Asma

AN ALLERGIC MODEL IN RATS PRESENTING AIRWAY EOSINOPHILIA, LUNG EOSINOPHIL ACTIVATION AND PULMONARY HYPERREACTIVITY.

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The aim of this study was to examine antigen-induced lung cell migration, eosinophil activation and pulmonary reactivity to a new sensitization technique. Wistar rats were sensitized with a single subcutaneous implant of a fragment of heat coagulated hen egg-white (EWI, 200 mg) and challenged 21 days later with an intratracheal injection of heat-aggregated ovalbumin (2 mg). For comparison, a group of rats was sensitized with ovalbumin (OVA, 20 µg) using alumen (10 mg) as adjuvant, boosted on day 14 and challenged on day 21 postimmunization with soluble OVA. Twenty four hours later, the EWI sensitized animals presented bronchopulmonary inflammation with a marked increase in the number of eosinophils in the BAL, increased levels of eosinophil peroxidase activity in cell free BAL and in homogenates of lung tissue and increased levels of thromboxane B2 and prostaglandin E2 in the BAL. In the OVA/AL sensitized group the eosinophil infiltration was significantly less intense and the levels of prostanoids were lower. The EWI, but not the OVA/AL group, developed increased pulmonary reactivity to serotonin (i.v.) 24 h after challenge. The extent of eosinophil infiltration and activation into the lungs and the pulmonary hyperreactivity induced by this novel sensitization procedure without adjuvants represent a significant improvement over existing experimental models of asthma.

EFFECT OF NITRIC OXIDE ON PULMONARY HYPERREACTIVITY AND AIRWAY INFLAMMATION IN ALLERGIC RAT MODELS.

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Our study evaluated the role of nitric oxide (NO) in two allergic rat models of lung inflammation. In the first model (OVA/AL group), rats were sensitized with an i.p. injection of ovalbumin (OVA; 10µg) using aluminium hydroxide (10 mg) as adjuvant and challenged with OVA (100 µg i.t.) 21 days later with one booster injection of 20 µg of OVA i.p. in the 14th day. In the second model (EWI group), rats were sensitized with an implant of solidified egg-white (200 mg, s.c.) and 21 days later challenged with heat-denatured ovalbumin (20 mg i.t.). After challenge, a predominant neutrophilic infiltration was observed in the lungs of OVA/AL group. Increased *in vitro* bronchial reactivity to carbachol and serotonin was also observed at 8 h but not 24 h after challenge. In the EWI group, pulmonary hyperreactivity to serotonin (i.v.) and intense eosinophil migration were observed 24 h after antigen challenge. Pretreatment of the rats with NO synthase inhibitors L-NAME or aminoguanidine (100 mg/kg; s.c.) during 3 days before antigen challenge did not affect the increased pulmonary reactivity nor the neutrophil infiltration in the OVA/AL group whereas it totally inhibited eosinophil migration and pulmonary reactivity in the EWI group. Our results suggest the involvement of NO in eosinophil-dependent lung allergic inflammation.

ANTISENSE OLIGODEOXYNUCLEOTIDES SPECIFIC FOR MURINE EOTAXIN AND INTERLEUKIN-5 PARTIALLY REDUCES ANTIGEN-INDUCED LUNG EOSINOPHILIA.

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Eosinophils are proinflammatory cells thought to make an important contribution to tissue damage and bronchial hyper-responsiveness in allergic inflammatory diseases such as asthma. Interleukin-5 (IL-5) and the eosinophil-selective chemoattractant eotaxin act cooperatively to induce blood and tissue eosinophilia in vivo (J Exp Med 1995;182:1169). In this study, we evaluated the possibility that i.v. administered antisense oligodeoxynucleotides (ODNs) specific for eotaxin and/or IL-5 could reduce eosinophil accumulation in the lungs of ovalbumin (OA)-sensitized CD-1 mice exposed to aerosolized OA. Eotaxin and IL-5 antisense ODNs, formulated with unilamellar cationic liposomes composed of DOPE/DOTAP (1:1 mol ratio), were administered i.v. 2 h after challenge of OA-sensitized mice by inhalation of an aerosol of OA for 20 min generated from a solution of 2% OA in saline. Eosinophil numbers in lung tissue were quantitated by measuring eosinophil peroxidase. Total lung eosinophils (bronchoalveolar lavage and lung tissue) were reduced by 58% from 75×10^5 to 3.8×10^5 eosinophils/lung in animals treated with control and both eotaxin and IL-5 antisenses respectively ($n = 3$; $p < 0.05$). These results correlated with a reduced IL-5 mRNA density (expressed as a percent of GAPDH), as measured by a semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) assay. The results suggest that this approach may be a potentially useful strategy to reduce lung tissue eosinophilia.

