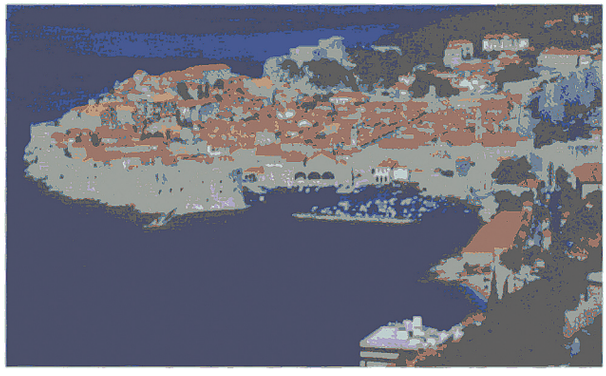
ABSTRACT BOOK



7th Symposium on Experimental Rhinology and Immunology of the Nose (SERIN)

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EAACI

ABSTRACT

1

IMMUNOLOGIC EVALUATION OF PATIENTS WITH RECURRENT EAR, NOSE AND THROAT INFECTIONS

Sina Abdollahzade,[1] Asghar Aghamohammadi,[1] Alireza Karimi,[2] Mohsen Naraghi[2] and Amir Arvin Sazgar[2]

[1]Department of Pediatrics, Children's Medical Center Hospital, Immunology, Asthma and Allergy Research Institute
[2]Department of Ear, Nose and Throat,
Medical Sciences/University of Tehran, Tehran, Iran

In this study, we aimed to evaluate the frequency of possible underlying immunodeficiency responsible for susceptibility to ear, nose, and throat (ENT) infection.

One hundred and three (72 males and 31 females) consecutive children and adult patients with history of recurrent or chronic ENT infections, referred by otolaryngologists to the Department of Allergy and Clinical Immunology, Children's Medical Center, Tehran University of Medical Sciences (Tehran, Iran), were enrolled to the study from March 2003 to March 2006. For each patient, demographic information and medical histories of any ENT infections were collected by reviewing the patient's records. We measured immunoglobulin isotype concentrations and immunoglobulin (Ig) G subclasses by nephelometry and enzyme-linked immunosorbent assay methods, respectively. Of 103 patients, 75 received unconjugated pneumococcus polyvalent vaccine, and blood samples were taken before and 21 days after vaccination. Specific antibodies against whole pneumococcal antigens were measured using enzyme-linked immunosorbent assay method. Existence of bronchiectasis was confirmed in each patient using high resolution computed tomography scan.

Among 103 patients, 17 (16.5%) patients were diagnosed to have defects in antibody-mediated immunity including 6 patients with immunoglobulin class deficiency (2 common variable deficiency and 4 IgA deficiency), 3 with IgG subclass deficiency (2 IgG2 and 1 IgG3), and 8 with specific antibody deficiency against polysaccharide antigens. In our series, bronchiectasis was detected in 5 cases associated with primary immunodeficiency.

Long-standing history of ENT infections could be an alarm for ENT infections associated with primary antibody deficiency.

2

UPPER-LOWER AIRWAY INTERACTION - MEASURED BY LOCAL AND SYSTEMIC INFLAMMATORY PARAMETERS (INFLAMMOMETERS) IN ADULT ASTHMA AND RHINITIS

Sonja Badovinac, Andrea Vukić-Dugac, Dijana Krmpotić, Bojana Butorac-Petanjek, Marta Koršić and Sanja Popović-Grle

University Clinic for Lung Diseases Jordanovac, Zagreb, Croatia

Close association and significant coexistence between allergic asthma and rhinitis is already defined. The airway inflammation of both diseases has the same immune response path with the similar inflammatory parameters involved. NO is one of the parameters of local inflammation involved in both allergic rhinitis and asthma. ECP is the parameter of systemic inflammation, while FEV1 is parameter of bronchial obstruction. The objective of this retrospective study was to investigate if there is a difference in local and systemic inflammation level as well as in bronchial obstruction level in asthma patients with coexisting rhinitis compared with those with asthma alone.

The level of FeNO, FEV1 and ECP was evaluated in two groups of patients - allergic asthma and allergic asthma with rhinitis. All patients were examined by ENT specialist and pulmonologist.

The total number of 47 patients was analyzed, 33 patients had coexisting allergic asthma with rhinitis and

15 patients had allergic asthma alone. In this study there was no difference found in levels of FeNO, FEV1 and ECP in evaluated groups.

In this retrospective study the nasal inflammation did not affect the level of inflammatory - and bronchial obstruction - parameters in asthma patients.

Inflammatory response could have been modified with treatments, smoking status and phase of the disease (exacerbation, stabile phase) and therefore further prospective studies should follow.

3

STUDY OF A LONG-TERM EFFECT OF TOPICAL CAPSAICIN TREATMENT IN NASAL POLYPOSIS

Tomislav Baudoin,[1] Hrvoje Ćupić,[2] Josip Hat[3] and Livije Kalogjera[1]

[1]Department of Otorhinolaryngology and Head and Neck Surgery, [2]Department of Radiology and [3]Department of Pathology, Sestre Milosrdnice University Hospital, Zagreb, Croatia

The aim of this study was to estimate efficacy of topical capsaicin treatment in patients with nasal polyposis during a 2-year period.

Twelve non-allergic patients with massive sinonasal polyps who were not willing to undergo surgical treatment were included in this study. Six male and 6 female, 7 previously operated, all after ineffective topical steroid treatment. For 3 consecutive days the patients received 0.5 ml 30 mumol/l capsaicin solution sprayed in each nostril, and 100 mumol/l of capsaicin solution on days 4 and 5. The treatment scheme was repeated every 3 months during a 2-year period.

The questionnaire was completed right before the first application, a month after the first application, and every 3 months during a 2-year period. Scored symptoms were following: congestion, postnasal drip, nasal secretion, headache, sneezing, cough, facial swelling and olfaction. Symptoms were scored for intensity from 0 to 3, and for frequency from 0 to 4. Punch biopsies of the polyps were performed before the application, half an hour after last application and 3 weeks later. Specimens were stained with HE and Giemsa, and immunohistochemically with IgE and IgA monoclonal antibodies. Control specimens were nasal polyps from the operated patients who were not treated with capsaicin. The correlation between some histopathological parameters (IgE, IgA, mastocyte and eosinophil infiltration) and subjective scores was analyzed. Conservative treatment of a massive nasal polyposis with a repeated topical capsaicin application during a 2-year period was estimated.

Eight patients had a statistically significant improvement in total symptom scores and in majority of single scores during the observed period. Three patients underwent surgical procedure (FESS) because of a poor improvement after capsaicin therapy. One patient was lost to control.

Topical capsaicin treatment was found to be an efficient therapy in the majority of treated patients according to selection criteria in this study, and could be a substitute if other medical or surgical treatment was found inefficient. Because of the small number of patients we are not able to make a conclusion about a possible impact of the observed histopathological parameters on subjective symptom scores.

4

IL-8 PRODUCTION AND APOPTOSIS OF NASAL EPITHELIAL CELLS IN RESPONSE TO DEXAMETHASONE

Sonja Bobic,[1] Ina Callebaut,[1] Valerie Hox,[1] Jan L. Ceuppens,[1] Cornelis M. Van Drunen,[2] Wytske J. Fokkens[2] and Peter W. Hellings[1]

[1]Laboratory of Experimental Immunology, Catholic University Hospitals, Leuven, Belgium [2]Academic Medical Center, Amsterdam, The Netherlands

IL-8 production by epithelial cells represents one of the key features of chronic airway inflammation as seen in chronic rhinosinusitis with/without nasal polyps. The immunologic regulation of IL-8 production and the effects of the anti-inflammatory drug dexamethasone on IL-8 production by epithelial cells remain unknown.

Nasal epithelial cells (NEC) were freshly isolated from nasal mucosa of patients with NP (n=12) and from nasal mucosa of healthy donors (n=19). 10^5 epithelial cells were incubated with IL-1â (10ng/ml), TNF-á (10ng/ml), dexamethasone (10, 100, 1000 μg/ml) or combinations. After 24h, IL-8 levels were determined in supernatants of epithelial cells by ELISA. Finally, hNECs isolated from healthy nasal mucosa and incubated with increasing doses of dexamethasone were stained with trypan-blue and annexin-FITC/PI to estimate the degree of apoptosis.

NECs from healthy and nasal polyp patients produced equal levels of IL-8 upon IL-1â stimulation with

TNF-á having similar, but less potent effects in both groups. Dexamethasone did not alter IL-8 production by epithelial cell stimulated with both inflammatory cytokines. Dexamethasone appeared to induce a dose-dependent IL-8 production by epithelial cells at the low dose, with the induction of apoptosis of epithelial cells at the highest doses.

IL-8 production by NECs does not differ between epithelial cells of NP and healthy individuals under baseline conditions or after stimulation. Despite its anti-inflammatory effect, dexamethasone induces the secretion of IL-8 from nasal epithelial cells in a dose-dependant fashion, with abrogation of IL-8 production at high dose due to the induction of apoptosis in NECs.

5

ROLE OF VEGF AND PLGF IN ALLERGIC AIRWAY INFLAMMATION

Sonja Bobic,[1] Vanessa De Vooght,[2] Ina Callebaut,[1] Valerie Hox,[1] Jan L. Ceuppens[1] and Peter W. Hellings[1]

[1]Laboratory of Experimental Immunology
[2]Laboratory of Pneumology
Catholic University Hospitals, Leuven, Belgium

VEGF-A (vascular endothelial growth factor-A) has been implicated in upper airway diseases such as allergic rhinitis and chronic rhinosinusitis with nasal polyps. The role of other members of VEGF family such as PlGF (placental growth factor) in upper airway diseases has not yet been evaluated. The aim of this study is to estimate the role VEGF and PlGF in a mouse model of allergic airway inflammation.

In the mouse model of experimental asthma expression levels of VEGF, PlGF and their receptors were determined by real time RT-PCR in bronchial and nasal tissue and ELISA in BALF, NLF and/or lung homogenates. Differential white blood cell counts were performed on BALF. Bronchial responsiveness to metacholine was determined using Flexivent technique. Additionally, effect of neutralizing PlGF on the outcome of airway inflammation was evaluated.

Constitutive expression of VEGF was relatively higher in the nose than in bronchi (NLF: 395,8±186,2 vs BALF: 221,8±47,8 pg/ml, P=0,55) whereas PlGF was undetectable in both lavages. Surprisingly, VEGF and its receptors were downregulated in bronchi of mice with experimental asthma while their levels remained unaltered in nasal tissue. In contrast, PlGF levels were strongly increased in bronchi of OVA-allergic mice (65,4±6,8 pg/mg) compared to controls (17,8±1,2 pg/mg, p<0,005). PlGF neutralization reduced airway hyperresponsiveness (24,4±3,9 to 18,9±0,9, P=0,25) and the number of BAL-infiltrating neutrophils in mice with experimental asthma.

Unlike VEGF, PlGF is upregulated in bronchi of mice with experimental asthma and may account in part for the influx of neutrophils and concomitant airway hyperresponsiveness.

6

ALLERGIC RHINITIS AND ASTHMA IN CHILDREN

Katerina Boškovska, Jane Buzarov, Slavica Kostadinova, Lidija Petruševska and Marija Maneva

Institute for Respiratory Diseases in Children, Kozle, Skopje, FYR Macedonia

Allergic rhinitis and asthma often coexist in the same patients. The aim of the study was to show the prevalence of allergic rhinitis (AR) in asthmatic children.

In our institution 115 children with astma were examined. 56,5% were male, mean age 8.3 yrs. Intermittent asthma was present in 30.9% of patients, 44.,8% had a mild persistent, and the others had a severe persistent asthma. Diagnosis of AR was based od anamnestic data, skin prick test, main symptoms and ORL status.

AR was displayed in 68.7% of children. Intermittent rhinitis with moderate-severe degree was demonstrated in 14 of them, the others had persistent AR and in 41.5% of them it was with a moderate to severe degree. Nasal symptoms preceeded asthma in 65.8% of children. Dermatophagoides pteronyssinus sensitivity was confirmed in 2/3 of the patients with skin prick test. Over 80% of them were with persistent AR and asthma. Pollen sensitivity had 20.9% and all of them were with intermittent AR. Dominant symptom in 68.3% of the patients was nasal blockage, most of them with Dermatophagoides sensitivity. In this group also concomitant sinusitis was present. Sneezing and rhinorrhea were main symptoms in 22.8% patients, all of them with pollen sensitivity. Most of these children also had associated conjuctivitis.

The study confirmed that there is a strong association between allergic rhinitis and asthma in children.

7

THE EFFECTS OF *STAPHYLOCOCCUS AUREUS* ENTEROTOXIN B ON GRANULOCYTE CHEMOTAXIS AND SURVIVAL THROUGH THE ACTIVATION OF EPITHELIAL CELLS

Ina Callebaut I,[1] Kristien Reekmans,[1] Sonja Bobic,[1] Greet Hens,[1] Dominique Bullens,[1] Jan Ceuppens,[1] Claus Bachert[2] and Peter W. Hellings[1]

[1]Division of Clinical Immunology, Katholieke Universiteit Leuven, Leuven
[2]Department of Otorhinolaryngology, University Hospital Ghent, Belgium

The pathophysiologic role of Staphylococcus aureus in nasal polyps and allergic airway inflammation remains incompletely understood. The immunologic interaction between Staphylococcus aureus enterotoxin B (SEB) and airway epithelial cells remains elusive so far. The aim of this study relates to the immunologic interaction between SEB and epithelial cells and the resulting granulocytic inflammation.

Human nasal epithelial cells were freshly isolated from nasal turbinates of healthy, non-allergic individuals, and incubated for 24 h without/with different doses of SEB. The supernatans were analyzed for chemokines and growth factors related to airway inflammation using ELISA. The capacity of the supernatans to attract granulocytes was studied in vitro using a Boyden chamber. FACS analysis for annexin and propidium iodide expression in these granulocytes was used for evaluation of their survival.

SEB showed a dose-dependent induction of IP-10, MIG, RANTES, MCP-1 and G-CSF production by human airway epithelial cells. Supernatans of SEB-stimulated nasal epithelial cells attract significantly more granulocytes (13,73 ± 0,5745) than the unstimulated supernatants of epithelial cells (11,54 ± 0,806; $p<0,0001$). There is a trend towards reduced expression of propidium iodide in granulocytes cultured with SEB-stimulated supernatans from epithelial cells.

