expression of ECM proteins in ASM cells following co-culture with eosinophils when compared with medium alone. Interestingly, blocking the activity of cysteinyl Leukotries using antagonists inhibited eosinophil-derived ASM proliferation.
Conclusions: Eosinophils enhances the proliferation of ASM cells. This role of eosinophil does not seem to depend on ASM derived ECM proteins nor on Eosinophil derived TGF-β or TNF-α. Eosinophil seems to induce ASM proliferation via the secretion of Cysteinyl Leukotrienes.

128

Colour Change in the Human Histamine Wheal; a Sign of Desensitized Histamine Vasoconstrictory Receptors

Lars Malm, MD, PhD. *Clinical Science, Lund University, Lund, Sweden.*
Background: The aim was to find the cause and consequences of a colour change in histamine wheals found after ordinary histamine skin prick tests (SPTs) (10 mg/mL). A rapid change to a darker red colour from the 18th and to the 20th minute has been demonstrated by using a digital image-processing technique called LYYN and ImageJ to yield numerical values.[1]
Methods: Repeated histamine SPTs in the middle of the site for earlier performed histamine SPTs in humans. Calculations of the sizes of photographed wheals. Histamine solutions perfused in isolated rabbit ears.
Results: Histamine SPT performed 90 minutes or 6 hours apart from initial histamine SPTs evoked a ring of wheal peripherally around the site of the initial wheal or no wheal at all. The initial wheals had at those times disappeared. Histamine perfusion in isolated rabbit ears indicated first vasoconstriction and after a mean of 17 minutes vasodilatation in post-capillary vessels despite continued histamine perfusion.
Conclusions: The results indicate that total desensitization of histamine-1 receptors in the wheal is the cause of the colour change in human histamine SPTs and that such desensitization lasts long time. If histamine released at allergen provocations also evokes such a long-lasting desensitization and post-capillary vasodilatation it opens new aspects on vascular events in allergic reactions.

REFERENCE

1. Holm O, Lindell E, Malm L. Colour change in the human histamine wheal made visible by LYYN: a technique to enhance colour differences. *Skin Res Technol.* 2010;16:385–389.

129

Involvement of the Hypoxia-Inducible Factor-1 Transcription Complex in the Inflammatory Responses of Human Mast Cells and Basophils

Inna Yasinska, PhD, Vadim Sumbayev, PhD, and Bernhard Gibbs, PhD. *Medway School of Pharmacy, University of Kent, Chatham Maritime, United Kingdom.*
Background: We recently found that hypoxia-inducible factor 1 (HIF-1) plays a crucial role in the pro-allergic functions of human basophils by transcriptional control of energy metabolism via glycolysis as well as directly triggering the expression of the angiogenic cytokine vascular endothelial growth factor (VEGF). Here, we investigated whether there is an overarching principle of HIF-1 involvement in controlling the synthesis of angiogenic and inflammatory cytokines from various human effector cells stimulated by IgE-dependent or innate immune triggers.
Methods: LAD2 human mast cells,[1] primary human basophils, and THP-1 human myeloid cells were used for investigations of FcεRI and Toll-like receptor (TLR) ligand-induced responses. Quantitative real-time PCR, Western blot analysis, ELISA, fluorometry, luminometry and fluorescence microscopy were used to run the assays.
Results: In contrast to basophils, LAD2 mast cells expressed high background levels of HIF-1α, which was largely independent of the effects of stem cell factor (SCF).[2] Both mast cells and basophils expressed TLR2 and 4, albeit weakly compared to THP-1 cells. Cytokine production in mast cells following TLR ligand stimulation was markedly reduced by HIF-1α knock-down in LAD2 mast cells. In contrast, although HIF-1 is involved in IgE-mediated IL-4 secretion from basophils, it was not clearly induced by the TLR2 ligand PGN.
Conclusions: HIF-1α accumulation is fundamentally important for sustaining human allergic effector cell survival and function. This transcription complex facilitates the generation of both pro-angiogenic and inflammatory cytokines in mast cells but has a differential role in basophil stimulation comparing IgE-dependent triggering with innate immune stimuli.