COMPARING SCINTIGRAPHIC PULMONARY DEPOSITION USING A JET AND AN ULTRASONIC NEBULIZERS.

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Purpose: The aerosols have become essential therapy in reversion of airway obstruction and other conditions that affect the respiratory tract. There are various controversies about the aerosol generator and its deposition in the tracheobronchial tree. The purpose of this study was to analyze the pulmonary deposition of the aerosol particles in the airways generated by a jet nebulizer compared to the ones generated by an ultrasonic nebulizer. Methods: 10 young (mean age = 23.1 ± 1.79) and healthy volunteers were submitted to pulmonary scintigraphy in two phases. The phase one was performed by a jet nebulizer (flow = 8 l/min) and the phase two with an ultrasonic nebulizer. The deposition was analyzed considering the total numbers of counts in each lung comparing the results in the two phases. Results: the average of the total number of counts for the right posterior ROI was 184873 + 31618,08 for the jet nebulizer and 115017,60 + 22072,85 for the ultrasonic nebulizer. For the left posterior ROI we had an average total number of counts of 177932,40 + 32764,80 for the jet nebulizer and 113873,50 + 24679,24 for the ultrasonic. The total

number of counts in both lungs were significatively greater when using the jet nebulizer ($p < 0.0001$). Conclusion: the results suggest a better aerosol deposition generated by the jet nebulizer compared to the ultrasonic.

MEASURING DEAD VOLUME WITH RATE FLOW VARIATION FROM FIVE DIFFERENT JET NEBULIZERS.

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Purpose: inhalation techniques are widely used aiming its deposition into the respiratory tract. The goal of this study was to assess the influence of flow rate variation on dead volume (DV) in different jet nebulizers. Methods: five different jet nebulizers (from four different brands) were tested, which principle of aerosol generation is promoted by the Bernoulli effect. After being weighted nebulizers were attached to an oxygen cylinder and connected to a manometer and a flow meter. Solution of NaCl 0,9% was used and the duration of nebulization was 20 min. With different flow rate variation (5, 7, 11 and 13 L/min). After 10, 15 and 20 minutes all of them were reweighted. Dead volume was considered as a difference between the solution placed into the nebulizer chamber before and after nebulization. Results: raising the airflow rate from 5 to 7 L/min dead volume variation was found between 1.16 ± 0.3 to 2.81 ± 0.2. Nebulizers 2 and 5 didn't reach flow rate of 11 L/min. It could be a result from jet collar attachment and design of the device. The nebulizer 4 presents a jet collar shaped like a cone, possibly permitting increased dead volume. Conclusion: increasing the duration of nebulization DV rose suggesting that nebulizers should be used for a period of time less than 10 minutes and flow rate variation between 5 to 7 L/min. Jet collars and nebulizer design were determinants to raise DV.

ANALYZING SCINTIGRAPHIC AEROSOL DEPOSITION COMPARING DIAPHRAGMATIC AND 3 TIMES FRACTIONAL INSPIRATION PATTERNS.

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Purpose: the regional ventilation through muscular ventilatory patterns (MVP) is used for respiratory physical therapy as a therapeutic way in front of pulmonary disease. The goal of this study was to assess quantitatively the VMP through muscular ventilatory pattern with three fractional inspiration using pulmonary scintigraphy to analyze the regional distribution in patients with both sexes and different shapes of the thorax. Method: 20 healthy subjectives were studied, 10 men and 10 women, mean of age 24.85 ± 5.11, subdivided in 4 groups and classified according to Godin Thoracic Index (GTI). This study was divided in 2 parts: Phase A, control/diaphragmatic inspiration pattern – Phase B, ventilatory pattern with three fractional inspiration. Between both phases there was an interval of 3 days. It was used ^{99m}Tc-DTPA, inhaled during 10 min. Sitting and submitted were limited to 200.000 counts. Results: the significant pulmonary segments analyzed were: 1/3 left lower segment of group A ($p < 0.0002$), 1/3 right upper segment of group B ($p < 0.01$), 1/3 right lower segment group C (p,0.03) and 1/3 left upper segment of group D ($p < 0.01$). Conclusion: the scintigraphic results obtained suggest that MPV were found as reexpansive and subjects with higher GTI seemed to use 1/3 upper of both lungs the diaphragmatic pattern.

