aspartic acid for valine: KIT-D816V (>93%) has been identified. Cutaneous Mastocytosis (CM). Classified in Urticaria Pigmentosa (UP), solitary mastocytoma, diffuse, and telangiectasia macularis eruptiva perstans (TMEP). The most common is the Urticaria Pigmentosa as fixed, reddish brown macular or papular, urticate in physical irritation (Darier’s sign). WHO Diagnostic Criteria for cutaneous Mastocytosis: Presence of at least 1 of skin lesions with Focal dense MC infiltrates (>15 M Cs per cluster) or diffuse (>20 cells per high-power field).

Methods: We report 2 cases of patients with this disease who were not diagnosed at first. A 51 years old female, who noticed 20 years ago, the appearance of tichy “spots” in thorax, abdomen and extremities, progressively increasing in number and size, receiving unspecified treatments without improvement. On examination, we found brown macules with sharp borders, 0.3 to 0.5 cm erythema and Darier’s sign, disseminated lesions on thorax, shoulders and extremities. A 45 year old female, who noticed 2 years ago, the appearance of freckles in neck, arms, thorax and legs progressively increasing in number, who in stress are itchy. Receiving multiple treatments without improvement. On examination disseminated brown macules with sharp borders <0.5 cm with Darier’s sign.

Results: In both patients, the biopsies taken had findings compatible with mastocytosis (inflammatory infiltrate with perivascular lymphocytes, histiocytes and mast cells). Mast cells were not quantified. We realized a genetic study in search of c-kit mutation. Once the diagnosis was considered and treated accordingly, they had a good control of symptoms.

Conclusions: Mastocytosis is diagnosed by clinical features and histological infiltrate of mast cells. The skin is the organ most frequently affected. These patients previously received multiple treatments with no clinical improvement suggest inadequate diagnosis. Histologically, compatible although no quantificate mast cells, but a mutation of c-kit was found. It is important to consider this disease in the differential diagnosis of pruritic skin disorders since an appropriate treatment with an improvement in quality of life also must be aware of the risk of anaphylaxis and its potential triggers.

MECHANISMS OF ASTHMA AND ALLERGIC INFLAMMATION

500 Human Mononuclear Phagocytes Are Regulated by a Cross-talk with Epithelial Cells
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Background: Cell-cell interactions are particularly important for modulating the monocyte to macrophage transition in tissue compartments. Both cell membrane contacts and soluble signals from the environment might be involved in these interactions. The aim of our study was to characterize gene expression profiles of human mononuclear phagocytes induced by a co-culture with epithelial cells.

Methods: Human THP-1 macrophages were co-cultured with A549 epithelial cells either directly or separated by a filter insert. At different time points, THP-1 cells were aspirated and the mRNA expression was evaluated by multiplex Real-time RT-PCR, the release of selected cytokines was evaluated by Lumines technology or ELISA. The phenotype of both cultured cells was evaluated by flow cytometry.

Results: Co-culture with epithelial cells induced a number of cytokine genes (IL-1 beta, IL-6, IL-10, TNF alpha, IL-19, GM-CSF, ...etc) together with upregulation of genes associated with NFkappaB activation including REL, RELB, transcription co-activator BCL3, MALT gene, and NFKB1 subunit. Our recent study has confirmed the role of NFk signalling by inhibition of IL-6 release from co-cultured cells by p65 siRNA transfection. Phenotypic pattern of THP-1 cells co-cultured with epithelial monolayers showed maturation and activation associated changes such as CD14 upregulation associated with higher release of the soluble form (sCD14) from macrophage membrane.

Conclusions: Our data suggest that properties of human mononuclear phagocytes in tissues are highly influenced by their immediate interactions with other, e.g. epithelial cells. These factors might be of particular importance in final steps of differentiation of monocytes/macrophages into fully competent effector cells.