Staphylococcus aureus enterotoxin B exerts a direct pro-inflammatory effect on human nasal epithelial cells, with the induction of chemokine and growth factor release. In this way, SEB may contribute to the granulocytic inflammation in chronic airway disease.

8

ALLERGIC RHINITIS AND BRONCHIAL ASTHMA, A SINGLE DISEASE?

Gabriela Ramona Cioaca, Miltiade Staicus and Virgil Paunescu

University of Medicine and Pharmacy Victor Babes Timisoara, Romania

Rhinitis and asthma commonly coexist in allergic patients, both being the opposite poles of a single disease affecting the respiratory system, and an impaired systemic immune response to allergens. The aim of study was to evaluate the prevalence of allergic rinithis in asthmatic patients.

Study group consisted of 220 chronic coughers (1.8 to 71 years old), evaluated for pulmonary function and skin prick tests for the main aeroallergens (pollens, mites, dander and molds - HALCIS).

Asthma was assessed in 76 (37%) cases, 52 (25%) extrinsic and 24 (12%) intrinsic forms. In 43 asthmatics, allergic rhinitis was associated. Bronchial hyperreactivity was assessed in 43 asthmatics. Allergy was positive in 119 (61.3%) cases, especially polysensitiveness, most frequent allergens being mites, molds and pollens.

There is a high prevalence of allergy in asthmatics (especially indoor allergens) and a high association with allergic rhinitis. Their coexistence requires a complex treatment addressing to all pathogenic chains, with a longer duration, as well as clear primary prophylaxis measures against allergens.

9

CLINICAL AND IMMUNOLOGICAL EVOLUTION OF NON-ALLERGIC RHINITIS

Inmaculada Doña Diaz,[1] Carmen Rondon Segovia,[1] María José Torres Jaén,[1] José Juan Romero Andreu,[1] Dolores Ruiz Ros,[1] José Luis Rodríguez-Bada[2] and Miguel Blanca Gómez[1]

[1]Allergy Service, Carlos Haya Hospital, Malaga
[2]Research Laboratory, Carlos Haya Hospital-Fundacion IMABIS, Malaga, Spain

Non-allergic rhinitis (NAR) represents a significant number of patients in our daily clinical practice. In most cases, symptoms persist over time, and since an allergic mechanism has been ruled out, the majority of these patients would not be followed. The aim of the study

was to do a follow-up of subjects diagnosed with NAR in our clinic four to seven years before.

A representative sample of NAR patients diagnosed during 2000-2004 were randomly selected from a nameless database and re-evaluated. All subjects had rhinitis symptoms and negative skin prick test (SPT) and specific IgE at the moment of the first visit. Clinical questionnaires, skin prick test, total and specific-IgE to aeroallergens and spirometry were performed in all subjects.

One-hundred and fifty patients were followed, mean age was 43.79 years, 61% female, non-smoking (75%), with family history of atopy (31%) and city dwelling (60%). According to ARIA criteria 88% had persistent symptoms and 12% intermittent. Hyposmia was present in 15% of subjects. Re-evaluation of NAR patients showed sensitization to aeroallergens in 24% of subjects detected by SPT and/or specific-IgE. Severity of symptoms increased in 52% of subjects. New co-morbidities occurred in 25% of patients (18% asthma, 17% conjunctivitis, 5% sinusitis, 4% nasal polyps and 3% aspirin sensitization).

Sensitization to aeroallergens and an increase of comorbidities and symptom severity may occur in NAR patients over time. A re-evaluation of NAR may be useful in order to diagnose and treat these patients properly.

10

SUBCLINICAL INVESTIGATIONS BY MEDICAL MYCOLOGISTS WITHIN HUMAN HABITATIONS BENEFICIAL IN THE CASE DIAGNOSIS OF CHRONIC RHINOSINUSITIS WITH NONDESCRIPT CAUSE

Josef Dumanov,[1] Dennis Hooper,[1] Ritchie Shoemaker[1] and Michael Rudenko[2]

[1]Mycological Institute for the Study of Fungal Mold in Human Habitations, NJ, USA
[2]Clinical Center "EuroDon", Rostov-on-Don, Russia

Mycological Institute was contacted by Liana J. 48-year-old woman. She had a history of a progressive chronic fatigue and skin rash, headache, severe upper respiratory congestion and blurry vision, she had been coughing and sneezing while staying in her office. According to her history, number of clinical examinations and laboratory was used: blood test, urine test, immunologic tests, total and specific IgE, prick tests. Total IgE was increased (247 kU/ml) but all specific IgE tests were negative (Mucor, Alternaria and Cladosporum), skin prick tests were negative (including two fungal allergens Alternaria and Cladosporum).

It was recommended that the client relocate temporally fromthe place where the suspect mold-like appearance was present. To find specific reason of her symptoms a subclinical investigation was conducted and inspection by medical mycologists of the office: inside and on the ceiling and walls we found numerous colonies of dark green and black color fungi. A building had suffered water damage and possessed an odor. The office also had a mold-like appearance on ceilings and wall. Surface and airborne samples were collected and taken for identification by medical mycologists. In those samples spores and hyphae of Aspergillus's fumigatus were identified. The mycologists' findings and review of pathology slides identifying this fungi present. First morning urine collection detected mycotoxins of Aspergillus including Ochratoxin, aflatoxin and tricothecenes as confirmed by toxicological assay. This is an example how subclinical indoor environmental investigations by medical mycologists can benefit to clinical practice. This investigation helped to make a correct diagnostics.

11

EFFICIENT PURIFICATION OF EOSINOPHILS FROM HUMAN TISSUES: A COMPARATIVE STUDY

Alon H. Nissim Ben Efraim, Ariel Munitz, Francesca Levi-Schaffer and Ron Eliashar

Department of Pharmacology, School of Pharmacy and Department of Otolaryngology/Head and Neck Surgery, The Hebrew University School of Medicine, Hadassah Medical Center, Jerusalem, Israel

Eosinophils (EO) are key effector cells in allergy and in other inflammatory diseases. Although they carry out their function in the tissues, most data concerning their biology was obtained from peripheral blood EO. No efficient method exists that allows consistent purification of tissue EO for culture studies.

To isolate eosinophils from human polyp tissue.

Human nasal polyp tissue was obtained from patients undergoing polypectomy. The tissues were enzymatically digested and mesh-filtered. The filtrate was subjected to three methods of purification: (1) positive magnetic selection (PS) by CCR3, (2) or negative magnetic selection (NS) by CD3/CD14/CD16. Cell yield

and purity were evaluated by FACS analysis. Tissue EO were cultured in the presence of either IL-3, IL-5 or GM-CSF and their survival was evaluated by FACS analysis (PI). Surface receptor analysis was performed by FACS.

PS of CCR3+ cells consistently yielded highly pure (>95%) EO but at 80% viability. In contrast, NS yielded viable cells that were less pure (75-95%).

We showed that NS of CD3+/CD14+/CD16+ cells is an efficient and consistent method for human nasal polyp EO purification suitable for culture studies.

12

FUNCTIONAL ENDOSCOPIC SINUS SURGERY (FESS) IMPROVED ASTHMA SYMPTOMS AS WELL AS PEFR AND OLFACTION IN PATIENTS WITH NASAL POLYPOSIS

Anders Ehnhage, P. Olsson, Karl Gustav Kölbeck, Maria Skedinger, Barbro Dahlén, M. Ålenius and Pär Stjärne

Department of Clinical Science, Intervention and Technology, Division of Otorhinolaryngology, Karolinska Institute, Stockholm, Sweden

Nasal polyposis is a disease known to be associated with asthma. The management is anti-inflammatory, with topical and oral corticosteroids as the first-line treatment. The effect of surgical treatment on lower airway inflammation has not been sufficiently studied.

The aim of the study was to investigate the effects of FESS as well as fluticasone proprionate nasal drops (FPND) 400 micrograms b.i.d. on nasal and lower airway parameters in patients with nasal polyposis and asthma.

This was a prospective 21-week study of 68 patients with asthma and nasal polyposis, on the benefits of FESS on nasal (butanol test, subjective olfaction, PNIF (peak nasal inspiratory flow, congestion, rhinorrhoea and polyp score) and on lower airways parameters (dyspnoea, cough, mean daily PEFR, and lung function tests). It also included a randomized, double-blind, placebo-controlled 14 weeks phase on FPND.

FESS significantly improved mean asthma symptom scores and daily PEFR as well as all nasal parameters including subjective and objective olfaction tests. This is the first study that shows the benefits of FESS on butanol tests in patients with nasal polyposis. We found no statistically significant differences between topical treatment with FPND or placebo in the nasal or lower airways variables.

FESS improved nasal as well as asthma symptoms in patients with nasal polyposis. FESS could be considered early in the natural course of nasal polyposis with concomitant asthma, as well as a second-line treatment in nasal polyposis patients with a reduced sense of smell. The potential benefits of FPND 400 micrograms b.i.d. were probably overshadowed by FESS.

13

VASOACTIVE INTESTINAL PEPTIDE IS A POTENT PRIMER FOR MAST CELL RECRUITMENT TO THE ALLERGIC AIRWAY: A NOVEL BIOPHYSICAL ASSOCIATION OF F-ACTIN

Amr El-Shazly

Department of Oto-Rhino-Laryngology and Head and Neck Surgery,
Liege University Hospital, Liege, Belgium

The novel neuro-immuno-cooperation between vasoactive intestinal peptide (VIP) and fracktalkine (FKN) in recruiting human mast cells to the allergic airway demonstrated a classical example of VIP induced priming of mast cells to FKN induced mast cell chemotaxis. However, little is known about the role of the cytoskeletal element, the F-actin in this scenario.

Mast cell (HMC-1) chemotaxis and chemokinetics were performed in 48-well microchemotaxis chambers. Changes in HMC-1 intracellular calcium concentration were assessed using the Ca2+-sensitive probe indo-1 (calbiochem). As for Actin reorganization and content assessment, after each challenge, HMC-1 were fixed in cold methanol for 20 minutes and permeabilized with 0.1% saponin (Sigma-Aldrich) for another 30 minutes. The cells were then stained with phalloidin-FITC (Sigma=Aldrich) labeled for 1 hour in the dark, in ice, and analyzed by FACS or confocal microscopy.

In the present study, it is shown that VIP primed human mast cells to exaggerated migratory response to FKN through calcium independent change in the signal transduction that is linked to a novel physical F-actin intracellular reorganization.

VIP is a potent primer for human mast cell chemotaxis that highlights the important role of this neuropeptide in promoting allergic inflammation. Therefore, an-

tagonists at the level of cell receptors may provide a therapeutic target for F-actin in treating mast cells in allergic inflammation.

14

SKIN PRICK TESTING - HELP IN TREATMENT OF CHRONIC ALLERGIC RHINOSINUSITIS?

Mohamed Eletriby, Ola Mohamed and Ali Hamed

El Mansoura Hospital, Egypt

Allergic rhinosinusitis (AR) has been a major health problem for most of the population. It does affect 35%-50% and may be accompanied with other allergic diseases. The prevalence of AR has been increasing. The principle features of AR are nasal itching, sneezing, watery rhinorrhea, nasal obstruction and sometimes eye redness. Skin prick testing is usually the first test recommended when an allergy is suspected.

Testing was done at El-Mansoura Hospital over 3 months and followed up from January 2004 to December 2005. Skin prick tests were done showing a positive result of 100 patients. Skin prick test concerned house dust mite, grass, cat, dog, cotton, wool, cockroach, tobacco, HD & also some foods as milk and maize.

Skin prick test has shown a positive result in 100 patients - 43% males and 57% females.

From the patients with a positive skin test (100), 34 have never received a previous anti allergy treatment, 32 received a single nasal steroid, and 14 had tried many nasal steroids. Twelve patients tried an antihistamine alone. Another 8 had tried both antihistamines and nasal steroids.

The most common allergens in our study were house dust mite, grass, cat, dog and cotton dust, wool, cockroach, tobacco, HD and maize.

Skin prick test can be taken as a reliable test, according to this study, to diagnose allergy, and for sure this would be more beneficial for securing the right treatment.

15

DETECTION OF INKT CELLS IN THE NASAL SECRETION OF ALLERGIC RHINITIS PATIENTS

Mohammad Fereidouni, Farahzad Jabbari Azad, Reza Farid Hossini, Abdol Reza Varasteh and Lena Al-Harthi

Immunology Research Center, Mashhad, Iran - Rush Medical College, Chicago, USA

Allergic rhinitis is the most common allergic disorder around the world and characterized by inflammation of nasal mucosa. Many studies revealed the importance of local immune response rather than systemic responses which lead to migration and accumulation of inflammatory cells. Recently detection of iNKT cells in bronchoalveolar lavage of asthmatic patients was reported. Because of similarities between asthma and allergic rhinitis the aim of this study was to evaluate the presence of iNKT cells in nasal secretion of allergic rhinitis patients.

Nasal secretions were taken from 9 patients with allergic rhinitis during active phase of rhinitis. Samples examined for the presence of iNKT cells by RT-PCR using primers for Va24 and Vb11 genes which are specific for natural killer t-cell receptor, as well as CD3 and GAPDH. The relative expression of genes was measured by densitometry.

Both va24 and Vb11 genes were detected to varying degree in nasal secretion. There was not significant difference between va24 and Vb11 but the ratio of iNKT TCR specific genes to CD3 gene was vary among samples and at least two samples showed a high rate iNKT TCR genes expression.

Our study shows that iNKT cells may play important role in the pathogenesis of allergic rhinitis by secreting Th2 cytokines and increased infiltration of inflammatory cells to the nasal mucosa.

16

EFFECTS OF PROINFLAMMATORY STIMULI ON GLUCOCORTICOID RECEPTOR ACTIVATION IN AIRWAY FIBROBLASTS

Laura Fernandez-Bertolin,[1] Laura Pujols,[1] Mireya Fuentes,[1] Isam Alobid,[2] Cristina Embit,[2] Jordi Roca-Ferrer,[1] Joaquim Mullol[2] and Cesar Picado[2]

[1]IDIBAPS, Hospital Clinic, Barcelona and [2]Hospital Clinic, Barcelona, Spain

Poor response of nasal polyps to glucocorticoids (GC) may be due to abnormal glucocorticoid receptor (GR) function. The aim of this study was to evaluate the effect of dexamethasone (Dex) on GR translocation kinetics, function and expression, and its modulation by proinflammatory stimuli.