REFERENCES

1. Kirshenbaum AS, Akin C, Wu Y, Rottem M, Goff JP, Beaven MA, et al. *Leuk Res.* 2003;27:677–682.
2. Gibbs BF, Yasinska IM, Oniku AE, Sumbayev VV; *PLoS One.* 2011:In press.

PATHO-BIOLOGY OF NASAL POLYPS

130

Mutiplex Analyses of Cytokine and Chemokine Release From the Cultured Fibroblast of Nasal Polyp: the Effect of IL-17A

Katsuhisa Ikeda, MD. *Otorhinolaryngology, Juntendo University Faculty of Medicine, Tokyo, Japan.*
Background: Nasal polyps of chronic rhinosinusitis (CRS) are characterized by epithelial damage, basement membrane thickness and subepithelial fibrosis. The fibroblast, one of the main cell types making up nasal polyps, is thought to be a target cell of various cytokines. The role of IL-17A in immunoresponse in the nasal poly fibroblast has not yet elucidated.
Methods: Subcultured fibroblasts were established from human polyp biopsy tissues in addition to normal mucosal membranes of sphenoid sinuses (controls).
Results: The IL-17A receptor was expressed at similar levels in all 3 groups. Simultaneous quantification of 27 kinds of cytokines and chemokines in culture supernatants was performed with a human multiplex cytokine assay system. In the eosinophilic group, basal secretion levels of IL-6 were significantly higher than those in the control and non-Eo groups. Basal secretion of MCP-1 in both the non-eosinophilic and eosinophilic groups was also higher than that of the control group. Both IL-9 and G-CSF secretion were remarkably enhanced by IL-17A stimulation in all 3 groups. The receptor-mediated response by IL-17A significantly upregulated IL-6 release alone in the non-eosinophilic and eosinophilic groups as compared with the control group. Only the basic FGF secretion was decreased by stimulation of IL-17A in all groups.
Conclusions: Our results demonstrate for the first time a potentially enhanced secretion of IL-6 and MCP-1 from nasal polyp fibroblasts, and a remarkable upregulation of IL-9 and G-CSF from nasal fibroblasts by IL-17A stimulation, which might contribute to nasal polyp formation and airway remodeling.

131

Expression of Chemokine Receptors CCR1, CCR3, CCR4, CCR5, CCR8 and CXCR3 in Human Nasal Polyps (NP); Comparison With NP From Allergic Patients With Aspirin Intolerance

Petra Fundova, MD,[1] David Funda, MD, PhD,[2] Daniel Kovar, MD,[1] and Helena Tlaskalova-Hogenova, MD, DSc[2]. *[1]ENT Department, Central Military Hospital, Prague, Prague 6, Czech Republic; [2]Immunology & Gnotobiology, Institute of Microbiology, Prague 4, Czech Republic.*
Background: Inflammatory processes play an important role in development of nasal polyps (NP), but the etiology and to a great degree also the pathogenesis of NP is not known. Several cytokines and chemokines such as eotaxin, IL-3, IL-5, IL-6, IL-8, RANTES may influence development of NP

by regulation of migration, activation and survival of the chronic inflammatory cellular infiltrate.

Methods: In this study we investigated expression of selected chemokine receptors in human NP and non-affected human nasal mucosa and carried out a comparison with NP from allergic patients with aspirin intolerance. Biopsies of NP were obtained from 20 patients and 4 patients with NP and aspirin intolerance. Mucosal biopsy specimens of the inferior turbinate were obtained from 12 NP patients and 4 healthy controls. Using indirect imunohistochemistry, frozen tissue sections were stained for CCR1, CCR3, CCR4, CCR5, CCR8 and CXCR3.

Results: Numbers of infiltrating cells expressing CCR3, CCR8 and to a lesser extend also CCR1 were significantly higher in biopsies of NP compared to healthy nasal mucosa. Only a slight increase in CCR5 expressing cells was detected in NP compared to nasal mucosa. No differences in expression of CCR4 and CXCR3 were found in NP compared to nasal mucosa. There were no significant differences between NP of patients with or without aspirin intolerance.