MURINE AIRWAYS SHOW ENHANCED RESPONSES TO THE ASSOCIATED ALLERGEN AND LPS, WHICH MAY BE RELEVANT TO HUMAN DISEASE.

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The intranasal (i.n.) instillation of antigen (ovalbumin, OVA) to hyper-IgE BP2 Biozzi mice induces bronchopulmonary hyperreactivity (BHR) (Eum et al., PNAS, 92: 12290, 1995; Hailé et al., AJRCMB, in press). Since LPS increases asthma severity (Michel et al., AJRCCM, 154: 1641, 1996) and induces BHR (Lefort et al., JI, 161, 474, 1998) we developed a model for LPS-antigen synergism, which is extensible to other pollutants. BP2 mice immunized s.c. with 100 µg OVA in Al(OH)3 at days 1 and 7 were challenged intranasally (i.n.) at day 14 with OVA (1-10 µg) and/or *E. coli* 055:B5 LPS (0.01-0.1 µg). The association of subeffective doses of OVA (1 µg) or LPS (0.01 µg) induced intense BHR and 30-100 fold increased titers of TNF-α in the BALF after 3-24 hours, titers of IL-5 and IL-4 being unaffected. Neutrophil and eosinophil counts in the BALF showed a tendency to increase. OVA and LPS were next instilled sequentially, at a 3 h interval, with readings at 6 or 24 h, to verify which primes for which. Both BHR and the production of TNF-α were markedly enhanced for the sequence OVA-LPS, but were as controls for LPS-OVA. The possibility that OVA turns more LBP available to LPS by augmenting vascular permeability was shown to be unlikely. mrTNF-α, one of the mediators of LPS, instilled i.n. at 10 ng did not induce BHR but did so if associated to 1 µg OVA. Anti TNF-α antibodies suppressed the enhanced effects of OVA by TNF-α, but not those of OVA and LPS, suggesting the participation of other mediators. A model was thus developed in which synergism between antigen and LPS for BHR and TNF-α production was shown, TNF-α replacing LPS as a co-stimulator with OVA, but not accounting for the synergized effects. Subeffective doses of antigen may synergize with other pollutants, with far-reaching environmental consequences.

ASTHMATIC INDIVIDUAL'S AWARENESS AND LIFE QUALITY.

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Asthma, a chronic respiratory disease in which the therapeutic approach has been submitted to a change since inflammatory component was evidence. Nevertheless, although the morbidity and mortality rates have always been low, these changes were expected to promote a better life quality (LQ) to these individuals. Conversely, the latest epidemiological studies have shown a slight but progressive gradual increase of these rates. One of the many questions the asthma initiative programs aim at answering is the importance of the asthmatic individuals participation in these rates. In order to evaluate the impact of asthma awareness (AA) a protocol has been developed; a group of 67 asthmatic individuals classified with intermittent (I) or persistent (P) asthma levels – I mild (n = 16); P mild (n = 29); P moderate (n = 10); P severe (n = 12) (ATS, 1997) were submitted to a metacholine bronchoprovocative test and to questionnaires of life quality (Woolcock, 1992) and asthma awareness (Gibson, 1995). Out of a maximum score of 2000 pts, the life quality was classified as good (75%) satisfactory (SAT < 75%/> 50%); non satisfactory (NON SAT. < 50%). The results were compared by variance analysis and correlation rank test of Spearman ($p < 0.05$).

LQ	Good	Sat.	Non-sat.	Value
N	29	21	17	$p < 0.05$
PC_{20}	6.48 mg/ml	8.9 mg/ml	1.51 mg/ml	$p < 0.02$
LQ x CP_{20}	–	–	–	r = NS
LQ x AA	–	–	–	r = NS
Minimal AA	+ +	+ +	Absent	$p < 0.02$

Conclusion: no correlations were found between AA and asthmatic levels. The highest bronchial responsiveness was in the NON-SAT. LQ level group. LQ level loss was related to the absence of minimal awareness.

