SUNDAY

483 Frequency Of Grass Pollen-specific Il-4, Il-10 And Dual Il-4 And Il10 Positive Cells In Atopic And Non-atopic Individuals In And Out Of The Pollen Season

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RATIONALE: Seasonal variability of grass pollen-specific Th2 cells and IL-10+ T cells may be important in disease expression in hayfever. We hypothesized that the frequency of IL-4+ and IL-4+IL-10+ cells in atopics is increased compared to non-atopics in and out of the pollen season; and that IL-10+ cells are increased and protective in non-atopics. Furthermore, the proportion of CD4+CRTh2+ cells and IL-4 receptor (CD124) expression on these cells is increased in atopics.

METHOD: PBMCs were obtained from six atopics and six non-atopics in and out of the pollen season. Cells were cultured with 10ug/ml of Phleum pratense for 42 hours. IL-4, IL-10 and IL-4/IL10 secreting cells were quantified by fluoroSpot assay. Allergen-specific proliferative responses were determined. CD4+CRTh2+CD124+ cells were evaluated by flow cytometry.

RESULTS: Significantly higher numbers of IL-4+ cells were shown in atopics (201.9±25.91 spots/106 cells) when compared to non-atopics (3.7±1.3). This was further enhanced during the pollen season in atopics ($p<0.03$). In contrast, higher frequency of IL-10+ cells was only observed in non-atopics ($p<0.002$). IL-4+IL-10+ cells were increased in atopics but not in non-atopics ($p<0.001$). Increased proliferative responses were demonstrated in atopics vs. non-atopics out of season ($p<0.001$), which was reduced in season ($p<0.004$). CD4+CRTh2+CD124+ cells were decreased in season compared to out of season in atopics ($p<0.02$).

CONCLUSIONS: Our findings confirm elevated numbers of grass pollen-specific IL-4+ cells in atopics compared to non-atopics during the pollen season. Protective IL-10+ cells are elevated in non-atopics. The role of IL-4+IL10+ cells in regulating allergic inflammation is yet to be investigated.

484 *In vivo* Exposure of Mice to Alternaria Alternata Induces IL-18 Release into the Airways: A Cause of Allergic Asthma?

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RATIONALE: Multicenter epidemiologic studies such as CAMP and NHANES, have reproducibly shown that allergic asthma is associated with allergic sensitization to Alternaria extract (ALT-E). To determine the mechanism by which Alternaria may contribute to asthma, here we examined the cytokines released in airways following ALT-E challenge of naïve mice.

METHODS: Naïve Balb/c mice were intranasally challenged by PBS or ALT-E. Bronchoalveolar lavage (BAL) was performed 1 hour later. IL-4, IL-9, IL-13, IL-18, IL-25, IL-33 and TSLP were measured in BAL fluids by ELISA.

RESULTS: Compared to mice challenge with PBS, ALT-E challenge induced a 9-fold greater increase in IL-18 levels in BAL fluids ($p< 0.01$). However, ALT-E challenge failed to increase the levels of IL-4, IL-9, IL-13, IL-25, IL-33 and TSLP in the BAL fluids.

CONCLUSIONS: Our results indicate that *in vivo* exposure of naïve mice to ALT-E selectively induces the release of IL-18 into the airways. We have previously shown that intrapulmonary administration of IL-18 with an allergen promotes allergic sensitization to that allergen, and induction of allergic airway inflammation. We suggest that rapid release of IL-18 into the airways by ALT-E challenge may contribute to allergic sensitization and allergic airway inflammation associated with allergic asthma.

485 Low Levels of Cytokines and Growth Factors in Serum of Allergic and Non-Allergic Top Atletes

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RATIONALE: Several changes of the immune cells and of the cytokine profile have been reported after an acute exercise challenge. However, in spite of the high prevalence of allergic diseases and upper respiratory tract infections reported in athletes, the immune response to an intense and prolonged training has been for less investigated.

METHODS: In the framework of an International study of Olympic athletes (www.ga2len.net) sera from 92 athletes of the Italian delegation in the Beijing Olympic Games and from 49 healthy non-allergic sedentary controls were tested for a broad cytokine and growth factor array. On the basis of data available through clinical history, an allergy specific questionnaire for athletes (AQUA), skin-tests, pulmonary function tests and serum total IgE determination, athletes were classified as allergic (n=41) or non-allergic (n=51). A Cytokine bead luminometric assay was used to measure the following cytokines and growth factors: IL-1ra, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, Il-17, IFNg, TNFa, RANTES, IP-10, MCP-1, MIP-1a, MIP-1b, Eotaxin, G-CSF, PDGF and VEGF.

RESULTS: For all cytokines and growth factors measured, apart from IL-13, TNFa and MIP-1a, serum levels in athletes were significantly lower ($p<0.05$) than in controls. This was particularly evident for IL-1ra, IL-8, IL-12, MCP-1, IFNg, PDGF and VEGF ($p<0.0001$). No difference was observed between allergic and non-allergic athletes except for G-CSF which was significantly lower ($p=0.01$) in allergic athletes.

CONCLUSIONS: The increased prevalence of allergic diseases and URTI reported in top athletes after a long-term intensive physical training is associated with a general down-regulation of the cytokine and growth factor profile.

486 Allergen-induced Ccl20 Release From Bronchial Epithelial Cells Requires Ion Transporter-mediated Chloride Export

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Asthma is a chronic inflammatory disease of the lung mediated by TH2 cells. Recent evidence suggests pulmonary dendritic cells (DCs) are important in driving initial T cell activation. We previously found that an important chemokine in the recruitment of immature DCs, CCL20, is released by bronchial epithelial cells (BECs) within the first few minutes after exposure to the common allergen house dust mite (HDM). CCL20 release from BECs was not affected by inhibitors of protein synthesis, suggesting mobilization of preformed stores. In this study, we sought to further define the mechanism of rapid CCL20 release from BECs. Others have shown that chemokine release from BECs may be mediated by chloride transporters, therefore we hypothesized that HDM-triggered CCL20 release may be ion transporter-dependent. We found that HDM-induced CCL20 release was inhibited by the chloride transporters, but not other transporters. Also, anion replacement experiments showed that it followed an ion selectivity pattern similar to most chloride channels. After HDM exposure, we measured decreased concentrations of intracellular chloride, and showed that intracellular CCL20 pools are also reduced soon after HDM exposure. These data suggest that early HDM exposure causes transporters to export chloride from the cell, which contributes to the release of intracellular pools of CCL20 from BECs.