Primary lines of fibroblasts were obtained from healthy nasal mucosa (NM, N=12) and from nasal polyp (NP, n=12) tissues from asthmatic patients. LPS (10 mg/ml), cytomix (IL-1 b), TNF a, and IFN g (10 mg/ml each) or culture medium was added 24h previous to Dex stimulation. Dex (100 nM) was added for 1, 2 or 3 hours for the translocation study and results were analysed by fluorescent immunocytochemistry. Total GR expression was analysed by Western Blot and GR function was analysed by ELISA. Data is shown as median and 25th-75th percentile.

At 1h, Dex induced maximal GR translocation in both NM and NP which remained significantly higher at 3h. Only in NP fibroblasts, LPS increased significantly GR translocation at every time point studied compared to cells treated with Dex alone. Incubation with cytomix did not alter GR translocation. LPS or cytomix exposure did not alter GR expression. LPS increased IL-6 production in both NM and PN fibroblasts which was inhibited by Dex in a dose-dependent manner in both cell types.

The finding of a greater GR translocation after LPS stimulation in fibroblasts from NP, suggests that the underlying inflammation present in NP may render these fibroblasts more susceptible to Dex-induced GR translocation when submitted to additional inflammatory insult.

17

CAN HISTOLOGICAL FINDINGS PREDICT SURGICAL OUTCOME IN PATIENTS OPERATED FOR NASAL POLYPS?

Marko Velimir Grgić,[1] Tomislav Baudoin,[1] Hrvoje Ćupić[2] and Mirjana Kujundžić Tiljak[3]

[1]Department of Otorhinolaryngology and Head and Neck Surgery, Sestre Milosrdnice University Hospital, Zagreb
[2]Department of Pathology, Sestre Milosrdnice University Hospital, Zagreb
[3]Andrija Štampar School of Public Health, Zagreb, Croatia

Nasal polyposis is known for its tendency to recur after operation. Clinical experience shows wide spectrum of outcomes: some patients are "cured" with one operation, while others require several operations due to rapid recurrence. The aim of this study was to determine whether IL-5, VEGF, IgE and eosinophil count in operated specimen can predict clinical outcome in operated patients.

Thirty patients with nasal polyps have been included. Every patient had preoperative endoscopic and CT staging and subjective score (SS) questionnaire. Removed polyps have been analyzed histologically for eosinophil count and immunohistochemically (IHS) for IL-5, VEGF and IgE positivity. Patients have been followed up for one year, by nasal endoscopy and questionnaire. The pathohistology and IHS score were correlated with clinical outcome.

One year postoperatively 19 patients had no recurrence, 3 had miminal recurrence (Malm 1), 6 moderate (Malm 2) and 2 patients had gross recurrence (Malm 3). SS correlated with clinical score, so patients with no clinical recurrence had best SS (average 88% improvement) and patients with recurrent polyps had average SS improvement of 36%. When correlating pathohistology and IHS score with clinical improvement, patients with higher IL-5, IgE, and eosinophil score had more recurrences and lesser SS improvement. VEGF did not show such a negative correlation with SS or clinical improvement.

This study leads to several conclusions: 1. The applied SS questionnaire is a valuable tool for assessment of postoperative improvement and nicely correlates with clinical endoscopic evaluation; 2. Though not statistically significant, some histologic and IHS parameters could predict clinical outcome after surgical treatment; 3. larger group of patients and different cut-off points for IHS scoring could improve predictive value of those scores.

18

CORRELATION BETWEEN SUBJECTIVE AND OBJECTIVE PARAMETERS OF NASAL POLYPS

Valerie Hox,[1,2] Ina Callebaut,[1] Ellen Dillissen,[1] Jan L. Ceuppens[1] and Peter W. Hellings[1,2]

[1]Laboratory of Experimental Immunology and [2]Department of Otorhinolaryngology, Head and Neck Surgery
University Hospitals, Faculty of Medicine, University of Leuven, Leuven, Belgium

Chronic rhinosinusitis with nasal polyposis (NP) represents an invalidating disorder causing mainly nasal blockage and loss of smell. Little is known about correlation between the severity of symptoms and objective parameters of disease.

The aim was to investigate the correlation between the extent of NP and subjective and objective parameters of disease.

Sixty five patients with NP were evaluated for the size of NP using a 0 to 10 scoring system, and were asked to score nasal blockage and loss of smell on a VAS scale. In addition, peak nasal inspiratory flow (PNIF), Sniffin Sticks (SS), smell tests, and blood analysis for eosinophilia and total IgE were performed.

NP scores using nasal endoscopy correlated with VAS scores for nasal blockage ($p<0.01$) but not with VAS scores for loss of smell. VAS scores for nasal blockage correlated inversely with PNIF values ($p<0.0001$), as was the case with VAS scores for smell dysfunction and SS scores. Therefore, NP scores showed an inverse correlation with PNIF values and SS scores (both $p<0.05$). NP scores failed to correlate with systemic parameters of disease severity like blood eosinophilia and total IgE levels. Of note, blood eosinophilia correlated with smell reduction ($p<0.05$) whereas this wasn't the case for total IgE.

The extent of NP disease evaluated with nasal endoscopy correlated with the reduction of PNIF and SS scores, but did not correlate with blood eosinophilia and total IgE. Concerning the subjective parameters, we found only a correlation between NP scores and VAS for nasal blockage.

19

STAPHYLOCOCCUS AUREUS ENTEROTOXIN B FACILITATES ALLERGIC SENSITIZATION

Wouter Huvenne,[1] Ina Callebaut,[2] Bert Verbinnen,[2] Greet Hens,[2] Jeroen Vanoirbeek,[3] Paul Van Cauwenberge,[1] Phillipe Gevaert,[1] Claus Bachert,[1] Jan L. Ceuppens[2] and Peter W. Hellings[2]

[1]ENT-Department, Ghent University Hospital, Upper Airway Research Laboratory, Ghent
[2]Laboratory of Experimental Immunology, UZ Gasthuisberg, Leuven,
[3]Department of Lung Toxicology, University Hospitals, Faculty of Medicine, Leuven, Belgium

Staphylococcus aureus Enterotoxin B (SEB) has immunomodulatory effects in allergic airway disease. However, little is known about the potential contribution of SEB to the sensitization process in allergic airway disease.

Mice (BALB/c) received repeated nasal application of OVA or saline and/or SEB or saline. On d 13, 4 groups of mice (Sal/Sal, Sal/SEB, OVA/Sal, OVA/SEB) were sacrificed for evaluation of OVA-specific IgE in serum, bronchial inflammation in bronchoalveolar lavage fluid (BALF) and in bronchial tissue on H&E stained histologic sections and for evaluation of cytokine production by peribronchial lymph node (PBLN) cells in vitro. Airway responses to inhaled metacholine (MCh) were measured using a forced ventilation technique.

A significant bronchial inflammation with cellular influx of mainly eosinophils and lymphocytes was found in OVA/SEB mice. Sensitization to OVA occurred only in mice where SEB had been applied in the nose at the time of OVA exposure, as reflected by the induction of OVA-specific IgE only in OVA/SEB mice. Upon stimulation with OVA, PBLN cells of the OVA/SEB group produced higher protein levels of the typical Th2 cytokines IL-4, IL-5, IL-10 and IL-13 compared to the 3 other groups of mice. Simultaneous application of OVA and SEB resulted in a significant increase in bronchial responsiveness to increasing doses of nebulized methacholine compared to all other conditions.

Our study highlights a crucial role of SEB in the sensitization process to allergens in a mouse model. As SEB is capable of facilitating sensitization to proteins like OVA, these data shed new light on the concept of the hygiene hypothesis.

20

THE EFFECT OF DOXYCYCLINE-COATED STENTS ON POSTOPERATIVE HEALING QUALITY AFTER SINUS SURGERY

Wouter Huvenne,[1] Nan Zhang,[1] E Tijsma,[2] A Driessen,[2] B Hissong,[3] J Huurdeman,[2] G Holtappels,[1] S Claeys,[1] Paul Van Cauwenberge,[1] H Nelis,[4] T Coenye[4] and Claus Bachert[1]

[1]Upper airway research laboratory (URL), ENT-Department, University Hospital, Ghent, Belgium
[2]Medtronic Bakken Research Center, Maastricht, The Netherlands
[3]Medtronic ENT, Jacksonville, FL, USA
[4]Laboratory of Pharmaceutical Microbiology, Ghent, Belgium

Surgical management of chronic rhinosinusitis without polyps (CRSsNP) and chronic rhinosinusitis with nasal polyposis (CRSwNP) is indicated after failure of medical treatment. However, persistent inflammation and/or bacterial colonization can result in poor post-operative healing, linked to high concentrations of matrix metalloproteinase-9 (MMP-9). The frontal recess is especially vulnerable to re-stenosis, and frontal sinus stents have been used to overcome this problem. However, the long-term success rate still is controversial and may be poor.

We evaluated the effect of doxycycline-coated stents – which deliver an active agent against MMP-9 locally – on postoperative MMP-9 levels, healing quality and symptom scores.

Patients (n=10) suffering from CRSw/sNP were recruited at the ENT-department, Ghent University Hospital. After functional endoscopic sinus surgery, a doxycycline-releasing stent (DC) was placed in the frontal recess/ostium on one side, while a non-releasing stent was placed on the other side, based on a random code. Healing quality was endoscopically evaluated using visual analogue scales (VAS) and symptom scores. Local levels of MMP-9 were measured using ELISA.

MMP-9 concentrations were significantly lower at the side of the DC-releasing stent (3413±582 ng/ml,) compared to the contralateral placebo stent (9172±2564 ng/ml) ($p<0.05$). VAS for the frontal region was significant better at month 3 (median value 7.5 vs 5.3, p = 0,0007) compared to its placebo counterpart. All frontal sinuses were accessible at month 3 at the side of the DC-releasing stent, whereas 3 out of 10 sides of the placebo stent were occluded.

Compared to placebo stents, doxycycline-releasing stents improve postoperative healing quality after functional endoscopic sinus surgery, as is shown by significantly better VAS at the DC side. Moreover, lower levels of MMP-9 were found, objectifying the MMP-decreasing activity, which results in better postoperative wound healing.

21

EOTAXIN-1, -2, AND -3 IMMUNOREACTIVITY AND PROTEIN CONCENTRATION IN THE NASAL POLYPS OF EOSINOPHILIC CHRONIC RHINOSINUSITIS PATIENTS

Katsuhisa Ikeda, Toru Yao, Yuko Kojima, Akemi Koyanagi, Hidenori Yokoi, Tatsuya Saitoh, Kenji Kawano, Masayuki Furukawa and Takeshi Kusunoki

Department of Otorhinolaryngology Juntendo University School of Medicine, Tokyo, Japan

Eosinophilic chronic rhinosinusitis (CRS) is characterized by the accumulation of numerous eosinophils in the sinus mucosa and nasal polyps, which are frequently difficult to control, even with surgery. The present study was designed to evaluate the expression and localization of eotaxins, which are well known to be potent and selective chemoattractants for eosinophils in CRS.

The patients were classified into eosinophilic and non-eosinophilic groups. Histopathological profiles of the nasal polyp were observed with hematoxylin-eosin staining. Eotaxins1-3 were immunohistochemically stained in the nasal polyps. Furthermore, the protein content of eotaxin subtypes inside the nasal polyp and sinus effusion was measured using enzyme-linked immunosorbent assay (ELISA).

In the nasal polyps, immunoreactivities of the eotaxin subfamily, eotaxin-1, -2, and -3, were noted in most of the infiltrating eosinophils, as well as in othe with non-eosinophilic CRS groups, eosinophilic CRS groups had a significant expression of eotaxins in their eosinophils. The eotaxin concentrations of nasal polyp and sinus effusion as measured by ELISA were significantly increased in the eosinophilic CRS group compared to the non-eosinophilic CRS group.

The present findings suggest that enhanced eotaxin family production by eosinophils results in the recruitment of eosinophils into the tissue by a self-amplifying process.

22

CAVEOLAR TRANSPORT THROUGH NASAL EPITHELIUM OF BIRCH POLLEN ALLERGEN BET V 1 IN ALLERGIC PATIENTS

Sakari Joenväärä, Pirkko Mattila, Jutta Renkonen, Sanna Toppila-Salmi, Antti Mäkitie and Risto Renkonen

Transplantation Laboratory, Haartman Institute, University of Helsinki, Helsinki, Finland

Previous work in type I pollen allergies has focused on aberrant immunoresponses. Our system level analyses explore the role of epithelium in early pathogenesis of type I allergic reactions.

We began top-down analyses of differences in human nasal epithelial cells and biopsies obtained from birch allergic patients and healthy controls in resting state and after intranasal *in vivo* birch pollen challenge. Immunohistochemistry, immuno electronmicroscopy, mass spectrometry, transcriptomics and integration of data to a pathway were conducted.

Bet v 1 allergen bound to epithelium immediately after *in vivo* birch pollen challenge during winter only in allergic individuals. It also travelled through epithelium with caveolae to mast cells. 16 unique proteins were found to bind to Bet v 1-column only in lysates from allergic epithelial cells; 6 of these were caveolar and 6 cytoskeleton proteins. The nasal epithelial transcriptome analysis from allergic and healthy subjects differed during the winter season and responded also differentially to birch pollen challenge. Within this pollen-induced response, gene ontology categories cytoskeleton and actin cytoskeleton were decreased in allergic patients, while the actin-binding category was enriched in healthy subjects. Integration of microscopy, mass spectrometry and transcriptomic data to a common protein-protein binding network showed how these were connected to each other.

We put out a hypothesis of caveolar-dependent uptake and transport of birch pollen allergen only in epithelium of allergic patients. Application of discovery driven methodologies can provide new hypotheses worth further analyses of complex multifactorial diseases such as type I allergy.

23

THE RELATIONSHIP BETWEEN ALLERGIC RHINITIS AND PERSONALITY TRAITS USING SYMPTOM CHECKLIST-90 REVISED QUESTIONNAIRE

Ebrahim Karimi, Mona Heidarali and Mehdi Bakhshaee

Otorhinolaryngology Research Center, Tehran University of Medical Sciences, Tehran, Iran

Allergic rhinitis is an important public health problem. Growing psychoneuroimmunology evidence shows stressful events affect physical health and concerning the role of psychological factors in the etiology and symptomatology of allergic conditions is equivocal. We opted to investigate the relationship between allergic rhinitis and personality trait in patients with proven allergic rhinitis status.