SUBMUCOSAL COLLAGEN CONTENT IN PERIPHERAL AIRWAYS OF FATAL ASTHMA.

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Subepithelial fibrosis due to collagen deposition is a well established feature of asthma. The functional consequences of the subepithelial thickening are not fully understood. It could represent a protective mechanism against excessive airway narrowing, but on the other hand it could contribute to the increase in thickness of the airway wall and potentialize the effects of smooth muscle (ASM) contraction on lumen caliber. Lack of distensibility has been reported in asthmatic airways. Submucosal fibrosis could contribute to such a feature. This region of the airway is involved in the asthmatic inflammatory process and there is no reason to believe that remodeling would not happen at this level. Conversely, scarring type fibrosis is not observed in asthmatic airways as in other inflammation lung diseases, and it has been suggested that antifibrinogenic pathways could also be activated in asthma. Increase in submucosal type III and V collagens in large airway of biopsies of asthmatic patients has already been reported. In this study we analyzed the collagen content in the submucosal region of peripheral airways (PA) in 20 cases of fatal asthma (FA) and 8 control subjects (C), Slides from PA were stained with Sirius Red, that stains selectively the fibers of the collagenous system. Submucosal collagen content was assessed in the region underneath the BM to the internal limit of the ASM, by means of image analysis. Results: there was no statistically significant difference between collagenous fibers content between FA and C (3.73 ± 0.46 µ and 3.24 ± 0.43 µ, respectively, $p = 0.52$). Our results show that there is no increase in submucosal collagen content in PA of FA. We suggest that collagen deposition in the submucosa is not uniform in the asthmatic airway and could be centered in large airway.

NEUROKIN RELEASE INDUCES EOSINOPHIL RECRUITMENT IN THE AIRWAY WALL: ROLE OF NK_1 AND NK_2 RECEPTOR ANTAGONISTS.

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Capsaicin (Cap) administration results in release of neurokinins (NK) such as substance P and NKA and their effects are mediated mainly through NK1 and NK2 receptors. There are some evidence that NK may affect inflammatory cell function in allergic diseases. We assessed the airway wall kinetics of eosinophil recruitment 30 min, 2 h, 6 h and 24 h after Cap infusion (10 µ/kg) in anesthetized guinea pigs (GP). Control animals were studied after vehicle (Ve) infusion. In the group studied after 30 min of Cap infusion, we also evaluated the effects of non-peptide antagonists of NK1 (SR140333) (300 nmol/kg) and NK2 (SR48968) (100 nmol/kg) receptors. GP were anesthetized, tracheostomized and mechanically ventilated. Tracheal pressure and airflow were recorded and respiratory system resistance (Rrs) was obtained. A histochemical method for eosinophil peroxidase activity was performed. Maximal values of Rrs after Cap infusion were significantly attenuated by SR 140333 and SR48968 ($p < 0.001$). There was a significant increase of eosinophils around the airway wall after 30 min that persisted until 24 h of Cap infusion [medians and percentiles: 0.45 (0 to 0.9), 2.07* (1 to 3.3), 1.9* (1.3 to 4), 3.5* (2.7 to 5), 1.5* (0.6 to 3.7) cells/10^4 µm^2, respectively: Ve, 30 min, 2 h, 6 h and 24 h] (*$p < 0.001$) compared with Ve group. The eosinophil recruitment was lower in GP that received SR140333 ($p < 0.001$). Our results suggest that both NK1 and NK2 receptors exert a significant role on the increase in respiratory system resistance. However, the recruitment of eosinophils around the airway wall of GP was predominantly induced via NK1 receptor activation.

OUTCOME OF A QUESTIONNAIRE OF ASTHMA GUIDELINES UTILIZED BY MEDICAL STUDENTS.