Conclusions: We documented an increased expression of selected chemokine receptors within the cellular infiltrate of NP that may play an important part in the inflammatory pathogenesis of NP.

Supported by grant NS10054 from the IGA MZ, Czech Republic.

132
Characterization of 2 Epithelial Cell Air-Liquid Interface (ALI) Culture Models for Human Healthy Nasal Mucosa and Nasal Polyps

Fco de Borja Callejas,[1,2] Asunción Martínez-Antón, PhD,[1,2] Jordi Roca-Ferrer, PhD,[1,2] Julio Cortijo, MD, PhD,[2] César Picado, MD, PhD,[1,2,3] and Joaquim Mullol, MD, PhD,[2,4,5,6]. *[1]Clinical and Experimental Respiratory Immunoallergy, IDIBAPS, Hospital Clínic, Barcelona, Spain; [2]CIBER of Respiratory Diseases (CIBERES), Palma de Mallorca, Spain; [3]Allergy Unit, Pneumology and Allergy Department, Hospital Clínic, Barcelona, Spain; [4]Clinical and Experimental Respiratory Immunoallergy, IDIBAPS, Barcelona, Spain; [5]Rhinology Unit & Smell Clinic, ENT Department, Hospital Clínic, Barcelona, Spain; [6]Rhinology Unit & Smell Clinic, ENT Department, Hospital Clínic i Universitari, Barcelona, Spain.*

Background: Primary human airway epithelial cells, when submerged in culture, undergo a dedifferentiation with loss of many features of the in vivo airway epithelium. However, when cultured in an air-liquid interface (ALI), cells develop a well-differentiated, polarized, and pseudostratified epithelium. The aim of the current study was to characterize the mucociliary differentiation of human nasal mucosa and polyp epithelial cells cultured using an ALI system.

Methods: Nasal mucosa (NM, n = 3) and nasal polyps (NP, n = 3) were obtained from patients undergoing nasal corrective surgery and endoscopic sinus surgery, respectively. Epithelial cells were obtained from the explant method, and differentiated in ALI culture during 28 days. Cultures were studied at different time points (0, 7, 14, 21, and 28 days): tissue ultrastructure by scanning electron microscopy (SEM) and transmission electron microscopy (TEM); mucous (MUC5AC, MUC5B) and serous (lactoferrin) cell secretion by ELISA; and cytokeratin 18 (epithelial marker), β-tubulin IV (cilia marker), MUC5AC (goblet cell marker), and p63 (basal cell marker) expression by immunocytochemistry.

Results: In both NM and NP ALI cultures and at days 14 and 28, a pseudostratified epithelium with ciliated, mucus-secreting and basal cells was observed, and expression of cytokeratin 18, b-tubulin IV, MUC5AC and p63 was detected. In NP cultures, both MUC5AC (day 14: 2.2 ± 0.1-folds; day 28: 3.6-fold ± 0.7-fold) and MUC5B (day 14: 3.2-fold ± 0.6-fold; day 28: 3.1-fold ± 1-fold) increased over time compared to day 0 ($P < 0.05$). In NM cultures, only MUC5B (day 14: 3.9-fold ± 0.9-fold; day 28: 3.4-fold ± 0.4-fold; $P < 0.05$) but not MUC5AC increased over time compared to day 0 ($P < 0.05$). Secretion of lactoferrin was present but showed no changes over time in either NM or NP ALI cultures.

Conclusions: Epithelial cell ALI cultures provide a well-differentiated human nasal mucosa and polyp tissues that may be used as an in vitro model to study mucin regulation, inflammatory mechanisms of upper airways, and their regulation by antiinflammatory drugs.

