear if there is a redundant role for the $CysLT_1$ and $CysLT_2$ receptors in eosinophils and other cell types where both receptors are expressed. LTC_4 is about fivefold more potent for functional activation of the $CysLT_2$ receptor than for the $CysLT_1$ receptor; conversely, LTD_4 is about 40-fold more potent for functional activation of the $CysLT_1$ receptor than

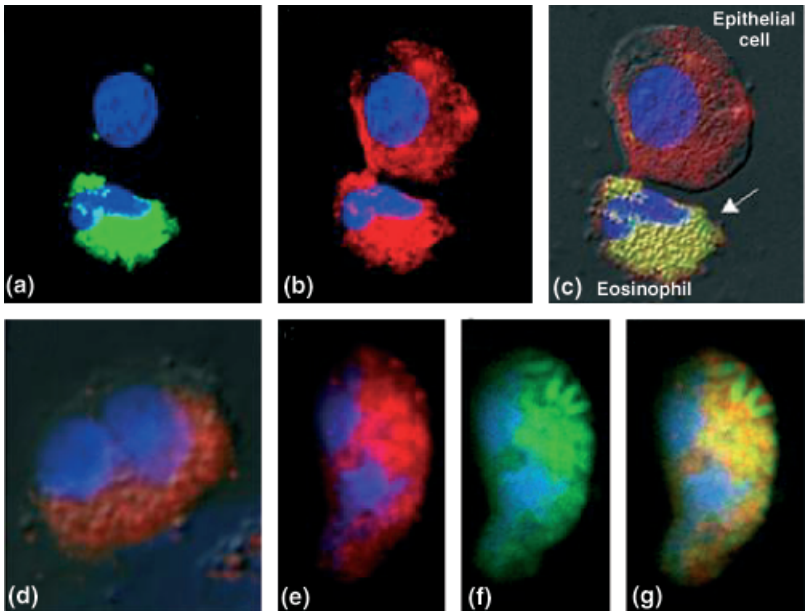

Fig. 4. Expression of the cysteinyl leukotriene receptors on eosinophils in nasal lavage samples. (a) $CysLT_1R$ protein is abundantly expressed on an eosinophil. The signal is visualized with green FITC and cell nuclei are counterstained with DAPI and seen as blue. (b) LTC4 synthase (and/or MSGT-II) immunoreactivity on the same eosinophil in (a) is visualized with Texas red. LTC4 synthase (and/or MSGT-II) also highlights an adjacent epithelial cell. Nuclei are seen as blue (DAPI). (c) Differential interference contrast microscopy DIC combined with double immunohistochemical detection of LTC4S (red) and $CysLT_1R$ (green) demonstrate co-expression of $CysLT_1R$ and LTC4 synthase on the eosinophil (yellow). Cell nuclei are stained with DAPI (blue). (d) $CysLT_2R$ receptor protein on an eosinophil adjacent to an epithelial cell visualized by Texas red immunohistochemistry. (e) $CysLT_2R$ protein detected with fluorescence (Texas red) on an eosinophil (f) $CysLT_1R$ receptor protein detected with FITC (green) on the same eosinophil (g) Double exposure shows that both receptors are co-expressed on the eosinophil. The combined signal is seen as yellow. Nuclei are blue DAPI.

