response related to cytokine storm, mainly characterized by higher levels of IFN and other proinflammatory cytokines causing fatal infections or severe clinical outcome. Until now, is unknown the cytokine profile involved during AH1N1 infection, thus it was the aim of this study.

**Methods:** Serum samples were obtained from 39 infected patients and their close contacts from the first wave outbreak of influenza AH1N1 and were storage at −80°C until cytokine determination, Human Inflammatory and Th1/Th2/Th17 Cytometric Bead Array Kit was used to determine cytokine concentration in serum samples. Cytokine protein arrays were performed to know relative expression of 36 cytokines with the Proller Array Membrane from R&D Systems. The results are reported on relative units (RU). It was performed with ANOVA test, considering 𝑃 < 0.05 as statistically significant.

**Results:** We did not find significative statistical differences between patients and their close contacts in IFN-γ, IL-1β, IL-2, IL-4, IL-6, IL-10, IL-12p70, IL-17A, and TNF evaluated with cytometry. However, the proteomic analysis showed a significant increased (𝑃 < 0.05) in G-CSF, I-309, sICAM-1, IL-1Ra, IL-16, IL-23, IL-27, I-TAC, MIP-1α, and E1-Serpin compared with the close contacts.

**Conclusions:** AH1N1 patients showed higher levels of IL-27. We observed more RU of sICAM expression in patients and closed contacts, possibly due to the IL-27 signaling. IL-23 were increased in AH1N1 patients, this cytokine is involved in the IFN-γ secretion and in the generation of memory CD T cells. These results suggest that AH1N1-patients developed an immune response characterized by IL-27 and IL-23. We need more studies to determine if this cytokines are related with the memory response induction.

### 506 Basal T Cell Subpopulations of Normal Humans Vary by Stress Hormone Receptor Polymorphisms

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**Background:** Psychological stress has been correlated with allergy and asthma activity although there are clearly individual differences in the responses to the same stressor. These individual differences could be influenced by stress hormone receptor binding affinity, which could be altered by single nucleotide polymorphisms (SNPs).

**Methods:** We categorized differences in immunoregulatory profiles from peripheral blood mononuclear cells (PBMC) of 207 normal volunteers according to various glucocorticoid (GCR) and beta-2 adrenergic receptor (B2AR) polymorphisms. Subjects were genotyped for SNPs by real time RT-PCR, and Th1, Th2, Th1/Th2 ratio, and CD3+CD4+CD25hiFoxp3+ cell numbers were measured using flow cytometry. Each immune parameter in the SNP groups was compared to the wild-type (WT) gene.

**Results:** Significant differences were observed in B2AR SNPs Gly16Arg for Th2 (means: WT gly/gly, 1.89; arg/arg, 2.58; P = 0.003) and Th1/Th2 ratio (medians: WT gly/gly, 10.18; arg/arg, 6.89; P = 0.004) and Gln27Glu for Th1 (means: WT gln/gln, 17.21; gln/glu, 19.4 P = 0.031; glu/glu, 19.82 P = 0.049). No differences were observed based upon GCR SNPS tested (BCL1; NC363S; TH1111; A3669G).

**Conclusions:** These data suggest that SNPs from various components of the stress-immune network (such as hormone and cytokine promoters and receptors) may be useful for subgrouping of immune responses to more accurately evaluate psychoneuroimmunological components of stress risk in individual subjects. This approach has significant clinical potentials in identifying those patients who may be most susceptible to stress effects on their immune balance.

### 507 The Protective Effects of Exogenous IGFBP-3 on Allergic Airway Inflammation through Blockade of vegf Production

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**Background:** Bronchial asthma is a chronic airway inflammatory disease that is usually accompanied by increased vascular leakage, resulting in plasma exudation. Vascular endothelial growth factor (VEGF) plays as a pro-inflammatory mediator as well as a vascular permeability factor in bronchial asthma. Insulin-like growth factor (IGF)-1 is also involved in the inflammatory process associated with bronchial asthma and it has been demonstrated to stimulate VEGF expression. The IGF binding proteins (IGFBPs) are a complex family of proteins which bind IGFs with high affinity. IGFBPs, especially IGFBP-3, display distinctive properties and can interfere with various biological processes. However, there are little data on the effect and the molecular basis of IGFBP-3 on allergen-induced bronchial inflammation and airway hyper-responsiveness.