One hundred allergic rhinitis patients were selected based on medical history, physical examination, allergic skin test, and standard questionnaire. Patients were evaluated with the symptoms checklist-90 revised questionnaire (SCL-90R), a multi-dimensional self report inventory consisting of 90 items covering 8 dimensions of psychological stress: phobic anxiety, anxiety, depression, somatization, obsessive-compulsitivity, distrust and interpersonal sensivity, hostility, and insomnia. Each item describes physical or psychological symptoms that are pointed on a five-point scale.

The male to female ratio was 35:6. Younger patients were found to have more personality traits (mean age: 24.15; range: 18-38 years). In male subjects the score of personality traits from maximum to minimum compared with normal subjects were: somatization (+1.44), obsession (+0.66), aggression (+0.47), anxiety (+0.28), and the lowest score was phobia (-0.32). In females the scores were somatization (+0.59), obsession (+0.52), anxiety (+0.2), phobia (+0.15) and the lowest one was paranoid with the score of (–0.20).

Our findings confirm a high rate of somatization and depressive disorders in patients with allergic rhinitis. It seems personality traits are more common in subjects with allergic rhinitis and patients with allergic rhinitis have poorer psychological function compared with the non-allergic subjects.

24

INHIBITORY ACTION OF ROXYTHROMYCIN ON HISTAMINE RELEASE AND PROSTAGLANDIN D2 PRODUCTION FROM BETA-DEFENSIN 2-STIMULATED MAST CELL

Kaori Kase

Tokyo, Japan

The long-term, low-dose therapy with the 14-membered macrolides is well known to be effective in the treatment of chronic airway inflammation. Although the mode of macrolides on neutrophils, monocytes, epithelial cells, etc. have been reasonably investigated, the effect of macrolide on mast cell function is sparsely reported. We first examined the effect of roxithromycin (RXM) on mast cell functions activated by human beta-defensin-2 (hBD-2).

Using rat peritoneal mast cells stimulated with hBD-2, histamine release, prostaglandin D2 (PGD2) production, and intracellular Ca2+ concentration ([Ca2+]i) were measured in the absence or presence of RXM.

RXM at the dose of 12.5 and 25 mg/ml significantly inhibited the histamine release from mast cells (*p*<0.05). PGD2 production induced by hBD-2 was significantly reduced by RXM at 6.25 (*p*<0.05) and 12.5 mg/ml (*p*<0.01). Furthermore, the rise of [Ca2+]i in mast cells caused by hBD-2 was inhibited by 6.25 and 12.5 mg/ml of RXM (*p*<0.05).

The present findings suggest that RXM modulates mast cell activation induced by hBD-2 at least via a Ca2+ signal pathway, thereby possibly alleviating inflammatory reactions of chronic rhinosinusitis.

25

TYPE I AND TYPE III INTERFERON EXPRESSION DURING RHINOVIRUS INFECTION

Musa R. Khaitov,[1] Vasile Laza-Stanca,[2] Michael R. Edwards,[2] Ross P. Walton[2] and Sebastian L. Johnston[2]

[1]NRC Institute of Immunology, Moscow, Russia
[2]Department of Respiratory Medicine, National Heart and Lung Institute & Wright Fleming Institute of Infection and Immunity, Imperial College London, Norfolk Place, London W2 1PG, UK

Rhinoviruses are a major cause of common colds and asthma exacerbations worldwide. It is known that rhinovirus infects and replicates in respiratory epithelial cells of lower respiratory tract inducing proinflammatory cytokines. Type I interferons such as IFN-a, IFN-b and newly discovered type III interferons - IFN-ls play vital role in innate immune response against viruses.

In this study we investigated the potential of different cell types such as human bronchial epithelial cells (BEC), BEAS-2B cells and PBMC to express and produce various type I and type III interferons upon respiratory virus infection by RT-RCR and ELISA.

In BECs we detected induction of IFN-a mRNA expression by 8 hours, induction of IFN-l1 mRNA by 24, IFN-l2/3 was induced by 8 and 24 hours. In BEAS-2B cells we detected induction of IFN-a mRNA expression by 8 hours, IFN-l1 mRNA from 8-hours with peak at 24 and induction of IFN-b from 24 hours. By ELISA we also observed production of IFN-l1 and IFN-b protein by 24 hours. IFN-l were induced either earlier and/or to a greater degree than the type 1 IFNs. In PBMC induction of IFN-a, IFN-l1 and IFN-b mRNA expression and protein production were all detected from 8 hours. However induction of IFN-a was to a much greater degree than -b or -l.

Lambda IFNs appear important in epithelial cell responses to rhinovirus infection, while alpha IFNs are the major IFNs released by macrophages.

26

CLINICAL MANIFESTATION OF ODONTOGENIC SINUSITIS

Soo Whan Kim, Ji Hong Kim, Jin Hee Cho, Jun Myung Kang and Sung Won Kim

Department of Otolaryngology - HNS College of Medicine, The Catholic University of Korea, Seoul, Korea

Sinusitis of odontogenic source is a common disease and accounts for about one tenth of cases of all maxillary sinusitis. Sinusitis from an odontogenic source is different from sinusitis of other origins in pathophysiology, microbiology, clinical manifestation, management. By analyzing features of odontogenic sinusitis, we tried to report the clinical manifestation of odontogenic sinusitis.

Material includes 15 patients who were diagnosed with odontogenic sinusitis between May 2000 to May 2008. Male to female ratio was 10:5 and average age was 41.8 years ranged from 17 to 87 years. All the medical records were reviewed retrospectively.

Seven cases from extraction, 2 cases from endodontic treatment, 3 cases from dental cary treatment, 2 cases from foreign body removal, and another 1 case was originated from odontogenic cyst removal. Oroantral fistula occurred in 6 patients, who underwent extraction or dental cary treatment. Most frequent symptoms were toothache and swelling, but nasal symptoms like rhinorrhea and nasal obstruction were rare. Follow-up period was average 11.5 months, no complication was observed.

Mostly odontogenic sinusitis resulted from a iatrogenic cause. Frequent symptoms of odontogenic sinusitis were toothache and swelling. A combination of a medical and surgical approach is generally required for the treatment of odontogenic sinusitis.

27

ASSESSMENT OF THE PREVALENCE AND POSSIBLE RISK FACTORS OF SELF-REPORTED ASTHMA AND ALLERGIC RHINITIS IN THE POPULATION OF A RURAL DISTRICT NEAR AMSTERDAM, A GA²LEN STUDY

Jurriaan Kok, Yvette Smulders, Cornelis M. Van Drunen and Wytske J. Fokkens

Department of Otolaryngology, Amsterdam Medical Centre, Amsterdam, The Netherlands

Asthma and allergic rhinitis (AR) are both worldwide physical and social health problems, with a big impact on the economy. Previous studies have shown that these two diseases frequently coexist and are possibly linked to each other, as stated in the unified airway concept. The purpose of the present study is to determine the prevalence of asthma and AR in the less studied age groups and to assess if occupation and smoking exposure are related to AR and asthma.

A random sample of 5000 subjects (aged 15–75 years) was drawn from the general population of a rural district near Amsterdam. A screening questionnaire, which assessed allergic diseases (asthma, AR, CRS and eczema), smoking exposure and occupation (healthcare workers and cleaners), was sent to the subjects. Categorical data was compared using the Chi square test. Independence of variables was assessed using the binominal logistic regression analysis and adjusted odds ratios (OR) with their 95% confidence intervals (CI) were calculated.

3192 questionnaires (63.84%) were used for analysis. Mean age of the study population was 46.98±15.33 years, female (54.76%). Prevalence of AR was significantly different in age group 56-75 (21.20%) compared to age group of 20-55 years old (30.38%; $p<0.001$). Statistically significant predictors for reporting AR were age (OR 0.99, for each year), pack years (OR 0.99, for each pack year) and work in healthcare (OR 1.52).

Prevalence of self-reported AR showed a decrease with progressing age and the amount of pack years. Occupational agents in healthcare could possibly trigger AR symptoms. Possible explanations for these findings are investigated in the currently running follow-up of this study.

28

EFFECT OF SUBCUTANEOUS IMMUNOTHERAPY ON SYMPTOM AND MEDICATION SCORES FOR RHINOCONJUNCTIVITIS: A 3-YEAR STUDY

Elena S. Korovkina and Marina A. Mokronosova

Mechnikov's Research Institute for Vaccines and Sera, Moscow, Russia

Allergic rhinitis is a common disease, affecting 25-35% of the population. Birch pollen is a significant cause of immediate hypersensitivity among sensitive subjects, affecting about 80% of the population in the central part of Russian Federation. The aim of the study was to investigate the efficacy of SCIT for allergic rhinoconjunctivitis for birch pollen using the Average Rhinoconjunctivitis Total Symptom Score (ARTSS) and Average Rescue Medication Score (ARMS).

54 subjects with IgE-mediated seasonal allergic rhinoconjunctivitis with or without seasonal asthma were enrolled; 10 patients with birch pollen allergy were included in a control group. All patients were sensitized to birch pollen-allergens. SCIT was performed by a birch extract standardized in IR and absorbed onto calcium phosphate (Phostal, Stallergenes, France) using the conventional build-up phase in 12 weeks and a maintenance treatment with monthly injection for three years. Patients were asked to complete diary during the birch pollination season.

Reduction in ARTSS of 37% ($p = 0.004$) and reduction in ARMS of 41% ($p = 0.036$) was found in the birch pollen season for subjects treated with the SCIT com-

pared with controls after one year of treatment, and after three years of treatment the ARTSS was 71% lower in SCIT groop ($p = 0.003$) and the ARMS was 93% lower in actively-treated groop ($p = 0.038$); well days increased by 87% ($p = 0.002$) after three years of treatment. After one year of SCIT 5 patients (9%) vs no control reported a reduction of OAS symptoms ($p < 0.001$), and after three years of treatment reduction of OAS symptoms was shown in 11 patients (20%).

Immunotherapy for 3 years shows clinical effect on development of seasonal rhinoconjunctivitis. The decrease in ARTSS for Phostal-treated patients that occurred in parallel with a marked reduction in their requirement for ARMS provides additional evidence for the efficacy of SCIT over and above usual pharmacotherapy for rhinitis.

29

USE OF RHINOLIGHTŇ IN THE TREATMENT OF ALLERGIC RHINITIS

Edyta Krzych-Falta, Magdalena Arcimowicz, Adam Lusawa, Ewa M. Galkowska and Boleslaw K. Samolinski

Department of the Prevention of Environmental Hazards, Medical University of Warsaw, Poland

The main aim of this study was to evaluate the effectiveness of allergic rhinitis treatment using UV rays (UVA and UVB light – below 3%) emitted by a device called RhinolightÒ. Due to its properties, the phototherapy – a beam of "variable rays" - did not pose any risk of nasal mucous membrane damage, pain or local burns.

The sample was a group of 8 patients (5 women and 3 men) diagnosed with seasonal or perennial allergic rhinitis.

The test method employed in the study was RhinolightÒ. The spectrum of the light emitted by the device on nasal mucous membrane is visible light (85%), UVA (less than 10%) and UVB (less than 3%). Patients with seasonal allergy received the therapy for 2 weeks and those with perennial allergic rhinitis for 6 weeks (their nasal mucous membranes were irradiated for 2 minutes+ to 3 minutes in the final phase of the therapy, at average intervals of 3-4 days). Additionally, the patients received vitamin A (suspension) to grease their nasal mucous membranes in the event of their excessive dryness. Approval was obtained from the Bioethics Committee, Medical University of Warsaw (Approval No. KB/71/2006). Before and after the phototherapy, the patients underwent the following diagnostic tests: skin prick tests, nasal cytology, acoustic rhinometry and rhinomanometry, followed by an objective assessment of the nasal symptoms reported by each patient (on a 0-3 point scale).

The acoustic rhinometry, rhinomanometry and nasal cytology tests revealed no statistically significant changes in all the patients under study. In the objective assessment of nasal symptoms (amount of nasal secretion, number of sneezes, nasal obstruction, nasal itching) rated on a 3 point scale (0 – none, 1 – weak, 2 – medium, 3 – strong), a statistically significant correlation ($p<0.05$) was observed before and after the phototherapy, especially reduced nasal symptoms: a reduction in the number of sneezes, reduced nasal obstruction and a reduction in the amount of nasal secretion, with no statistically significant correlation for nasal itching ($p<0.058$).

In addition to the treatment methods of pharmacotherapy and immunotherapy, phototherapy can only help to treat allergic rhinitis.

30

CHRONIC RHINOSINUSITIS WITH NASAL POLYPOSIS – THE ROLE OF *ASPERGILLUS AND DEMATIACEOUS* FAMILY FUNGI IN PATHOGENESIS

Asen Kutsarov,[1] Vanya Tzvetkova,[2] Rumjana Koleva,[3] Albena Todorova,[3] Svetla Gecheva[2] and Tanja Aleksovska[2]

[1]ENT Clinic, [2]Clinic of Allergology and Clinical Immunology and [3]Oncology Center Cytopathology Laboratory[3] UMHAT "Georgi Stranski" Pleven, Bulgaria

Chronic rhinosinusitis (CRS) is a widespread disease. Some authors have recognized the leading role of Aspergillus and Dematiaceous family fungi in its pathogenesis. Eosinophils have a key role in the immune reaction.

The aim of this study was to determine the role of Aspergillus and Dematiaceous family fungi in pathogenesis of CRS with nasal polyposis.

We studied 34 patients (20 females and 14 males; 40-65 years old) with CRS and nasal polyposis and 20 healthy control patients (12 females and 8 males; 40-65 years old) during a 4-month period. Nasal discharge for cytological analysis was taken from all the patients and

the controls. Skin prick test and enzyme-linked immunosorbent assay (ELISA) for specific serum IgG to fungal allergens (Àspergillus, Àlternaria, Fusarium, Rhizopus, Đånicillium and Ìucor) were performed.