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Sanguinetti et al. (Chest 1997;11:A35S) have previously show that the education for a compliance of international guidelines for bronchial asthma, published in the Global Initiative for Asthma, is important for physicians. Purpose: we did a questionnaire about some items in asthma management for intern students in the sixth year to know if these students complied this guidelines and if they remembered their classes. Methods: 100 students were request to fill in a questionnaire about some items in asthma management. Results: they marked, in order of importance for each questions, about diagnosis procedures and the order to importance for each questions, about diagnostic procedures and the treatment of asthma attack.

Diagnosis	%	Diagnosis	%
History	94	Spirometry	43
Family history	56	Challenge	45
Physical signs	61	Prick tests	30
Chest x ray	69		
Treatment of asthma attack	**%**	**Treatment of asthma attack**	**%**
B2 Ai	96	Ci	18
B2 Ao	8	Cs	32
		Theophylline	24

Conclusion: more time, attention and exhaustive review would be necessary for this students to comply the guidelines and use them correctly.

THE DOCTORS AND THE STUDENTS KNOW HOW TO USE THE METERED DOSE INHALER WITH A CORRECT TECHNIQUE?

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Purpose: the aim of our study is to know if the intern students and doctors know to use the metered dose inhaler (MDI) with the correct technique. Methods: we studied 100 intern students of University of Santo Amaro – Brazil, and 100 doctors who work in this University and were requested to fill a questionnaire about how use the MDI. Results: the following results were observed and the right answer for each group in percentage is listed below:

Principles of good technique	Students (%)	Doctors (%)
1. Shake the inhaler	20	31
2. Remove the cap	68	82
3. Put the MDI between 2 to 4 cm to the mouth	44	40
4. Keep your mouth open	60	60
5. Sit upright	32	33
6. Exhale fully	33	42
7. Press the top of the MDI	40	35
8. Inhale slowly for five seconds	48	32
9. Hold the breath for ten seconds	27	16
10. Exhaling normally	14	11
11. Wait 30 seconds if you should be another dose	7	5

Conclusion: although the students and the doctors had knowledge on how to use the MDI, when we used questionnaire to check this, the answers were not precise and in many principles were incorrect.

MODULATION OF EOSINOPHILIC BRONCHITIS IN ASTHMATICS BY INHALED BECLOMETHASONE DIPROPIONATE (BDP) IS ACCOMPANIED BY INCREASED NEUTROPHILIA.

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In asthma, strong evidences point out to the central role that eosinophils play in the development of desquamative bronchitis, one of the key aspects of the disease. The aim of this study was to evaluate the effect of inhaled corticotherapy on eosinophilic bronchitis of asthmatics. We studied different markers of inflammation in induced sputum before and after the use of inhaled BDP. We included 21 stable asthmatics, adults, nonsmokers and without recent episodes of respiratory infections, that signed informed consent. The sputum was then dispersed with dithiothreitol, centrifuged, the supernatant collected in Epperndorfs and frozen, and the cell pellet washed twice. Cytospins were stained with Diff-Quik and differential cell counts of at least 400 non-squamous cells performed. Analysis of the cellularity of sputum 30 days after star of BDP showed a marked reduction in the proportions of eosinophils (13.4% ± 2.84 vs. 2.5% ± 2.41; $p = 0.001$). However, measurements of eosinophils cationic protein (ECP) in the sol phase failed to show significant change (173.0 ng/ml ± 15.0 vs. 133.4 ng/ml ± 12.6; $p = 0.078$). With recent reports that ECP can be produced by other cell types, including neutrophils, we compared the sum of eosinophils and neutrophils before and after treatment and found no change (31.9% ± 1.61 vs. 28.2% ± 4.75; $p > 0.05$). We concluded that inhaled BDP, despite a marked effect on eosinophils, induced increase in neutrophils in the airways. It has been recently suggested that this action could be due to a blockage in neutrophil apoptosis.

INDUCIBLE NITRIC OXIDE SYNTHASE (NOS2) EXPRESSION BY SPUTUM INFLAMMATORY CELLS OF ASTHMATICS: MODULATION BY BECLOMETHASONE DIPROPIONATE (BDP).