**Methods:** This study was aimed to investigate the related signaling regarding the action of IGFBP-3 on bronchial inflammation and airway hyper-responsiveness in allergic airway disease of mice.

**Results:** In this study with an ovalbumin (OVA)-induced murine model of allergic airway disease, the increases of HIF-1α/2α activity and VEGF protein levels in lungs after OVA inhalation were blocked substantially by the administration of IGFBP-3. We also showed that the increased numbers of inflammatory cells of the airways, airway hyper-responsiveness, and increased levels of IL-4, IL-5, IL-13, and vascular permeability in lungs after OVA inhalation were significantly reduced by the administration of IGFBP-3.

**Conclusions:** These results indicate that IGFBP-3 may attenuate antigen-induced airway inflammation and hyper-responsiveness through the modulation of vascular leakage and VEGF expression mediated by HIF-1α/2α in allergic airway disease of mice.

### 508 Antioxidants Attenuate Airway Remodeling by Regulating NF-κB, NRF2, and HIF in a Murine Model of Chronic Asthma

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**Background:** Reactive oxygen species (ROS) play a crucial role in the pathogenesis of acute and chronic respiratory diseases. Antioxidants have been found to ameliorate airway inflammation and hyperresponsiveness in animal models employing short-term exposure to allergen. However, little data are available on the effect of antioxidants on airway remodeling and signaling pathways in chronic asthma.

**Methods:** In the present study, we used a long-term exposure murine model of allergic airway disease to evaluate the influence of an antioxidant, l-2-oxothiazolidine-4-carboxylic acid (OTC) or α-lipoic acid (LA) on airway remodeling and to explore possible transcription factors and kinases involved in this effect.

**Results:** Long-term challenge of ovalbumin (OVA) increased ROS production, airway inflammation, and airway hyperresponsiveness, and developed features of airway remodeling such as excessive mucus secretion, subepithelial fibrosis, and thickening of the peribronchial smooth muscle layer. Administration of OTC or LA reduced these features of asthma including airway remodeling, which was accompanied by suppression of transforming growth factor-β1, vascular endothelial growth factor, and T-helper 2 cytokines. In addition, OVA-induced activation of nuclear factor-κB (NF-κB), nuclear factor erythroid 2p45-related factor-2 (Nrf2), hypoxia-inducible factor (HIF)-1α, and HIF-2α was reduced by OTC or LA. Our results also showed that OTC or LA down-regulated phosphoinositide 3-kinase activity and decreased phosphorylation of p38 mitogen-activated protein kinase but not extracellular signal-regulated kinase 1/2 or c-Jun N-terminal kinase.

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Conclusions: These findings demonstrate that OTC and LA can inhibit activation of NF-κB, Nrf2, and HIF and thus attenuate allergen-induced airway remodeling, suggesting that antioxidants may provide therapeutic benefit in chronic asthma and other airway disorders.

509 CCL3L1 Protein Did Not Affect IL-6 Expression, but Significantly Up-regulated IL-10 Expression in the Allergic Response
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Background: Previously, we found that the mean copy number of CCL3L1 in patients with asthma was significantly lower than that of control subjects (3.13 vs 3.75, P = 0.001). We investigated its possible molecular mechanism using a human monocytic cell line stimulated with house dust mite extract.
Method: The THP-1 human monocytic cells were stimulated with various concentrations of HDM extract. After stimulation, assay-on-demand gene expression products (Applied Biosystems) were used to evaluate mRNA expression of CCL3L1 (Hs 00609691_ml), IL-6 (Hs00174131_ml), and IL-10 (Hs00961622_ml) levels as measurement of mRNA levels by real-time PCR.
Results: Treatment of THP-1 cells with various concentrations of HDM extract induced marked up-regulation of the expression of cytokines IL-10 and IL-6, which indicated that allergic responses were efficiently induced. Recombinant CCL3L1 protein had no effect on cytokine expression of THP-1 Cells in absence of HDM extract stimulation. In the presence of HDM extract (10 µg/mL) stimulation, CCL3L1 protein significantly up-regulated IL-10 expression (Ratio to ng/mL CCL3L1) dose-dependently (0 µg/mL CCL3L1 + 0.3/12.4, 10 µg/mL CCL3L1; 15.8 ± 1.1, 50 µg/mL CCL3L1; 16.8 ± 0.3, 100 µg/mL CCL3L1; 18.0 ± 0.8, (P > 0.05), but did not affect IL-6 expression (P > 0.05).
Conclusion: The significantly elevated asthma risk in subjects with a low copy number of the CCL3L1 gene may be due to up-regulating IL-10 expression, not IL-6 expression.