Cytological findings of nasal smear showed presence of fungal elements (hyphies) in 8 controls (80%) and lack of eosinophils in all control subjects, while fungal elements were found in 24 (70,58%) patients and eosinophils in 27 (79,4%) patients. Skin prick tests were positive to one or several fungal types in 4 (11,8%) of the patients with polyposis and none of the control group. Both the patients and the control group had elevated specific serum fungal IgG (sIgG). The serum level of IgG in the control group was 2,5-40 μg/ml while in the patients group it was significantly higher serum leval fungal sIgG (>40 μg/ml) – in 28 (82,4%) patients respectively ($p<0,05$).

We suggest that Àspergillus spp. and Dematiaceous family fungi might play an important role in the development and maintenance of inflammation process in CRS with NP.

31

NON-ALLERGIC RHINITIS, AND NOT ONLY ALLERGIC RHINITIS, IS ASSOCIATED WITH INCREASED EXHALED NO LEVELS

Andrei Malinovschi,[1] Christer Janson,[2,3] Marieann Högman,[1,2,4] Kjell Torén,[5] Dan Norbäck,[2,6] Giovanni Rolla[7] and Anna-Carin Olin[5]

[1]Department of Medical Cell Biology: Integrative Physiology, Uppsala University, Uppsala, Sweden
[2]Asthma and Allergy Research Centre, Uppsala University, Uppsala, Sweden
[3]Department of Medical Sciences: Respiratory Medicine and Allergology, Uppsala University, Uppsala, Sweden
[4]Centre for Research and Development, Uppsala University/County Council of Gävleborg, Sweden
[5]Department of Occupational and Environmental Medicine, Sahlgrenska University Hospital, Gothenburg, Sweden
[6]Department of Medical Sciences: Occupational and Environmental Medicine, Uppsala University, Uppsala, Sweden
[7]Department of Allergy and Clinical Immunology, University of Turin and Mauriziano Hospital, Turin, Italy

Allergic rhinitis is consistently reported to be associated with increased exhaled NO levels. However, it is unclear if this increase is due to rhinitis per se or allergic sensitisation. There are available only small-sized studies regarding non-allergic rhinitis and these report no effect of non-allergic rhinitis on exhaled NO in univariate analyses.

Exhaled NO (FE_{NO}) was measured in 323 non-smoking, non-asthmatic subjects and with no reported asthma symptoms from the random sample of three centres (Uppsala and Gothenburg, both Sweden, and Turin, Italy) of European Community Respiratory Health Survey II with regard to their levels of exhaled NO and rhinitis status. Subjects who answered "yes" to the question: "Do you have any nasal allergies including 'hay fever'?" were considered to be rhinitic. The dichotomisation into allergic and non-allergic rhinitis was made on the basis of specific IgE measurements against cat, mite, timothy and mould.

Subjects with non-allergic rhinitis (n=32) had similar levels of FE_{NO} as non-atopic healthy subjects (n=205) while allergic rhinitis subjects (n=58) had 26% (6, 50%) higher FE_{NO} levels than non-atopic healthy subjects. There were more women in the non-allergic rhinitis group (66%) than in healthy non-atopics group (45%) or allergic rhinitis (38%) (both p-values<0.05). After adjustments for female gender, both rhinitis groups were characterized by increased FE_{NO} levels, with a FE_{NO} % increase of 26% (2, 55%) for non-allergic and 23% (4, 45%) for allergic rhinitis, when compared to healthy non-atopic subjects. These results remained consistent after further adjustment for other confounders of interest, as height, age, lung function, previous smoking and study centre.

Our results suggest that non-allergic rhinitis also is associated with increased exhaled NO levels. The apparent contradiction with previous studies in the literature is probably explained by lack of adjustment for female gender, that dominates among the non-allergic rhinitis subjects. Therefore both allergic and non-allergic rhinitis should be accounted in epidemiological studies looking at determinants of exhaled NO levels.

32

IMPAIRED LUNG FUNCTION IN CHILDREN WITH ALLERGIC RHINITIS WITHOUT ASTHMA

Iva Mrkić, Davor Plavec, Boro Nogalo and Mirjana Turkalj

Children's Hospital Srebrnjak, Zagreb, Croatia

Inflammatory markers present in asthma can be elevated in patients with allergic rhinitis. Aim of our study was to evaluate lung function together with level of ex-

haled NO (eNO) in patients with allergic rhinitis without asthma.

In a group of 51 children (31 male, 4-19 yrs) with diagnosed allergic rhinitis without asthma, we measured lung function, eNO, evaluated allergen sensitivity (skin prick tests, specific IgE) and therapy.

Twenty-two patients were allergic to pollen, 3 to mites, 10 to other perennial allergens, 12 to pollen and mites, and 3 were multisensitized. Spirometry showed restriction in 4% patients (FVC <80% predicted), while up to 13% had at least a distinct obstruction in the area of small airways (MEF_{25} or MEF_{50} <80%), and up to 27% in the area of large airways (MEF_{75} or PEF <80%). Patients receiving intranasal corticosteroid (INCS) therapy plus antihistamines had the best PEF. In 25 patients we found elevated eNO. The lowest eNO values were found in patients receiving only INCS, and the highest in patients receiving triple rhinitis therapy (INCS + antihistamines + leukotriene antagonists). Significant correlation between eNO and lung function (FVC, P=0.004; FEV_1, P= 0.006) was found. Better lung function was found in patients with higher level of eNO.

It seems that allergic rhinitis in children can be related to impaired lung function without clinical symptoms of lower airways. As these children may have increased risk for asthma they deserve further monitoring.

33

ALLERGIC RHINITIS IN CHILDREN

Carlos Nunes

Centre of Allergy and Immunology Algarve, Portimao, Portugal

There are no widely agreed criteria for the diagnosis of rhinitis, namely in children. Rhinitis is a common disease in pre-school, school children and adolescents, and its potential severity is still under-diagnosed and under-treated in a significant number of children population surveys. It is generally being accepted that the major symptoms of non-infectious rhinitis are sneezing, rhinorrea and nasal obstruction, in the absence of infectious symptoms.

There are some difficulties in children to determine how one diagnoses allergic and non-allergic rhinitis and to determine the minimum level of testing that is needed to differentiate allergic from non-allergic rhinitis; whether differentiating allergic from non-allergic rhinitis is important; the effectiveness of treatments in non-allergic and allergic rhinitis; and how treatment of allergic rhinitis impacts the development of asthma.

Total serum IgE may be as useful as specific allergy skin prick tests, which, in turn, are more useful than RAST-type testing in confirming a diagnosis of allergic rhinitis. Beyond skin testing and diagnosis by exclusion, there is a small number in literature on differentiating allergic from non-allergic rhinitis. In the majority of cases exclusion of allergic disease by an absence of positive allergy skin tests or negative results by RAST is the usual prerequisite criterion for diagnosing non-allergic rhinitis.

The data concerning treatment of non-allergic rhinitis is scant and no single agent is identified as being uniformly effective in controlling all the symptoms associated with this condition. In allergic rhinitis treatment, nasal corticosteroids are superior to antihistamines and there is no consistent difference between sedating antihistamines and non-sedating antihistamines for the relief of nasal symptoms during childhood. The majority of studies reported no major adverse events associated with current treatments. There is insufficient evidence to address the relationship between allergic rhinitis and the development of asthma or rhinosinusitis. However, it was estimated that ~50% of symptomatic allergic children with rhinitis for more than 10 years can develop asthma throughout their adult life.

Differentiation of allergic from non-allergic rhinitis is important if treatments are significantly different and if the outcomes of treatment including prevention of complications differ in response to those treatments. As seen in the evidence tables, similar treatments are frequently employed in the two conditions. However, what has been studied in the literature does not imply that differentiation might be important. It is generally believed that environmental control and immunotherapy have relevance only for treatment of allergic rhinitis. Therefore, differentiation is important for these two interventions.

Treatment with antihistamines, nasal corticosteroids, sympathomimetic agents, anticholinergic agents, and cromoglycate efficacy and leukotriene modifiers in the treatment of allergic and non-allergic will be considered.

Proactive programmes are needed in view of prevalence data of children rhinitis, to alert the medical community and general public to the need for early diagnosis and treatment of allergic diseases, which can have significant personal and social repercussions, even in very young children.

34

A DOUBLE-BLIND CROSS-OVER CONTROLLED STUDY OF PRANLUKAST, A LEUKOTRIENE RECEPTOR ANTAGONIST, AGAINST JAPANESE CEDAR POLLINOSIS USING THE OHIO CHAMBER

Kimihiro Okubo, Kazuhiro Hashiguchi, Minoru Gotoh, Shuichiro Endo, Hidenori Suzuki and Keisuke Masuyama

Department of Otolaryngology, Nippon Medical School, Tokyo, Japan

Only a few reports have described the effects of leukotriene receptor antagonists on Japanese cedar pollinosis and there has been no detailed verification of efficacy. In this study, the therapeutic effects of Pranlukast, a leukotriene receptor antagonist, on nasal symptoms in Japanese cedar pollinosis were examined in a pollen-exposure chamber (OHIO Chamber) that we developed for this purpose.

A double-blind cross-over controlled study was performed to investigate the efficacy of Pranlukast by exposing patients with cedar pollinosis to a specific amount of cedar pollen (8000/m3) in the OHIO Chamber during the non-pollen scattering season. The subjects underwent two exposures of 3 hours each at a 1-week interval. Pranlukast was administered orally after a meal before and after each exposure. The effect of Pranlukast was evaluated based on nasal symptoms, nasal airway patency, and impaired performance.

Among patients with cedar pollinosis who showed a positive intradermal reaction to cedar pollen, 39 in whom nasal congestion symptoms became more severe during the pollen scattering season for more than two years in a row were included in the study. One-day administration of Pranlukast suppressed nasal symptoms (sneezing, runny nose, and nasal congestion) and reduced the late-phase reaction of nasal congestion significantly in these patients.

Our results confirm a rapid effect of one-day administration of Pranlukast on Japanese cedar pollinosis, and we conclude that Pranlukast, a leukotriene receptor antagonist, is effective when administered at the early-stage and after pollen scattering.

35

NASAL INFLAMMATION AFTER NASAL PROVOCATION TEST IN PATIENTS WITH WORK-RELATED RHINITIS

Gianni Pala, Patriza Pignatti, Marta Pisati, Luca Perfetti, Gabriella Banchieri and Gianna Moscato

Allergy and Immunology Unit, Fondazione Salvatore Maugeri, IRCCS, Pavia, Occupational Immunology and Allergy Laboratory ISPESL, Rome, Italy

The monitoring of nasal inflammation after a nasal provocation test (NPT) is relevant to the objective confirmation of occupational rhinitis. In our study we aimed to identify a cut-off value for post-NPT eosinophil increase that is sensitive and specific for occupational rhinitis in subjects with work-related rhinitis symptoms.

We enrolled 103 healthy subjects, 30 allergic rhinitis patients and 29 subjects with work-related rhinitis symptoms. Nasal secretions of patients with work-related symptoms were collected before and 30 min, 4 and 24 hours post-NPTs. Samples were processed to solubilize mucus and cytospin preparations microscopically analyzed.

We obtained reference values for nasal secretion cells in healthy subjects. The cut-off of 1.5% eosinophils, regardless of the smoking habit of the subjects, clearly highlighted nasal eosinophilic inflammation in patients with allergic rhinitis. In work-related symptomatic subjects, the maximal eosinophil increase after NPT was significantly higher in NPT+ than in NPT- subjects ($p=0.006$). 4% and/or 1x104 eosinophils/ml was the cut-off for maximal eosinophil increase after NPT. Sensitivity of nasal secretion examination in defining occupational rhinitis was 84.6%, specificity 69.2%.

Nasal secretion evaluation is an easily reproducible method to assess nasal eosinophil inflammation. The proposed cut-off for a significant post-NPT eosinophil increase is sensitive and useful in supporting the diagnosis of occupational rhinitis.

36

THE EFFECT OF GINKGO BILOBA ON THE EXPRESSION OF INTERMEDIATE-EARLY ANTIGEN(C-FOS) IN THE EXPERIMENTALLY-INDUCED ANOSMIC MOUSE

Chan-Soon Park, Jin-Hee Cho and Soo-Hwan Kim

Department of Otolaryngology - HNS, the Catholic University of Korea, College of Medicine, Seoul, Korea

Treatment of olfactory dysfunction is very difficult and has limited modality. In the present study, the effect of systemic administration of dexamethasone and EGb 761 on damage to olfactory mucosa produced by zinc sulfate was examined.

After anosmic mice were made by bilateral intranasal irrigation with 0.2ml of 5% (0.17M) zinc sulfate, anosmia was confirmed by a food finding test. Four groups of anosmic mice were studied: a steroid group (steroid injection group, n=12), an EGb group (EGb injection group, n=12), a steroid-EGb group (steroid and EGb injection group, n=12), and a control group (anosmic mice and no Tx. n=12). The olfactory bulb and piriform cortex of four mice in each group were obtained at 1, 2, and 3 weeks after instillation of zinc sulfate by cardiac perfusion, and immunohistochemical staining for c-fos was also performed to evaluate brain activity.

In all experimental groups, c-fos (+) cells increased in a time-dependant manner. The combination treatment of steroid and EGb was the most effective and the no-treatment group the least effective 1 week later after zinc sulfate irrigation.

The combination treatment of EGb and steroid enhanced the regeneration of the olfactory pathway after olfactory mucosal injury by zinc sulfate. Our study suggests that EGb could be an effective treatment option for olfactory dysfunction.

37

AN UPDATE ON SPECIFIC IMMUNOTHERAPY FOR ALLERGIC RHINITIS

Giovanni Passalacqua

Allergy and Respiratory Diseases, Department of Internal Medicine, University of Genoa, Genoa, Italy

Although specific immunotherapy (SIT) was empirically introduced more or less one century ago, systematic rules on its use, indications and contraindications are a matter of the last ten years only (1). The injection route (SCIT), the traditional one, is supported by a large number of randomised controlled clinical trials, and its efficacy in reducing symptoms of allergic rhinitis is not questioned. The efficacy of SCIT has been recently revised in a large meta-analysis (2), involving more than 50 studies. This meta-analysis concluded for a significant effect over placebo for both symptoms and rescue medications usage. Nevertheless, there was a great heterogeneity among the trials, due to the wide variety of experimental designs, outcomes and type of patients enrolled. Another possible problem is that all but one study involved a very limited number of patients. Some concerns about the safety still remain. It is true that when correctly prescribed and administered, SCIT is reasonably safe, but it is also true that unpredictable severe adverse events may occur. For this reason, it is recommended that SCIT is given by a trained specialists, with the ability to promptly recognize and manage adverse events. In the last ten years, some special aspects of SCIT have gained the attention of specialists, being the most important one the preventive effect. It is well known that allergic rhinitis is a risk factor for the subsequent development of asthma (3), expecially in children, and that no drug is able to modify the progression of the disease. On the contrary, the big PAT study has consistently shown that SCIT can reduce by more than 50% the risk of developing asthma (4,5). This protective or preventive effect persists even 10 years after discontinuation of SCIT. Another important progress in the field of SCIT is the rapidly increasing knowledge on the mechanisms of action. In this sense, there are now consistent data about the effect of SCIT on regulatory T cells (6), which involves also the induction of allergen specific IgG4. These IgG4, resembling the old "blocking antibodies", are capable of inhibiting the IgE-facilitated antigen presentation (7).