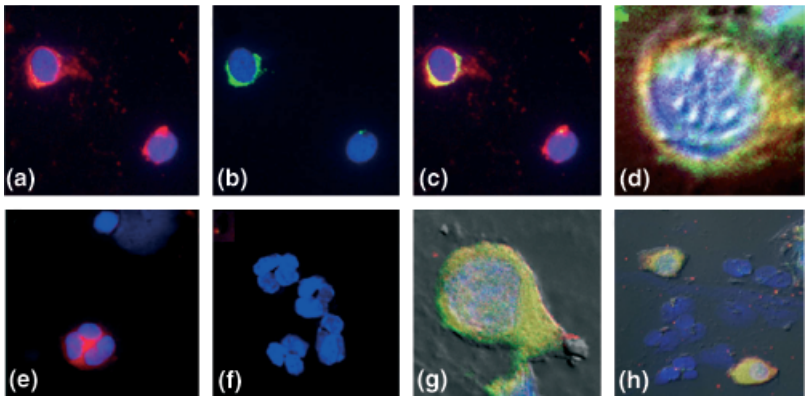

Fig. 5. Expression of cysteinyl leukotriene receptors on mast cells, neutrophils and mononuclear cells in nasal lavage samples. (a) $CysLT_1R$ protein immunoreactivity, detected with Texas red, is seen on a mast cell. (b) The mast cell, seen in panel (a), is identified with tryptase detected with FITC (green). (c) The $CysLT_1R$ protein (red) shows co-localization with tryptase (green) and the combined signal is visualized as yellow. (d) $CysLT_2R$ protein (Texas red) also shows co-expression (yellow) with mast cell tryptase FITC (green). (e) $CysLT_1R$ protein detected with Texas red on a neutrophil with polymorphonuclear morphology. Nuclei are stained with DAPI and seen as blue. (f) Immunocytochemical detection for $CysLT_2R$, with Texas red, shows no signal on polymorphonuclear cells. Cell nuclei are again seen as blue (DAPI). (g) $CysLT_1R$ positive and $CD64^+$ monocyte seen by double IHC. $CysLT_1R$ is detected with Texas red, CD64 with FITC and nuclei are blue DAPI. (h) CysLT2 receptor and CD64, highlighted with the same detection scheme, on a typical granulocytic monocyte.

for the $CysLT_2$ receptor [5, 6]. Since clinical studies have shown that topical nasal challenge with LTD_4 causes acute nasal obstruction and nasal secretion in patients with allergic rhinitis, this might suggest that the primary CysLT activation pathway is through the $CysLT_1$ receptor [22, 23]. It is possible that the $CysLT_2$ receptor is only activated in concert with the $CysLT_1$ receptor (perhaps in heterodimer formation) and thus $CysLT_1$ antagonists would block the activation of both receptors. Recently, it has been shown that aspirin-sensitive rhinosinusitis is characterized by a selective increase in expression of the $CysLT_1$ receptor on nasal submucosal $CD45^+$ inflammatory cells and that this expression is down-regulated after aspirin desensitization [40]. Aspirin-sensitive asthmatics have a greater response to LTE_4 than to LTC_4 and LTD_4. This agonist profile is not reflective of either a $CysLT_1$ or $CysLT_2$ receptor subtype and may be due to either a combination of receptors or a dysfunctional $CysLT_1$ receptor.

In our study, only subsets of mast cells and mononuclear cells express the $CysLT_1$ or $CysLT_2$ receptors and the CysLT synthetic proteins. It is not clear why only a subset of tryptase-positive mast cells or CD64-positive mononuclear cells express the CysLT pathway, but this may reflect their stage of differentiation or activation. It has been shown that expression of the $CysLT_1$ receptor can be enhanced by cytokines, including IL-4 and IL-13 [41, 42]. There are no studies on the regulation of the $CysLT_2$ receptor in inflammatory cells or on the functional role that this receptor may play in these cells. The techniques used in our study cannot determine in every case that it is the same subset of mast cells or mononuclear cells expressing the two receptors,