510 Caspase-9 is Involved in CD30 Activation Induced Eosinophil Apoptosis
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Background: We evaluated whether ligation of CD30 incite the apoptosis, and investigated the mechanisms of CD30 induced eosinophil apoptosis is dependent on caspase activation.
Methods: We purified eosinophils using MACS system. Expression of CD30 on eosinophils were measured and eosinophils were cultured in the wells pretreated with anti-CD30 mAb and isotype control IgG1, IL-5 and dexamethasone in RPMI 1640 media supplemented with 10% FBS, and the apoptotic rate were measured using flow cytometry. To evaluate whether caspase-9 is involved in CD30-induced eosinophil apoptosis, the apoptotic rate were evaluated with addition of caspase-9 inhibitor and the expression of procaspase-9 was also measured using Western blot.
Results: The apoptotic rates of eosinophils cultured in the presence of anti-CD30 mAb were significantly increased to 29.1 ± 6.1% and 47.3 ± 4.7% compared with 17.1 ± 6.7% and 29.4 ± 9.2% of the control at 4 and 24 hours, respectively (both P < 0.05). Caspase-9 inhibitor suppressed the mAb induced eosinophil apoptosis from 54.8 ± 6.9% and 71.5 ± 11.6% to 24.5 ± 6.0% and 47.8 ± 11.4% at 18 and 36 hours, respectively (both P < 0.001).

We also showed the expression of procaspase-9 with the mAb was diminished compared with that of the control and of IL-5.

Conclusions: This study showed CD30 activation enhances the eosinophil apoptosis and the effect is mediated by Caspase-9 activation.

511 Role of NLR (Nucleotide Oligomerization Domain (NOD)—like Receptor) on Allergic Inflammation in a Mouse Model of Allergic Rhinitis
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Background: Recently, a new set of pattern-recognition receptors, the nucleotide-binding oligomerization domain (Nod)-like receptors (NLRs), have emerged. Their activation, either by allergens or microbes, triggers an inflammatory response. Objective: To investigate whether recognition of bacterial microbial-associated molecular patterns in the nose may result in susceptibility to developing allergic reactions, and to understand the molecular mechanisms by which such triggers block natural tolerance.
Methods: Ligands of intracellular microbial-associated molecular pattern recognition receptors—the nucleotidebinding oligomerization domain (Nod)-like receptors, Nod1 and Nod2—were given intranasally with antigen, and their ability to modulate airway tolerance was analyzed. Seventy-two mice were randomly assigned to one of six groups: control (n = 12), pre NOD1 group (n = 12), pre NOD2 group (n = 12), post NOD1 group (n = 12), and post NOD2 group (n = 12). All mice except for the control group were sensitized by an intraperitoneal injection of ovalbumine (OVA) and aluminum hydroxide. Two weeks after sensitization, all sensitized mice were challenged intranasally with OVA. The control group was received phosphate buffered saline intranasally. The allergic symptom after the final challenge was recorded. Interleukin (IL)-5, interferon-γ (IFN-γ), and IL-10 levels in nasal lavage fluid (NALF), as well as serum OVA-specific IgE levels were measured. The number of eosinophils in lamina propria was evaluated. The levels of T-bet, GATA-3, and Foxp3 mRNA expression in splenic mononuclear cells were determined by real-time polymerase chain reaction.
Results & Conclusion: We show that a Nod-like receptor is a novel, previously unrecognized, pathway that adversely links innate and adaptive immunity and leads to allergic rhinitis.