Sublingual immunotherapy (SLIT), was introduced in clinical practice only 20 years ago, but there are currently more than 40 randomized controlled studies of its efficacy (8), so that 3 meta analyses could be performed (9-11) in adults and children. Based on these meta-analyses (and the results of controlled trials), SLIT is clearly effective in reducing symptoms and drug usage in adults and children with allergic rhinitis, although also in the case of SLIT there is a great heterogeneity in clinical trials that may limit the strength of meta-analyses. At variance with SCIT, several large trials, involving hundreds of patients, have been performed with SLIT (grass pollen) (12,13). These large trials have provided important information about the optimal dose, the dose dependency of the clinical effects and the safety. The safety still remains the distinctive feature of SLIT, which can be used also in younger children (14) and mixing two or three allergens (15) without increasing the risk of side effects. Also for SLIT, there are now experimental data indicating a preventive effect on the onset of asthma in rhinitis patients (16,17). On the other hand, there are less studies on the mechanisms of action, although the available observations suggest that an effect on T regulatory cells is involved (18). Another concern with SLIT is the wide variability of doses, schedules and pharmaceutical preparations. Also in this case, the general trend is to privilege high doses and once-daily schedules, without a build-up phase.

There are numerous opportunities to improve immunotherapy in the near future. These include the use of recombinant and engineered allergens, the association with bacterial adjuvants and the administration of allergenic peptides. Concerning SLIT, encouraging results have been obtained in the field of food allergy, atopic dermatitis and hymenoptera venom allergy.

In conclusion, SIT is now regarded as an essential component of the management strategy for allergic rhinitis. It must not replace drug therapy, or be used as a last choice, but rather in association with pharmacological treatments in order to take advantage of the curative and preventive action. When prescribing an immunotherapy (either SCIT or SLIT), the aetiological role of the allergen(s) and the IgE mediated mechanism of the disease must be clearly ascertained. In addition, it is essential to evaluate those individual factors that may affect the compliance, and the costs vs expected benefit ratio.

38

NASAL BECLOMETHASONE PLUS LORATADINE IN PATIENTS WITH RHINITIS AND CONCOMITANT ASTHMA

Željko Paunović

Emergency Center, Niš, Serbia

We sought to determine the efficacy and safety of loratadine (10 mg) once daily (L), plus nasal beclomethasone dipropionate (100 mcg) (Bdp) twice daily in patients with seasonal allergic rhinitis and mild asthma.

We conducted a randomized, double-blind, placebo-controlled trial of L/Bdp in 43 subjects during the fall allergy season. Nasal and chest symptoms, albuterol use, and peak expiratory flow rates were recorded daily for 6 weeks. Spirometry was measured at baseline and after 1, 2, 4, and 6 weeks of therapy, and health-related quality of life was rated at the beginning and end of the study.

Total rhinitis and asthma symptom severity scores were significantly reduced in patients receiving active therapy compared with those receiving placebo throughout the 6-week study. Peak expiratory flow rates improved significantly in patients treated with L/ Bdp during weeks 2 through 6 (peak effect [mean±SEM]: L/ Bdp, 28.13±4.32 L/min vs placebo, 8.67±3.53 L/min, $p = 0.002$) as did FEV1 (peak effect [mean± SEM]: L/ Bdp, 174±48 ml vs placebo, 21±41 ml, $p = 0.01$) at all clinic visits. In addition, select measures of asthma-specific quality of life improved significantly relative to placebo.

We conclude that L/Bdp significantly improved nasal and asthma symptoms, pulmonary function, and quality of life in patients with seasonal allergic rhinitis and concomitant mild asthma.

39

IS ASPIRIN THE REASON?

Erjola Piluri, Mesonjesi Eris and Qirko Etleva

University Hospital Center “Mother Teresa” Tirana, Albania

Nasal polyposis is an inflammatory condition of the nasal and sinus mucosa with a major impact on patients lives. NP is often coexisting with asthma, rhinitis and aspirin intolerance. Inflammation is characterized by congestion and loss of smell.

We report a case of a 58-year-old woman with persistent rhinitis, moderate asthma and NP. The rhinitis first appeared at the age of 43, asthma at age 52, NP at age 54 (4 years ago). She is an aspirin tolerant subject, but as a general rule, for all who have asthma, she stopped taking Aspirin, arbitrary 5 years ago. The treatment was with nasal topical corticosteroids, short term use of oral steroids approximately 5 times a year. Because the polyps do not regress and because of her low quality of life, she underwent polypectomia, combined with maintenance of nasal topical corticosteroid. Seven weeks after the surgery, our patient has the same moderate asthma, her rhinitis, her polyps and her low quality of life. But, for the last 5 months she is taking Aspirin again, 325 mg once daily.

NP was seen endoscopically. Spirometry was performed before and after the surgery. SPT with aeroallergens and specific IgE antibodies was negative. SF36 was used pre-post-operatively and recently.

Our patient had a gradually regress of her NP, improvement of symptoms, and her quality of life, after the intake of interrupted Aspirin. Was it really Aspirin? Or was it a coincidence? Was it a prevention of becoming an intolerant aspirin subject?

40

ASPERGILLUS SPP. ARE ONE OF TRIGGERING FACTORS OF NASAL POLYPOSIS

Pavel Protasov, Anna Antropova, Marina Mokronosova and Tatiana Zheltikova

Federal Scientific Clinical Centre of Otorhinolaryngology, Moscow,
Mechnikov Research Institute for Vaccines and Sera, Moscow, Russian Federation

Sensitization to Aspergillus spp. is one of triggering factors of nasal polyposis(NP).

The aim of the study was to estimate the influence of fungal genera Aspergillus on the patients(P) with NP.

1848 air samples were collected from 300 Moscow dwellings. 35 P with NP and 34 healthy individuals (HI) were included in the research. The galactomannan (GM), IgG-Ab to A. fumigatus(Asp.f.); IgE to Asp. f. (m3), rAsp f1, rAsp f2, eosinophil cationic protein (ECP) in homogenized nasal polyps tissue, nasal mucus and serum were analyzed.

In the air of Moscow dwellings fungal genera Aspergillus and Penicillium dominated. Mean fungal concentration was 90 CFU/m3 of air. The frequency and abundance of Aspergillus were 82% and 37%, respectively. Aspergillus spp. were detected in nasal cavity of 7% to 9% of P. GM was revealed in nasal mucus of 83%, and polyp tissue – of 33% of P. GM was detected in serum of only 2 P with NP. IgG to Asp.f. were detected in 12 P with NP and in 2 HI. IgE to Asp.f. were detected only in 3 P with NP (0,7-15 units). The IgE to rAsp f1 were detected in serum of only 1 P. The IgE to rAsp f1, rAsp f2 were detected in polyp of 1 P. The concentration of ECP in polyp tissue was 1386?107 ug/l at the average. The mean concentration of ECP in sera was 50,6 ug/l.

Aspergillus is one of dominant genera in Moscow dwellings. Correlation between the concentrations of ECP and IgG to Asp.f. in serum, the eosinophils count in nasal mucus, polyps in P with NP were found.

41

UPDATE ON GLUCOCORTICOID RECEPTOR REGULATION

Laura Pujols

IDIBAPS, Immunoallčrgia Respiratňria, Hospital Clínic i Universitari, Barcelona, Catalonia, Spain

Inhaled and intranasal glucocorticoids are the most common and effective drugs for controlling symptoms and airway inflammation in respiratory diseases such as allergic rhinitis, chronic rhinosinusitis with/without nasal polyps, and asthma, although some patients respond poorly to them. Glucocorticoid effects are mediated through the glucocorticoid receptor (GR). To understand why glucocorticoid treatment fails, it is essential to investigate the expression and regulation of the GR, its mechanisms of action, and its alterations in disease. In humans, one single GR gene gives rise to two main GR products, namely GRa and GRb, which are subject to translational and post-translational modifications. GRa is expressed in virtually all human cells and tissues and, *in vitro*, its expression is downregulated by glucocorticoids. GRa mediates the anti-inflammatory actions of glucocorticoids mainly by 1) activating transcription of anti-inflammatory genes through binding of GRa to glucocorticoid response elements (GRE) located in the promoter region of target genes (transactivation) and, 2) repressing transcription of pro-inflammatory genes through direct interaction between GRa and pro-inflammatory transcription factors, such as AP-1 and NF-kB

(transrepression). GRb acts as a dominant negative inhibitor of GRa-mediated transactivation and transrepression in certain *in vitro* studies with transfected cells. The GRb message is expressed at low levels in numerous tissues and its protein is only expressed in specific cell types. Increased GRb expression has been reported in bronchial asthma, nasal polyposis and inflammatory bowel diseases, and after incubation of cells with certain proinflammatory stimuli. However, the role of GRb in modulating glucocorticoid sensitivity *in vivo* is not yet clear. In addition to GRb, other proposed mechanisms explaining glucocorticoid resistance include alterations in GR binding to ligand, nuclear translocation, and binding to GRE, and/or a defective cross-talk with transcription factors and cofactors.

42

EFFECTS OF THE REVERSIBLE PROTEASOME INHIBITOR MG262 ON FIBROBLAST PROLIFERATION AND FUNCTION

Laura Pujols,[1] Mireya Fuentes,[1] Laura Fernández-Bertolín,[1] Isam Alobid,[2] N Agell,[4] Jordi Roca-Ferrer,[1] Joaquim Mullol[1,3] and Cesar Picado[1,3]

[1]IDIBAPS, Immunoallčrgia Respiratňria, [2]Servei d'Otorinolaringologia, [3]Servei de Pneumologia i Allčrgia Respiratňria, Hospital Clínic, [4]Department de Biologia Cellular, Facultat de Medicina, Universitat de Barcelona, Barcelona, Catalonia, Spain

Remodelling and fibrosis are hallmarks of inflammatory airway disorders, including asthma and chronic rhinosinusitis with nasal polyps. We evaluated the effects of the reversible proteasome inhibitor MG262 on fibroblast proliferation and function.

Fibroblasts from healthy nasal mucosa (NM, n=8) and nasal polyps from asthmatic patients (NP, n=8) were cultured in DMEM 10% FBS to subconfluence, growth-arrested for 1 day, and incubated with DMEM 0.5% FBS with/without MG262 (0.1-10,000 nM) or the apoptosis inhibitor z-VAD-FMK for different times. Cell proliferation was analysed with the XTT proliferation assay, cell cycle by propidium iodide staining, expression of the cyclin-dependent kinase inhibitors p21 and p27 by Western Blot, and collagen mRNA expression and IL-6 production by RT-PCR and ELISA.

At 24h, MG262 inhibited -without reaching 50% inhibition- cell proliferation of both NM and NP fibroblasts ($p<0.01$). MG262 inhibited proliferation (IC_{50}, mean ± SD nM) at 48h (NM: 10.3 ± 1, $p<0.05$; NP: 6.2 ± 0.4, $p<0.05$) and 72h (NM: 3.7 ± 1.2, $p<0.01$; NP: 3.6 ± 0.3, $p<0.001$) compared to non-treated cells. Z-VAD-FMK partially prevented MG262-mediated inhibition of cell proliferation. MG262 increased caspase-3 activity at 48h and 72h ($p<0.05$). MG262 decreased the percentage of cells in S and G2/M phases by 83% ($p<0.05$), increased those in G0/G1 by 53% ($p<0.05$), and provoked early (6h) accumulation of p21 and p27 up to 24h. MG262 (24h) dose-dependently inhibited collagen 1a1, 1a2 and 3a1 mRNA expression and IL-6 production ($p<0.01$).

Proteasome inhibitors might be therapeutically used to inhibit fibroblast proliferation and function in fibroproliferative diseases.

43

ASPIRIN BRONCHIAL CHALLENGE DECREASES PERIPHERAL BLOOD EOSINOPHILS BOTH IN NASAL POLYPOSIS/ASPIRIN ASTHMA AND HEALTHY INDIVIDUALS

Mira Radulović Pevec,[1] Branko Pevec[1] and Asja Stipić Marković[1]

[1]Department of Clinical Immunology, Pulmonology and Rheumatology Sveti Duh General Hospital, Zagreb, Croatia

The etiology of nasal polyps and aspirin induced asthma is still unclear. Therefore, etiological treatment is not possible, and patients often need sinus surgery. Nasal polyps are histologically characterized by massive edema and accumulation of eosinophils, and some preliminary data indicate that eosinophils could also be involved in aspirin-sensitivity mechanisms. Desensitization with 300 mg of aspirin daily, results in polyp-free nasal airways and improvement of pulmonary function, but is a life-long treatment. The aim of the present study was to determine possible changes in peripheral blood eosinophil kinetics after bronchial aspirin challenge.

Twenty five subjects were included in the study: 9 aspirin sensitive asthmatics (ASA) with nasal polyposis, 9 patients with nasal polyposis (NP) without asthma, and 7 healthy controls (C). Bronchial challenges were performed by 5 inhalations of the aspirin lysine solution in increasing concentrations (11.25 to 360.00 mg/ml), until a 20% fall in FEV_1 or the endpoint concentration. Periph-

eral blood eosinophil counts were determined prior to, 30 minutes, 2, 24, and 48 hours after the challenge.

The results showed a significant decrease of eosinophils in peripheral blood ($\chi^2 = 78,83$, $p = 0.000$) 30 minutes, 2 and 24 hours after the aspirin challenge. Eosinophil count was restored to baseline values after 48 hours. Similar results were obtained if ASA, NP, and C groups were analyzed separately. No differences were found between the groups.