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Nitric oxide (NO) is a key player in the pathogenesis of the inflammatory events accompanying asthma. NOS2, the enzyme isoform associated with the inflammatory process, has been identified in bronchial epithelial cells of asthmatics. The aim was to investigate expression of NOS2 by inflammation cells from airways of asthmatics and its modulation by inhaled BDP. We included 18 stable asthmatics (mild = 7; moderate = 8; severe = 3), all nonsmoking adults without history of recent respiratory infections or use of corticosteroids. After signing Informed Consent, they started inhaled BDP (1000 µg/day). Sputum was induced before the start of BDP and at days 30 and 90 of treatment. Cytospins were prepared and stained by APAAP with the polyclonal antibody NO53. Optical density of the staining was measured with the software *Image Pro Plus®*. ANOVA for repetitive measures and the Bonferroni test were used to calculate changes of densities with treatment. The optical density of the staining for NOS2 was significantly reduced at day 30, as compared with day 0 ($p < 0.01$). This finding persisted at day 90. This way paralleled by improvement of all clinical and functional parameters. Stratification by severity failed to show any difference between mild and moderate/severe case. These results demonstrate the inhibitory effect of BDP on the expression of NOS2 by inflammatory cells. They also reinforce the possible role of NO in the inflammatory events leading to deterioration of clinical and functional parameters of asthmatics.

RESPIRATORY FUNCTION IN NORMAL AND ASTHMATIC INDIVIDUALS: EFFECTS OF THE USE OF POSITIVE EXPIRATORY PRESSURE.

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Purpose: verify the effect of the use of PEP (positive expiratory pressure) associated to bronchodilator (Bd) inhalation. Methods: six healthy individuals (25.2 ± 1.6 years old) and six asthmatic patients (26.0 ± 5.2 years old) were studied through pulmonary function tests, topographic distribution and inhaled radioaerosol retention (RR). The groups were submitted to basal inhalation treatment (^{99m}Tc colloidal sulfar in 0.9% NaCl). 0.1 mg of terbutaline/kg was administered to the aerosol in the asthmatic for the studies with the use of Bd. During the use of PEP, both groups exhaled through pressure valve (10 cmH_2O) connected to the expiratory unidirectional valve of the mask. Patients inhaled radio aerosol for 10 minutes in sitting position. Results: it was observed that the spirometric values were significantly greater at 90 minutes than those at the first minute after the treatment with Bd associated to PEP. The ratio between the counts of the central and peripheral lung regions (C/P) was significantly greater in the asthmatic patients, with significantly higher in the inferior region only after the treatment with Bd associated to PEP. Conclusion: these results show an additional reduction of the resistance of the airway along the time even in the presence of a sufficient dose of Bd possibly by the higher RR basal lung regions.

FOLLOW-UP STUDY OF RETINOL SERUM LEVELS IN ASTHMA PATIENTS DURING DISEASE EXACERBATION AND STABILITY.

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Serum retinol, an essential nutrient for epithelial cell maintenance and repair, is reduced during the inflammatory process. In this study, serum levels of retinol, variables related to body composition and handgrip strength were evaluated in 11 asthma patients (age range = 17-67 years) during periods of exacerbation of the illness. Patients were evaluated on admission (D0), 24 hours (D1) and 10 days (D10) after starting treatment with corticosteroids (cort, given during 10 days), and 30 days after cort withdrawal, when patients were asymptomatic and only on aerosol maintenance medication. Nine healthy volunteers, matched for age and body mass index (BMI), were used as a control group. Results: serum retinol concentration (mean ± SD): D0 = 39.3 ± 16.5 µg/dL, D1 = 37.4 ± 13.5, D10 = 49.0µ16.5, D30 = 43.4 ±

13.5; values for normal subjects were 51.4 ± 37.4 µg/dL. Statistical analyses showed retinol levels at D10 to be significantly higher when compared to D0, D1 and D30 ($P < 0.05$). In comparison with the control group, lower levels were observed in asthma patients in D1. Values for body weight, BMI, fat-free-mass and handgrip strength did not show significant changes during the period of study. In conclusion, a fluctuation of the serum retinol levels was observed during this study; higher levels of retinol were seen after 10 days-treatment with oral corticosteroids. Low retinol levels as part of the inflammatory process might contribute to pulmonary dysfunction in asthma patients, due to impaired delivery of vitamin A to the lung.

A GENOME WIDE SEARCH FOR LINKAGE TO ASTHMA-ASSOCIATED TRAITS IN THE BIOZZI BP2 MOUSE MODEL OF ALLERGIC ASTHMA.