133
Corticosteroid Rreatment Reduces Tissue Eosinophilia and the Expression of Matrix Metalloproteinases (MMP-1, MMP-2, MMP-7, MMP-9) and Their Tissue Inhibitor (TIMP-1) in Nasal Polyps

Fco de Borja Callejas,[1,2] Asunción Martínez-Antón, PhD,[1,2] Jordi Roca-Ferrer, PhD,[1,2] Isam Alobid, MD, PhD,[3] César Picado, MD, PhD,[1,2,4] and Joaquim Mullol, MD, PhD,[2,3,5,6]. *[1]Clinical and Experimental Respiratory Immunoallergy, IDIBAPS, Hospital Clínic, Barcelona, Spain; [2]CIBER of Respiratory Diseases (CIBERES), Palma de Mallorca, Spain; [3]Rhinology Unit & Smell Clinic, ENT Department, Hospital Clínic, Barcelona, Spain; [4]Allergy Unit, Pneumology and Allergy Department, Hospital Clínic, Barcelona, Spain; [5]Clinical and Experimental Respiratory Immunoallergy, IDIBAPS, Barcelona, Spain; [6]Rhinology Unit & Smell Clinic, ENT Department, Hospital Clínic i Universitari, Barcelona, Spain.*

Background: Matrix metalloproteinases (MMPs) and their tissue inhibitors (TIMPs) may play an important role in both inflammation and remodeling of nasal polyposis. The aim of the current study was to compare the expression levels of MMPs and TIMP-1 between nasal mucosa and polyps, and to evaluate the effect of corticosteroid treatment in their expression in nasal polyps.

Methods: Nasal mucosa (NM, n = 12) were obtained from patients undergoing nasal corrective surgery while nasal polyp biopsies (NP, n = 33) were obtained from patients before (week 0) and after 2 (week 2) and 12 (week 12) weeks of corticoisteroid treatment (oral prednisone for 2 weeks and intranasal budesonide for 12 weeks). Matrix metalloproteases (MMP-1, MMP-2, MMP-7, MMP-9) and tissue inhibitor of metalloproteinases type 1 (TIMP-1) expression was evaluated by immunohistochemistry in tissue structural cells (epithelium, glands, vessels) and eosinophils.

Results: MMP and TIMP-1 expression were found in the epithelium, glands, vessels (in both NM and NP), and in eosinophils (only in NP). Expression of MMP-7 in epithelium (34% of tissues) and MMP-9 (19%) in glands was lower ($P < 0.05$) in NP than in NM (78 and 67%, respectively). Corticoisteroid treatment reduced tissue eosinophilia (Eos/5 fields) at week 2 (8.0 ± 2.9, $P = 0.001$) and week 12 (10.0 ± 2.3, $P < 0.003$) compared to week 0 (25.5 ± 8.4); and also decreased the expression of MMPs and TIMP-1 in eosinophils at week 2 and week 12 compared to week 0 ($P < 0.05$). In the epithelium, corticosteroids increased MMP-7 and TIMP-1 at week 2 and week 12, while decreased MMP-9 at week 12 ($P < 0.05$). In vessels, corticosteroids increased MMP-9 at week 2 and decreased MMP-1 at week 12 ($P < 0.05$). No effects were found in the glands.

Conclusions: Treatment of nasal polyposis with corticosteroids reduces both tissue eosinophilia and MMP expression in eosinophils while modifying the expression of remodeling markers in nasal polyp structural cells.

134
Expression and Localization of CYSLT2 Receptor in Human Nasal Mucosa

Shirasaki Hideaki, MD, PhD,[1] Etsuko Kanaizumi, MD, PhD,[1] Manabu Fujita, PhD,[2] and Tetsuo Himi, MD, PhD[1]. *[1]Otolaryngology, Sapporo Medical University, Sapporo, Japan; [2]Minase Research Institute, Ono Pharmaceutical, Osaka, Japan.*

Background: We have previously reported the localization of CysLT1 receptor by using immunohistochemistry and in situ hybridization (Shirasaki H et al. *Clin Exp Allergy*. 2002;32:1007–1012).

Methods: To clarify the expression of CysLT2 receptor in human nasal mucosa, we investigated CysLT2 receptor mRNA expression and its protein