The reduction of eosinophils in peripheral blood of both aspirin-sensitive and nasal polyposis patients, as well as of healthy individuals, shows a nonselective drug's anti-inflammatory property. Contrary to increased eosinophil counts observed in allergen-sensitive patients following bronchial challenges, aspirin-sensitive patients responded by different eosinophil kinetics. Further elucidation of aspirin effects in respiratory mucosa could help to understand the pathophysiologic mechanisms operative in aspirin-sensitive asthma and nasal polyposis.

44

INTRANASAL RECOMBINANT ALFA-2 INTERFERON TREATMENT OF PERSISTENT OFTEN RECURRENT HERPES VIRUS INFECTIONS

Michael Rudenko,[1] Andrey Simbirtcev,[2] Irene Rudenko,[1] Andrey Zabelev[1] and Andrey Shemshura[3]

[1]Clinical Center "EuroDon", Rostov-on-Don,
[2]The State Scientific Center State Research Institute of Highly Pure Biopreparations, Saint Petersburg
[3]Rostov Research Institute of Microbiology and Parasitology, Rostov-on-Don, Russia

Herpes infections affect more than one-third of the population worldwide and are responsible for a wide array of human diseases, ranging from mild localized infections to disseminated forms (N Novak, WM Peng, Clin. & Exp. Immun. 2005;142: 405–410). In a placebo-controlled study, patients with persistent often recurrent herpes virus infections were randomly assigned to receive nasal drops of recombinant alfa-2 interferon at 3 million IU in weekly decreased dilutions or placebo two times per day for 6 weeks in combination with valacyclovir 500 mg two times per day orally for 5 days. Clinical, laboratory {(PCR (blood, saliva, vesicles), ELISA (IgG, IgM, cytokines), flow cytometry (CD3+; CD4+; CD8+; CD16+; CD3+,CD25+; CD3+, CD95+; CD3+,HLADR+; CD20+), assessment of phagocytosis, immunoglobulins A, M, G, circulating immune complexes}, statistic methods were used. Study (n = 243), and placebo (n = 230) groups had comparable frequencies of documented recurrent herpes virus infections (CMV, HSV and EBV or their combinations), and mean durations of recurrences (156 to 192 h). In 2 weeks period after treatment interferon patterns were significantly higher in the study group versus placebo group. (INF alpha14.8±1.28 and 26.5±4.01, INF beta 9.24±0.71 and 11.6±1.26, INF gamma 36.5 ±3.12 and 49±4.99, respectively) ($p<0,05$). The median duration of symptoms tended to be longer in the placebo group (6 days) than the study group (4 days) ($p<0,05$). Interferon recipients had longer period of remission and less duration of recurrences than placebo recipients. This combined therapy showed its effectiveness in patients with persistent often recurrent herpes virus infections.

45

RELEVANCE OF EPITHELIAL DAMAGE AND BASEMENT MEMBRANE THICKNESS TO EOSINOPHILIC INFILTRATION IN NASAL POLYPS OF CHRONIC RHINOSINUSITIS

Tatsuya Saitoh, Hirotomo Honma, Takeshi Kusunoki, Toru Yao, Kenji Kawano, Yuko Kojima, Katsumi Miyahara, Junko Onoda, Hidenori Yokoi and Katsuhisa Ikeda

Department of Otorhinolaryngology, Juntendo University School of Medicine, Tokyo, Japan

Chronic rhinosinusitis (CRS) with nasal polyps has been characterized by eosinophilic infiltration. We hypothesized that a vicious cycle which aggravates the mucosal pathology of the remodeling is related to infiltrating eosinophils in the nasal polyps of CRS. To clarify the pathogenetic role of eosinophils in CRS with nasal polyps, we examined the relevance of epithelial damage and basement membrane (BM) thickening to the epithelial infiltration of eosinophils in nasal polyps.

The number of eosinophils infiltrated into epithelial and subepithelial layers of sinonasal tissues was counted. Staging of epithelial damage was revealed as a quantification of epithelial loss. The BM thickness was selected and calculated the 3rd fields from the top.

Both epithelial damage and BM thickness in CRS were significantly greater than in the control group. Both parameters were marked in CRS associated with asth-

ma as compared with non-asthma, manifested in that with aspirin-induced asthma as compared with non-aspirin-induced asthma, and correlated with the number of infiltrated eosinophils. There was a significant correlation of eosinophilic infiltration between subepithelial and epithelial layers.

It is suggested that eosinophilic infiltration into both epithelial and subepithelial layers plays a part in the processes of mucosal remodeling of CRS with nasal polyps.

46

ANALYSIS OF TLR2 PROMOTOR POLYMORPHISM IN CHRONIC SINUSITIS

Kathrin Scheckenbach, Adam Chaker, Matthias Hauer, Simone Hindersin and Martin Wagenmann

Department of Otorhinolaryngology, Heinrich-Heine-University, Düsseldorf, Germany

Toll-like receptors (TLR) are major players of the innate immune system and play an important role in chronic and acute immune reactions of nasal mucosa. TLR2 and TLR4 are expressed in the nasal mucosa. mRNA levels of TLR2 are described to be increased in the mucosa of patients suffering from chronic rhinosinusitis (CRS) compared to controls. Different expression of SNP-s (single nucleotide polymorphisms) of TLR-s are known to predispose individuals for immunologic diseases. Because the promoter region of each gene is crucial for its activation and expression, we investigated a SNP (rs4696480) of the promoter region of TLR2 in individuals suffering from chronic rhinosinusitis with polyps and healthy individuals.

Samples were collected from 134 patients (CRS with polyps: 87; healthy individuals: 47). DNA was extracted from tissue specimen or blood samples and direct sequencing of the promoter region of TLR2 was performed.

The SNP rs 4696480 showed a similar distribution in CRS patients with polyps compared to healthy controls (Chi-square test: p = 0,998) as well as compared to the normal distribution published for rs 4696480 of the Caucasian population (Chi-square test: p = 0,338).

The expression variants of the SNP rs 4696480 of the TLR2 promoterregion do not predispose to the development of CRS with polyps and probably do not influence the higher expression level TLR2 in this group of patients.

47

INCREASED IL-17 AND RANTES LEVELS IN INDUCED SPUTUM OF ALLERGIC RHINITIS SUBJECTS AFTER SINGLE NASAL ALLERGEN CHALLENGE

Aleksandra Semik-Orzech,[1] Adam Barczyk,[1] Ryszard Wiaderkiewicz[2] and Wladyslaw Pierzchala[1]

[1]Department of Pulmonology and [2]Department of Histology and Embryology, Medical University of Silesia, Katowice, Poland

Interleukin-17 is produced by Th17 cells and was recently implicated in the development of the Th2 cell response. RANTES, among other chemokines plays a crucial role in chemotaxis of eosinophils into airway mucosa. According to the „united airway“ hypothesis, markers of inflammation in allergic diseases are elevated both in the upper and lower airways.

The aim of this study was to assess the impact of a single nasal allergen challenge on levels of IL-17 and RANTES in induced sputum of patients with allergic rhinitis.

Eighteen patients with the history of allergic rhinitis due to grass pollen confirmed by positive skin prick test and 10 control subjects entered the study. Initially, all patients underwent sputum induction. A single nasal placebo challenge was performed 24 hours later with repeated sputum induction 24 hours post-challenge. After a wash-out period of 4 weeks, the above described procedures were repeated with allergen challenge. Differential cell count in sputum was determined and concentrations of IL-17 and RANTES were measured by ELISA.

Levels of IL-17 and RANTES significantly increased ($p=0.032$ and $p=0.007$, respectively) in sputum of allergic rhinitis subjects after allergen (but not placebo) challenge. Postallergen levels of both cytokines in sputum were positively correlated ($r=0.570$, $p=0.016$). Allergen challenge led to the increased total inflammatory cell count ($p=0.005$) and eosinophil count ($p=0.028$) in induced sputum of allergic rhinitis patients.

The study shows that nasal allergen challenge induces the enhanced secretion of IL-17 and RANTES in lower airways of nonasthmatic allergic rhinitis subjects.

48

IMPACT OF POSTTRAUMATIC STRESS DISORDER IN PATIENTS WITH CHRONIC RHINOSINUSITIS ON SYMPTOM SCORES IN SINONASAL OUTCOME TEST

Dražen Shejbal,[1] Siniša Stevanović,[2] Elvira Koić[3] and Livije Kalogjera[4]

[1]Division of Otorhinolaryngology, Pakrac General Hospital, Pakrac
[2]Division of Otorhinolaryngology and [3]Division of Psychiatry, Virovitica General Hospital, Virovitica
[4]Department of Otorhinolaryngology and Head and Neck Surgery, Sestre Milosrdnice University Hospital, Zagreb, Croatia

Posttraumatic stress disorder (PTSD) and chronic rhinosinusitis (CRS) are diseases with a strong impact on the quality of life. Literature data indicate certain comorbidity between PTSD and chronic fatigue syndrome (CFS), as well as high CFS scores in certain patients with CRS. The common hypothesised pathogenic background of PTSD and CRS is reduced hypocampal volume and impaired synthesis of endogenous opioids. This was, however, not confirmed in CRS patients.

The hypothesis of this study is that a lower pain threshold, sleeping disorder and higher fatigue scores in PTSD patients deteriorate the total symptom scores in standard tests for CRS, like sinonasal outcome test *22* (SNOT-*22*), which leads to classification of patients with PTSD and CRS comorbidity into severe rhinosinusitis group, although there is no objective confirmation of such a severe disease with CT scans or endoscopy findings.

The study includes 30 patients who were divided in two groups: 1. PTSD/CRS group and 2. CRS group. Criteria for CRS group include either obstruction or hypersecretion/postnasal drip, + hyposmia or facial pressure/headache longer than 3 months, confirmed either by endoscopy and/or CT score Lund-Mackay greater than 5. Therefore, endoscopy and CT scan were performed in all patients. They were also tested by medical outcome survey short form (SF-36), SNOT-*22* and VAS scale of present symptoms adjusted to all sinonasal and some general symptoms. Significantly different results of the health perception and sinonasal symptoms were obtained for the two groups in all tests. General health perception tested by SF-36 is 64.5% for CRS group comparing to 31.96 for PTSD/CRS group. SNOT-*22* score for CRS is 48.4 and 57.8 for PTSD/CRS group. VAS is 38.6 for CRS and 56.4 for PTSD/CRS group. CRS patients mostly complain of nose congestion, postnasal drip and thick discharge, opposite to PTSD/CRS patients who mostly complain of sleep disorder and night awakening. These results confirmed our hypothesis.

49

PREVALENCE OF SYMPTOMS INDICATIVE OF ACUTE AND CHRONIC RHINOSINUSITIS AND THE INFLUENCE OF PERSONAL, ENVIRONMENTAL AND OCCUPATIONAL CIRCUMSTANCES, FIRST REPORT ON THE GA²LEN SURVEY

Yvette Smulders, Jurriaan Kok, Cornelis M. Van Drunen and Wytske J. Fokkens

Department of Otolaryngology, Amsterdam Medical Centre, Amsterdam, The Netherlands

Acute and chronic rhinosinusitis (ARS and CRS) are highly prevalent diseases, yet accurate epidemiologic studies are limited. The aim of this study was to determine the prevalence of symptoms indicative of ARS and CRS according to the EP3OS 2007 guidelines, in a general population. We also studied which personal, environmental and occupational conditions are associated with the occurrence of symptoms.

The GA²LEN screening questionnaire was sent to 5000 unselected individuals living in a suburb of Amsterdam, 3200 questionnaires were returned. The survey comprises questions on symptoms of ARS and CRS and questions regarding personal (age, gender), environmental (smoking history) and occupational (healthcare or cleaning) characteristics. To determine which conditions have a significant influence on the prevalence of symptoms, we used crosstabs, Pearson's r tests and stepwise logistic regression analysis.

The questionnaire results of 1444 males and 1748 females were included (mean age 46.98 ± 15.33 (sd)). Complaints indicative of ARS and CRS were reported by 27.5% (n=3153) and 13% (n=3164) of subjects respectively. Symptoms of ARS and CRS occurred significantly more often in subjects working as cleaners and less often above the age of 55, compared to younger age groups. Female gender was associated with higher reporting of symptoms of ARS, but not CRS. Smoking was associated with higher prevalence of both diseases.

This is the first report on the GA²LEN survey in the Netherlands indicating a prevalence of acute and chronic

rhinosinusitis of 27% and 13% respectively. The distribution of reported symptoms varies with age, gender, occupation and smoking history.

50

SUBJECTIVE VS. OBJECTIVE SMELL ASSESSMENT IN DIFFERENT SUBGROUPS OF PATIENTS WITH IMPAIRED OLFACTION

Darko Solter, Davor Vagić, Tomislav Baudoin and Livije Kalogjera

Department of Otorhinolaryngology and Head and Neck Surgery, Sestre Milosrdnice University Hospital, Zagreb, Croatia

Major causes of olfactory disorders are sinonasal disease, viral upper respiratory tract infection, head trauma and old age. Olfactory testing is an important part of objective assesment of the severity of the disease in chronic rhinosinusitis and nasal polyposis. The aim of this study was to correlate results of objective smell testing with subjective scores on olfaction and other nasal symptoms in different subgroups of patients with smell impairment.

Data were extracted from questionnaires filled by patients sent to smell testing by the ENT specialist. For the analysis data were divided in subgroups: 20 with chronic rhinosinusitis (CRS), 24 patients operated for nasal polyps, 7 patients with nasal polyps on medical treatment, 7 with hyposmia after common cold, 4 with anosmia and 11 controls with no smell impairment. Smell testing was done by using the Sniffin' Sticks 12 complete odorant. This test is based on the assessment of odor identification abilities for 12 standard odors with forced choice. Subjective scores on (0-4) were taken for olfaction, nasal obstruction, hypersecretion and headache.

The correlation between subjective score for olfaction and olfactometry score was significant for the whole group (r – 0.682), and was similar when anosmic and controls were excluded. Subjective olfaction and olfactometry score did not correlate with any other symptom. Comparing correlation coefficients between the subgroups, it seems that patients with CRS and non-operated nasal polyp patients have better correlation between subjective and objective smell assesment than patients operated for nasal polyps and those who had olfactory lesion following viral infection.

Although correlation between subjective and objective smell assesment is significant, some subgroups of patients overrate their ability to recognize odors.