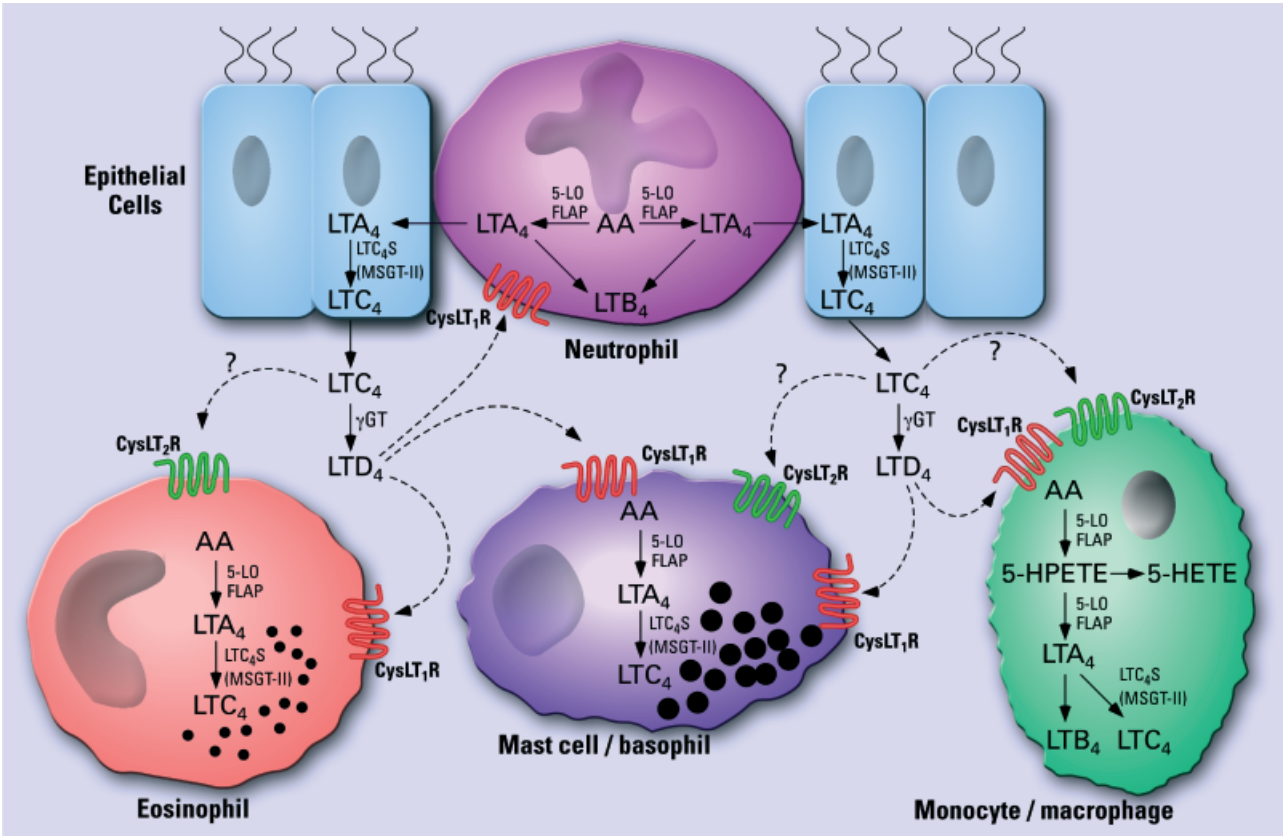

Fig. 6. Summary diagram of CysLT synthetic and signalling pathways in nasal lavage cells from patients with active seasonal allergic rhinitis. Activated neutrophils, eosinophils, mast cells, monocytes and macrophages migrate and adhere to epithelial cells in the nasal epithelium. Activated neutrophils have the capacity to synthesize large amounts of LTA_4, which may be transported to the adherent epithelial cell or to other myeloid cells. Epithelial cells can convert LTA_4 to LTC_4 and this can be actively transported into the blood, where it is converted to LTD_4. LTD_4 can bind with high affinity to $CysLT_1$ and $CysLT_2$ receptors on eosinophils or subsets of mast cells, neutrophils, monocytes or macrophages and potentially activate the release of cytokines, toxins and small-molecule inflammatory mediators. Eosinophils and mast cells independently can produce large amounts of CysLTs for autocrine or paracrine cell activation, resulting in an amplification of inflammation.

but in the case of eosinophils they must be expressed together on the majority of cells.

In the present study, we show that about 40% of neutrophils express the $CysLT_1$ receptor, a result that is discordant with our previous work showing minimal expression of the $CysLT_1$ receptor protein in non-activated peripheral blood neutrophils [7]. $CysLT_1$ receptor mRNA in peripheral blood neutrophils and within the nasal mucosa of obstructed patients has been shown by others to express the $CysLT_1$ receptor protein [8, 27]. We propose that $CysLT_1$ receptor-positive cells may be selectively recruited into sites of inflammation, or that receptor expression is increased by the cytokine milieu in tissues such as allergic nares [40–42].

Nasal lavage fluids from patients with active allergic rhinitis contained numerous epithelial cells presumably dislodged from the mucosa. We were intrigued by the strong expression of LTC4S (or MSGT-II) in nasal epithelial cells. In Fig. 6, we hypothesized the epithelial conversion of neutrophil-derived LTA_4 into LTC_4. Transcellular biosynthesis of leukotrienes has been demonstrated both *in vitro* and *in vivo* [43]. Neutrophils are found in large numbers in nasal lavages, second only to monocytes in number, from patients with active seasonal rhinitis and may contribute to the production of CysLTs via transcellular metabolism of LTA_4 [31–33, 43]. Figure 6 depicts a proposed epithelial cell transcellular CysLT pathway in nasal mucosa. However, other cell types such as endothelial cells may also take part in the transcellular metabolism of LTA_4. CysLTs produced by inflammatory cells may result in autocrine or paracrine activation, depending on whether the cell type synthesizing CysLTs expresses the CysLT receptors. This transcellular metabolism is enhanced as increased numbers of neutrophils penetrate the epithelial layer and the production of LTs results in amplification of the inflammatory cycle.

Here, we show the distribution of the CysLT synthetic and signalling proteins in the key inflammatory cells in active seasonal allergic rhinitis, namely eosinophils, mast cells, neutrophils and mononuclear cells. These cells are also the key inflammatory cells in allergic asthma. The advantage of the seasonal allergic rhinitis nasal lavage model described here is that it allows sampling of these inflammatory cells in patients experiencing active rhinitis symptoms upon natural exposure to airborne allergen. Our study complements recent clinical studies that have shown that the $CysLT_1$ antagonist montelukast has a clinical benefit in this inflammatory disease and has been approved for use in this indication [25, 26]. In addition, our CysLT pathway expression data in active allergic patients support the premise that CysLT-modifying

agents may be of benefit in the treatment of 'one airway' symptoms for patients suffering from concurrent asthma and allergic rhinitis [44–46].

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