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501 Activation of PAR-2 Induces Myofibroblast Transformation via a TGF-β and GSK-3β/β-catenin Dependent Pathway in Tissue Remodeling in the Asthmatic Lung
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Background: Asthma is a chronic inflammatory lung disease, and airway remodeling denotes the pathophysiologic modifications of normal airway wall structure, including changes in the composition and organization of the airway wall’s cellular and molecular constituents. These structural alterations are largely irreversible in chronic severe asthma and lead to symptoms associated with chronic airflow limitation. However, the pathogenetic mechanisms leading to these responses remain unclear. According to recent reports, lung-resident fibroblasts and smooth muscle cells have been implicated in the pathogenesis of airway remodeling. Myofibroblasts are proposed to be the primary effector cells of lung fibrotic responses and are characterized by expression of α-smooth muscle actin (α-SMA) stress fibers. Transforming growth factor (TGF)-β is known to induce the transformation of fibroblasts to myofibroblasts. Protease activated receptor (PAR)-2, a G-protein-coupled receptor activated by serine proteases such as trypsin and mast cell tryptase has been recognized as a key molecule in inflammation and fibrotic changes. We hypothesized that activation of PAR-2 induces TGF-β and α-SMA expression and hence may be one of the potential mechanisms of airway remodeling in asthma.

Methods: Cultured human lung fibroblasts (MRC5) were exposed to trypsin (5 nM) or a specific activating peptide, PAR-2AP. Secreted TGF-β was measured using ELISA. Cell associated α-SMA was assessed by Western blot analysis and immunostaining and activation of downstream signaling pathways was assessed by Western analysis.

Results: Activation of PAR-2 by trypsin or PAR-2AP induced TGF-β secretion that peaked between 4 and 8 hours. These were correlated with activations of c-fos and cjun. Induction of α-SMA expression peaked between 4 and 24 hours. Treatment with trypsin or PAR-2AP also induced phosphorylation of GSK-3β on serine 9 and nuclear translocation of β-catenin.

Conclusions: Activation of PAR-2 induces TGF-β secretion through the AP-1 transcription factor complex leading to myofibroblast transformation via the GSK-3β/β-Catenin Pathway.

502 Plasminogen Activator Inhibitor-1, Fibrinogen and Lung Function in Adolescents with Asthma and Obesity
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Background: Obesity promotes a low-grade systemic inflammatory state that may act on the lungs to exacerbate asthma. There is little information on the relationship between systemic inflammation and lung function in children and adolescents.

Methods: One hundred and seventy-eight adolescents (boys and girls) were involved, 4 groups were divided according to their diagnosis: non-obese and non-asthmatic controls (n = 38), non-obese asthmatics (n = 31), obese non-asthmatics (n = 62), obese asthmatics (n = 47). The levels of PAI-1 and fibrinogen were determined in blood samples. The lung function was evaluated by measuring forced expiratory flow in 1-second (FEV1) and forced vital capacity (FVC1).

Results: Compared to healthy controls, obese adolescents with or without asthma showed higher levels of fibrinogen (328.4 ± 54.9, 324.9 ± 68.9 and 289.2 ± 61.5 mg/dL, respectively) and PAI-1 (36.0 ± 17.3, 53.2 ± 22.3 and 52.6 ± 24.7 ng/mL, respectively) and reduced FEV1/FVC ratio (87.7 ± 6.2, 81.6 ± 8.6 and 81.7 ± 6.9, respectively). In the whole studied subjects, FEV1/FVC ratio showed significant inverse correlation with PAI-1 (r = -0.185), fibrinogen (r = -0.157), BMI (r = -0.303), insulin (r = -0.198) and HOMA (r = -0.173). In the 78 asthmatic subjects, FVC correlated positively with BMI, no significant correlation was observed between FEV1/FVC ratio and BMI, HOMA, PAI-1 or fibrinogen.

Conclusions: Our data demonstrated that the degree of systemic inflammation and the degree of obesity in the whole studied groups correlated to the reduced lung function. Further studies are needed to identify the pathophysiologic mechanism for such association.

503 Peculiarities of Immune Response in Young Children with Recurrent Wheezing
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Background: Wheezing is a very common symptom in young children. However some questions of immune response are unclear for subsequent prediction in recurrently wheezy children. We studied immunological status of kids with recurrent wheezing.