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Following immunization and challenge with ovalbumin (OVA), the Biozzi BP2 mouse shows features of human asthma which include airway inflammation, eosinophil infiltration and bronchial hyper-responsiveness. In order to identify genetic loci influencing these asthma-associated traits, a cross was made between BP2 and Balb/c mice. An F1 intercross produced 217 F2 mice. Mice were immunized with OVA subcutaneously, and challenged intranasally with OVA in NaCl solution. Airway resistance (Penh, Enhaced Pause) was measured in a whole body plethysmograph chamber. Lungs from 112 of the F2 mice were submitted for histology and counting of eosinophils in the bronchial epithelium (EP). Genetic linkage was sought to a panel of 180 microsatellites. The results of the genome screen were:

Trait	Mouse chromosome	Lod score	% variance explained	Human homology	Human synteny & candidate genes
Penh	9	2.5	5.2	11q23	Chr11q23IL-10R
Penh	10	3.8	8.3	12q22-24	Chr12q22-24IFN -R
EP	11	3.4	12.9	incl.5q31	Chr5q21Il4 cluster
Penh	11	3.65	7.5	17q21-22	iNOS, Eoxatin cluster
Penh	17	2.1	4.4	6p21	HLA, TNF

The study identified several loci influenced allergen-induced bronchial responsiveness and airway eosinophil infiltration which are synthetic to human loci influencing asthma.

SYSTEMATIC EVALUATION OF DIARY CARDS FOR DIAGNOSIS OF ASTHMA EXACERBATION.

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Aim: to detect which clinical marker among symptom score, peak expiratory flow and number of rescue inhalations (diurnal and nocturnal), usually collected on diary cards, should be a useful predictor for asthma exacerbation. Methods: diary cards from 64 moderate asthma patients, who participated in clinical trials were reviewed. Exacerbation was considered when a course of oral steroid was prescribed during the trial. A systematic evaluation of markers registered on routine diary cards, 7 days before the exacerbation (event) was compared with diary cards from patients participants of the same trial where the event did not occur (control). Results: 26 exacerbation events and 38 controls were analyzed. An average of rescue inhalations, an index of symptom score and percentage of variation of peak expiratory flow related to the best value of the patient was calculated. Each marker was classified present or absent, as a predictor of an exacerbation event. These independent variables were included in a logistic regression model to detect the best predictor of the event (exacerbation). A multivariate analysis (MVA), by SAS, identified the nocturnal symptoms score (chi-square = 18.58, p = 0.000) and the diurnal number of rescue inhalations (chi-square 7.46, p = 0.0063) as significant risk factors for exacerbation in patients with moderate asthma. Conclusion: nocturnal symptoms score associated with diurnal number of rescue inhalations are the best clinical markers for exacerbation in patients with moderate asthma and should be used indaily clinical practice.

INDUCED SPUTUM INDICES IN MILD INTERMITTENT ASYMPTOMATIC ASTHMA.

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Airway inflammation is a cause of symptoms and current severity of asthma. Mild intermittent asthma (MIA) refers to a group of patients with infrequent symptoms and normal post-bronchodilator FEV_1. They are regarded not to warrant any other asthma treatment than in inhaled short acting $_2$-agonist as needed. However, the presence and characteristics airway inflammation in this group have received little study. Nine subjects [mean (range) 24(11-36) years] with MIA were consecutively examined. All with MIA had a history of intermittent symptoms of asthma which were controlled only with 2 puffs of bronchodilator twice per week. They were atopic or non-atopic, nonsmokers and the asthma was objectively confirmed by an improvement in FEV_1, from predicted of 15% or 0.2 L after salbutamol (200 µg). None had history of respiratory infection in the last month. Sputum was induced with hypertonic saline and examined for total cell count (TCC), cell viability (V), neutrophils (N), eosinophils (E), macrophages (M), lymphocytes (L). Sputum was processed as previously described (Am J Respir Crit Care Med 1996;154:808-817). Median (interquartile range) values were:

	Spirometry*		Sputum					
	FEV_1%	FEV_1/VC	TCCx10^6/ml	V,%	N,%	E,%	M,%	L,%
MIA	102(12)#	82(4.7)#	3.3(5.4)	79(13)	24(62)	9.2(13)	38(56)	1.6(1.5)
Healthy**	101(10)	83(2)	3.1(4.0)	83(16)	24(27)	0.5(1.1)	63(30)	1.3(1.6)

* mean (SD); ** median values for healthy are given for comparison (AJRCCM 1996;154:808-17); # post-bronchodilator value.