51

STRESS EFFECTS ON ALLERGIC INFLAMMATION OF THE AIRWAYS

Pontus Stierna and Susanna Georen

Department of Otorhinolaryngology, Karolinska University Hospital, Stockholm, Sweden

We have used a mouse model to investigate the effects of endogenous GC synthesis and GC receptor inhibition, as well as acute stress, and different doses of exogenous administered GCs on inflammatory cells in different cellular compartments in allergic airway inflammation. including nerve growth factor (NGF) in the airways, during allergic inflammation.

First we investigated the effects of endogenous GCs on eosinophilic airway inflammation. Inhibition of GC release with metyrapone (ME) induced an increase of bone marrow eosinophilia and when the ME treatment was combined with a GC receptor antagonist (RU 486) the allergen-induced bone marrow eosinophilia was further enhanced.

The second study was focused on the effects of timing of a short acute stress on allergic airway inflammation in upper and lower airways. Short stress applied before an allergen challenge decreased the allergen-induced eosinophilia in bronchoalveolar lavage fluid and lungs and also the inflammation in the nasal tissue. No effects on eosinophilia or inflammation were seen when stress was applied after allergen challenge or as a double stress both before and after challenge.

Short stress increased the levels of NGF locally in the airways in both allergic and non-allergic mice. We also demonstrated that airway eosinophils decreased when stress was applied after allergen-challenge in a model of mild airway inflammation. The stress-evoked increase in NGF and decrease in eosinophilia in the airways were dependent on endogenous GC-synthesis, as evident from pre-treatment with ME.

These results indicate that endogenous GCs have a protective effect in both upper and lower eosinophilic airway inflammation. Our studies also demonstrate that the inhibitory effect of GCs on the allergic inflammation is timing-dependent.

The inhibitory effects on the eosinophilic airway inflammation during acute stress are GC-dependent. Also acute stress increased local airway NGF and this effect is mediated by endogenous GC synthesis. NGF may function as a mediator in the psychoneuroimmunological stress-response.

52

RELATION BETWEEN CELLULAR INFILTRATION AND MARKERS IN PATIENTS WITH CHRONIC RHINOSINUSITIS

Davor Vagić, Livije Kalogjera, Tomislav Baudoin, Krešo Zurak and Goran Geber

Department of Otorhinolaryngology and Head and Neck Surgery, Sestre Milosrdnice University Hospital, Zagreb, Croatia

The aim of the study was to show the difference in the pattern of inflammation between asthmatic and non-asthmatic patients with CRS. Eosinophil activation, local IgE levels in the sinus fluid and tissue, and the severity of inflammation were measured.

The maxillary sinus lavages, mucosal biopsies and bacteriological swabs were taken in 23 asthmatic and 36 non-asthmatic adult patients with CRS. The concentrations of IgE, eosinophil cationic protein (ECP), myeloperoxidase (MPO), and tryptase were analyzed and IgE+ cells, eosinophils, lymphocytes and plasma cells counted.

The granulocyte activation markers and IgE in sinus lavages, and the inflammatory and IgE+ cells counts were significantly higher in the asthmatics with the greatest difference in ECP and IgE concentrations. The tryptase concentrations did not differ, but only in the asthmatics they correlated significantly with the IgE concentrations and IgE+ cells count. Subjective scores were decreased after therapy in both groups of patients and without correlation to objective parameters of inflammation.

Asthmatic patients present a distinct subgroup among the patients with chronic rhinosinusitis (CRS). The levels of the cellular markers and IgE in the sinus fluid differ from those of non-asthmatic patients with CRS. The activation of granulocytes (especially eosinophils), local IgE concentrations and the inflammatory cells infiltration are significantly higher in the asthmatics.

53

A NEW METHOD OF STUDYING THE HUMAN NASAL MICROCIRCULATION *IN VIVO* USING SIDESTREAM DARK FIELD (SDF) IMAGING

Anne-Marije Van Kuijen,[1] Can Ince,[2] Cornelis M. Van Drunen[1] and Wytske J. Fokkens[1]

[1]Department of Ear, Nose and Throat Disorders and [2]Department of Anesthesiology, Academic Medical Centre, University of Amsterdam, The Netherlands

The aim of the study was to demonstrate a new, direct and noninvasive method to visualize the morphology of the human nasal microcirculation in vivo using Sidestream Dark Field (SDF) imaging.

The SDF imaging (Microvision Medical, Amsterdam) technique is incorporated into a small portable, handheld imaging device which emits green light. As this light is specifically absorbed by the hemoglobin containing red blood cells, the microcirculation can be studied in great detail. Ten healthy volunteers were included and images from the microcirculation of the nasal mucosa of their nasal septum and the head of the inferior turbinates were obtained. Also, the microcirculation was monitored after local cocaine application.

It appeared possible to obtain clear, real-time images of the nasal microcirculation by using the SDF imaging technique. The images show distinct architecture between the nasal septum and the inferior turbinate. Direct microcirculatory reactions to vasoactive agents can be visualized.

SDF imaging offers a noninvasive approach with unique imaging of the nasal vascularization, and allows us to assess the microcirculation *in vivo* at different places in the nose and under different conditions. Since it is a handheld noninvasive technique it is suitable to be used in a clinical setup. It provides us with new insights into the three dimensional organization and function of the nasal microcirculation. Further assessment of the microcirculation will afford us with detailed information into the pathophysiology of the nasal microcirculation.

54

IS NASAL HYPERREACTIVE RESPONSE TO NON-ISOTONIC AEROSOL RELATED TO THE LEVEL OF INFLAMMATION?

Krešo Zurak, Davor Vagić, Tomislav Baudoin, Darko Solter and Livije Kalogjera

Department of Otorhinolaryngology and Head and Neck Surgery, Sestre Milosrdnice University Hospital, Zagreb, Croatia

A significant increase in nasal resistance is observed in patients with allergic and non-allergic rhinitis after nasal inhalation of distilled water mist.

The purpose of the study was to compare hyperreactive response to nasal distilled water provocation in the patients with allergic and non-allergic hyperreactive rhinitis with the level of inflammatory cell activation markers prior to provocation.

The study was performed on a group of 78 patients with nasal hyperreactivity according to patient history, 48 patients with allergic rhinitis and 30 patients with non-infectious non-allergic rhinitis (NINAR). The concentrations of eosinophil cationic protein (ECP), myeloperoxidase (MPO) and tryptase (TRY) were measured in the nasal lavage prior to provocation. Basal nasal airway resistance (NAR) was measured by active anterior rhinomanometry, and nasal lavage with 5 ccm of saline was taken. The provocation was made by inhalation of 10 ccm of distilled water during 10 minutes.

The patients were divided in three subgroups according to NAR increase (0, 50%, 100%). Tryptase concentration in the nasal lavage and a relative eosinophil number were significantly higher in allergic than non-allergic patients. No correlation was found between the relative number of eosinophils and degree of nasal hyperreactivity. Likewise no correlation was found between any of the cellular markers and level of nasal hyperreactivity, and no difference in marker levels between the groups. The correlation between ECP and MPO in nasal lavage was significant.

Since there is no correlation between inflammatory cell activation and the degree of nasal hyperreactivity, it may be concluded that neural reflexes leading to nasal congestion after non-isotonic aerosol inhalation are not dependent on the level of inflammation measured by the activation of granulocytes and mastocytes.

55.

THE EUROPEAN POSITION PAPER ON RHINOSINUSITIS AND NASAL POLYPS (EP3OS)

Wytske Fokkens

Department of Otolaryngology and Head and Neck Surgery, Academic Medical Center, Amsterdam, The Netherlands

The European Position Paper on Rhinosinusitis and Nasal Polyps (EP3OS) group, a task force of the European Academy of Allergology and Clinical Immunology (EAACI), has issued updated guidance on the treatment of acute and chronic rhinosinusitis and nasal polyposis. Rhinosinusitis is a significant and increasing health problem which results in a large financial burden on society. The new guidelines offer the most comprehensive evidence-based recommendations on the diagnosis and management of rhinosinusitis and nasal polyposis for primary care physicians, specialists on ear, nose and throat (ENT), and non-ENT specialists.

Developed by a broad group of both primary care physicians and specialists to update the knowledge of acute and chronic rhinosinusitis and nasal polyposis, the EP3OS guidelines include a review of diagnostic methods and treatments, propose a step-wise approach to the management of the disease, explore new findings on how rhinosinusitis develops and consider how we can make progress with continued research in this area.

Rhinitis and sinusitis usually coexist and are concurrent in most individuals; thus, the correct terminology is now rhinosinusitis. Rhinosinusitis (including nasal polyps) is defined as inflammation of the nose and the paranasal sinuses characterised by two or more symptoms, one of which should be either nasal blockage/obstruction/congestion or nasal discharge (anterior/posterior nasal drip), +/- facial pain/pressure, +/- reduction or loss of smell; and either endoscopic signs of polyps and/or mucopurulent discharge primarily from middle meatus and/or; oedema/mucosal obstruction primarily in middle meatus, and/or CT changes showing mucosal changes within the ostiomeatal complex and/or sinuses. The paper gives different definitions for epidemiology, first line and second line treatment and for research. Furthermore the paper describes the anatomy and (patho)physiology, epidemiology and predisposing factors, inflammatory mechanisms, evidence based diagnosis, medical and surgical treatment in acute and chronic rhinosinusitis and nasal polyposis in adults and chil-

dren. Evidence based schemes for diagnosis and treatment are given for the first and second line clinicians. Moreover attention is given to complications and socio-economic cost of chronic rhinosinusitis and nasal polyps. Last but not least the relation to the lower airways is discussed.

56

IMMUNE MECHANISMS OF THE LINK BETWEEN ALLERGIC RHINITIS AND ASTHMA AND THE ARIA UPDATE 2008

Ruby Pawankar

Nippon Medical School, Tokyo, Japan

Allergic rhinitis (AR) and asthma are the most common atopic diseases with an increasing prevalence to epidemic proportions. IgE-mediated inflammation of the airways can either manifest as AR, asthma or both. However, many patients with asthma have rhinitis, and rhinitis is a major risk factor for asthma and allergic rhinitis often precedes asthma. The increasing evidence on the links between allergic rhinitis and asthma comes from epidemiologic, immunologic and clinical studies. Epidemiologically, up to 40% of patients with ArR also have asthma, and up to 80% of patients with asthma experience nasal symptoms. Moreover, AR is linked to other comorbid conditions: like rhinosinusitis, nasal polyps and otitis media with effusion.

Both AR and asthma are chronic inflammatory diseases of the airways and their inflammatory mechanisms are characterized by an inflammatory infiltrate made up of eosinophils, T cells, and mast cells that release several mediators, chemokines and cytokines, local and systemic IgE synthesis, and a systemic link via the bone marrow. Studies have shown that patients with AR exhibit bronchial hyper-responsiveness (BHR) and increase in inflammatory cells, and that nasal allergen challenge further increases this hyper-reactivity. Results of several retrospective studies indicate that, for patients with asthma, the presence of comorbid allergic rhinitis is associated with higher total annual medical costs, as well as increased likelihood of asthma-related hospital admissions and emergency visits. These findings highlight the potential for improving asthma outcomes by treating co-morbid AR. This potential link may be due to cross talk between the upper and lower airway, the direct impact of inflammatory mediators released locally and to the systemic link between the two. Moreover, the impact on costs, frequency of emergency room visits, hospitalization and quality of life is greater if AR is associated with asthma as compared to asthma alone. Treating AR in patients with asthma and co-morbid AR has shown to reduce hospitalization by 61%.

Based on the strength of the above, the World Health Organization (WHO) established the first ever evidence-based position paper on rhinitis devoted to the relationship between rhinitis and asthma and its therapeutic implications. This guideline entitled ARIA: 'Allergic Rhinitis and its Impact on Asthma' emphasizes the importance of treating allergic rhinitis and asthma globally as 'One Airway One Disease'. Management of allergic rhinitis comprises of patient education, allergen avoidance, pharmacotherapy and validated allergen specific immunotherapy. The pharmacological treatment of allergic rhinitis proposed by ARIA is an evidence-based and step-wise approach depending on the classification of the symptoms. The ARIA workshop report published in 2001 has been updated and the pharmacotherapy update includes new information since 2000 on existing therapies as well as newer therapies like anti-IgE mAb and antileukotrienes with a new level of evidence for pharmacotherapy. Successful management of both rhinitis and asthma requires an integrated view of the airways, and an integrated approach of treatment.

57

UPPER AIRWAY IN CF AND PCD: DIAGNOSTICS AND TREATMENT

Andrew Bush, Royal Brompton Hospital & Imperial College, London, UK

Upper airway disease is common in normal children; they may have >10 colds a year, with symptoms lasting >2 weeks, and >3 episodes of acute otitis media; so careful selection for diagnostic testing is needed. Upper airway presentations of PCD typically include early onset, persistent rhinitis; severe chronic secretory otitis media, with prolonged otorrhea if tympanostomy tubes are inserted; and sinusitis. Nasal polyps are rare in my experience. There may be additional clues to the diagnosis, including heterotaxy, chronic wet cough. A positive family history, and other manifestations of ciliopathy. In CF, the ears are largely spared, but nasal polyps are common, as is sinusitis; indeed, the sinuses are

rarely normal radiologically in CF. Additional clues include chronic wet cough, abdominal and other associated CF-related problems, and a positive family history. CF can usually be diagnosed on a properly performed sweat test, although there may be false positives with eczema, or if the operator is inexperienced. Ancillary testing includes CF genetics, and nasal potential differences. PCD is diagnosed by first eliminating other diagnostic procedures as appropriate; screening with nasal nitric oxide, the saccharine test or an *in vivo* test of mucociliary clearance; but definitive testing requires a nasal brush biopsy, with measurement of ciliary function by light microscopy, and structure using electron microscopy. In difficult cases, ciliary culture or genetic studies may help. It is essential to distinguish PCD from secondary ciliary dysfunction due to viral infection or pollution. Treatment of CF upper airway disease is topical nasal steroids for polyps, and largely medical for symptomatic sinusitis. In PCD, tympanostomy tube insertion should be avoided, and if hearing loss is severe, hearing aids may be needed for short periods; however, as the child gets older, chronic secretory otitis media will improve. Topical nasal therapy with saline douches may be beneficial. In both conditions, attention should be paid to any allergic upper airway disease, along conventional lines.

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Examples of reference citations are listed.

EXAMPLES OF REFERENCE CITATIONS

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