Methods: 31 children with acute episode of wheezing were included in this study (24 male, 7 female) aged from 1 to 5 years, admitted in Vladimir Children Clinical Hospital. These children were the main group. Control group included 6 children with no signs of chronic and acute inflammatory diseases. Examination included studying clinical data, detecting in blood serum subpopulations of lymphocytes, expression of Toll-like receptors (TLR-2, TLR-4) on surface of monocytes (mFMI) and number of pro-inflammatory monocytes (CD14+CD16+).

Results: We revealed high frequency of bronchial obstruction (more than 5 episodes) at 30% of children in main group. 30% of children had allergic diseases (atopic dermatitis, allergic rhinitis) in history. In group of children with recurrent wheezing revealed increased levels of pro-inflammatory monocytes (9,44 ± 1,55% vs 5,39 ± 0,79%) and expression of receptors TLR-2 (8,28 ± 0,44 conventional units vs 6,59 ± 0,98). In children with recurrent wheezing an inverse correlation was found between frequency of respiratory infections and level of expression of TLR-2. For frequency of acute respiratory infection up to 6 times in year the level of expression of TLR-2 was 9,11 ± 0,31 and for children with monthly episodes of acute respiratory infections level was 7,6 ± 0,25 (r = -0,380, P < 0,05). Expression of TLR-4 was also tended to lower level in sickly children (2,7 ± 0,16 and 2,7 ± 0,14), however, no significant differences weren’t revealed (r = -0,370, P > 0,05). At the same time in patients with allergic diseases showed significant reduction in expression levels of TLR-4 compared with patients without atopy (P < 0,05).

Conclusions: Increased levels of pro-inflammatory monocytes and expression of TLR-2 correspond to local inflammatory reaction and adequate immune response against background of acute respiratory illness in children with relapsing course of bronchial obstruction. Changes of TLR-2 and TLR-4 in group of children with recurrent wheezing confirm, on the one hand, risk of bronchial asthma in children with predisposition to atopy. On the other hand these changes can be the result of oppression of innate immunity in children with persistent character of bronchitis.

504 Participation of Invariant NKT Cells (Vα24Jc18) during Asthma Exacerbation in Children
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Background: Invariant NKT cells (or type 1 NKT cells) co-express CD3 marker and NK receptors (CD56, CD161) and use a single type of TCRα chain (Vα24Jc18 for humans), comprising CD4-CD8+, CD4+ and CD8+ subpopulations. Participation of these cells and their cytokines in asthmatic children, in stable conditions and under exacerbation, was studied.

Methods: Three groups on children (6–12 years old) were selected: 1) asthmatics under exacerbation attack (AE) within the first 24 hours after the attack and before starting any treatment; 2) asthmatics with stable asthma (SA), without symptoms for at least a month before bleeding; and 3) healthy controls (HC) without history of asthma, atopy and with normal lung function were selected in the Allergy and Clinical Immunology Service, Hospital Infantil de Mexico. Invariant NKT cells and subset levels as well as intracellular cytokines were evaluated in whole blood by 4-color flow cytometry (antibodies against CD3, CD4, CD8, CD161, Va24, IL-4 and IFN-g).

Results: Proportion of iNKT cells among total CD3+ cells in HC group was 0.9%, while in SA patients they were increased up to 2.6%; interestingly, during exacerbation such cells were diminished (1.8%). Concerning iNKT CD4+ cells were 0.6% in HC, 1.8% in SA, and 0.7% in AE, while iNKT CD8+ cells were 0.1% in HC, 0.7% in SA, and 0.4% in AE. Both iNKT cell subsets expressed intracellular IFN-g and IL-4 cytokines in AE, SA and HC but predominantly IFN-g in iNKT CD8+ cells from AE patients.

Conclusions: iNKT cells participation in asthma pathogenesis was confirmed. Increase of IFN-g production in patients with exacerbations, may provide a regulatory environment to stabilize the condition.

505 Cytokine Profile Characterization in Patients Infected with AH1N1 Influenza Virus
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Background: In 2009, an outbreak of severe respiratory infection caused by influenza AH1N1 virus affected Mexican people, previously in 1996 in Guangdong province (China) an outbreak of HSN1 influenza started spreading throughout Asia and the western Paleartic in 2004 to 2006. Chinese patients were studied and it was observed a deleterious immune