The results indicate that eosinophilic airway inflammation is present in subjects with asymptomatic MIA. The therapeutic implications of this requires further investigation.

CLINICAL SIGNS ARE BETTER IMPROVEMENT MARKERS THAN AIRWAY RESPONSIVENESS IN THE STEROID TREATMENT OF ASTHMATICS WITH RHINITIS.

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Background: patients with asthma frequently have associated rhinitis. Objective: compare the clinical changes with the responsiveness changes in mild to moderate allergic asthmatics with perennial rhinitis. Methods: 25 patients were treated in a randomized, double blind, parallel, four week course of topic nasal or oral beclomethasone (BDP). Histamine challenges and clinical diaries were analyzed before and after BDP. The histamine concentration causing a 20% fall in FEV_1 (PC20) and 25% drop in MIF50 (PC25MIF50) were used as thresholds of bronchial and extrathoracic responsiveness. Results: 12 patients (G1) received nasal BDP and 13 (G2) bronchial BDP. There was no

difference in the mean PC20 of both groups before and after treatment (G1:0.26 and 0.23 mg/ml; G2:0.17 and 0.2 mg/ml). In 60% of all patients the PC25 did not occur until the provocation challenge ended (20% drop of FEV_1). PC25 was positive – mean 0.03 mg/ml – in 4 patients (two of each group) and became negative after treatment. The sum of symptoms decreased after treatment except for nocturnal awake.

	cough b/a	sneeze b/a	wheeze b/a	itching b/a	dyspnoea b/a
G1	18/6	65/42	32/25	41/23	31/26
G2	41/13	43/30	27/9	29/19	43/17

Conclusion: although both groups showed clinical improvement, we could not demonstrate changes in intra or extrathoracic responsiveness.

ADJUVANTS AS INFLAMMATORY RESPONSE POTENTIATORS IN ASTHMA EXPERIMENTAL MODELS: ARE THEY HELPFUL?

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Several adjuvants are used to enhance asthma inflammatory response in experimental models, but the real effect and relative potency of them is not hitherto fully clarified. We compared: aluminum hydroxide ($AlOH)_3$. Complete and incomplete Freund adjuvants (CFA, IFA) in a asthma model in mice. $AL(OH)_3$ induces mainly IgE antibody production and CFA and IFA induce predominately IgG class. Adult A/sn mice were immunized i.m. with ovalbumin (OA) and divided in 4 groups injected i.m. either with $Al(OH)_3$, CFA, IFA or saline; 48 hours after a booster of OA, the animals were anesthetized and submitted to respiratory mechanical measurements during dose-response curves to methacholine i.v. (3 to 10.000 µg/kg). Bronchoalveolar lavage fluid was collected and stained by Leishman method for cytological quantification of eosinophils, neutrophils, macrophages and ciliated cells.

	$ED_{70\%}$Grs	$ED_{70\%}$Cdyn	%Eos	%Ne	%M	%Ci
Sal/1mgOA	2.26 ± 0.4	2.32 ± 0.67	4	1	31	65
Sal/50µgOA	1.34 ± 0.45	1.78 ± 0.53	1.5	6.25	43.5	30.5
$Al(OH)_3$50µgOA	2.49 ± 0.38	1.76 ± 0.73	10.3	7.2	69.4	5
ACF 1mg AO	0.9 ± 0.35*	1.07 ± 0.45	47.5*	7.33	40.3	16.3
AIF 1mg AO	0.9 ± 0.4*	1.68 ± 0.47	48.4*	5.2	39.8	10.2

Values are expressed as means ± SEM of log of 70% of maximal respiratory system conductance ($ED_{70\%}$ Grs) and dynamic compliance ($ED_{70\%}$Cdyn) compared to basal values and percentage of eosinophils (Eos), neutrophils (Ne), macrophages (M) and ciliated cells (Ci). *$p < 0.05$. Our results suggest that CFA and IFA are better adjuvants than $Al(OH)_3$ in this murine experimental model of asthma.