197.5

Erk and PI-3 kinase pathways are involved in IL-13 induced chemokines production in Lung tissue cells

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Chemokines mediate migration and activation of eosinophils and other leukocytes, which are important features of airway inflammation and hyper-responsiveness in the pathogenesis of asthma. IL-13, a Th2 cytokine, has been shown to induce airway inflammation and hyper-responsiveness in a mouse model of asthma. To understand the pathological roles of IL-13 in asthma, we analyzed the effect of IL-13 on chemokine induction in human primary lung tissue cells in vitro. IL-13 induced the production of Eotaxin in bronchial smooth muscle cells, MCP-1 in lung fibroblasts and IL-8 in epithelial cells. In these cells, IL-13 induced tyrosine phosphorylation of Stat-6, activation of Erk and PI-3 kinase. Using specific kinase inhibitors, we further analyzed the signal transduction pathways of MCP-1 induction in airway fibroblasts and IL-8 induction in airway epithelial cells. PD98059, an inhibitor of Erk pathway, and Ly294002, an inhibitor of PI-3 kinase pathway, blocked IL-13 induced chemokine production. These inhibitors had no effect on the tyrosine phosphorylation of Stat-6. Our data suggest that the optimal induction of chemokines production by IL-13 requires multiple signal transduction pathways in addition to the activation of Stat-6.

197.6

Mechanism of Allergic Cross-Reaction-Mutagenesis, Screening, Expression and Purification of Single Chain Fv antibody Fragments with Specificity for Trinitrophenyl

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In order to demonstrate a multispecific model for allergic cross-reaction, two of the mouse monoclonal IgEs, LA2 and LB4, were cloned and sequenced. It has been found that an overabundance of solvent-exposed aromatic residues in the combining regions is responsible for the multispecific binding characteristic of these IgEs. We report here the mutagenesis and expression of ScFv of LA2 clone, which has been selected using for multispecific studies. Seven aromatics in CDRs of LA2 were selected for mutagenesis because they were identified in the computer models as potential contact sites. Meanwhile, two residues (L:52S, H:34W) were mutated for control. We designed and synthesized the mutant primers. The site-directed the mutation has been done by Eliminating a Unique Site method. We expressed all the ScFv fragments by the recombinant phage antibody system. The phage and soluble antibodies were screened by ELISA. As there is a 13 amino acid peptide tag(E Tag) gene followed the ScFv gene, we purified the ScFv soluble antibodies from the supernatant by using the anti-E tag affinity column. The purified proteins were obtained to facilitate future binding studies. Our further aim is to examine the contribution of aromatics to antibody specificity and stability of the antibody-antigen complex.

197.7

Topical Skin Exposure to Dry Trimellitic Anhydride (TMA) Powder Induces Specific IgE

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TMA is known to produce occupational asthma that is associated with its ability to acylate proteins and to induce production of TMA-specific IgE. Though the respiratory tract is considered to be a major exposure route leading to sensitization, the potential role of dermal exposure is not known. Animal studies have shown that topical application of TMA dissolved in organic solvents can lead to specific IgE production. The present study examined the ability of dry TMA to dose-dependently sensitize Brown Norway rats when applied to the skin. A patch of hair was carefully clipped with scissors on the rat's back. Dry TMA powder (1.25, 5 and 20mg) was applied once per week for 4 weeks and the area occluded with dermal adhesive tape overnight. Residual powder was collected and analyzed by proton nuclear magnetic resonance (NMR) for both TMA and its hydrolysis product, trimellitic acid. Some TMA hydrolysis occurred, but TMA was still the predominant component of the powder after application. Blood was taken 2 weeks after the last TMA application and anti-TMA IgE measured by ELISA. All doses of TMA elicited production of anti-TMA IgE and the titers were dose dependent. Specific IgE to TMA was not found in either unexposed controls or trimellitic acid exposed rats. This data suggest that dry, reactive chemicals can penetrate the epidermis (possibly suspended or solubilized in skin oils), react to proteins and induce production of specific IgE.

197.8

Sensitization of Asthmatics to KLH

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Asthmatics respond differently (more serum antigen specific IgG4) than control subjects to intrapulmonary exposure to keyhole limpet hemocyanin (KLH), a neoantigen (Immunology 91:167-175, 1997). We wished to determine if this difference extends to measures of cell mediated immune response. We immunized both asthmatics and control subjects with 1000 ug KLH via the intrapulmonary route and performed intradermal skin tests with 1 and 10 ug KLH. Induration was measured at 24, 48 and 72 hours. We found that compared to control subjects (induration 8.3 ± 3.1 mm, mean, sem), asthmatics exhibited larger delayed type skin tests (induration 1.0 ± 0.8 mm) at 72 hours ($p<.05$). We also examined positive skin tests from other KLH immunized subjects at 48 and 72 hours using traditional histologic and immunoperoxidase analysis. Skin tests, regardless of biopsy time, showed a superficial and deep perivascular and perifollicular infiltrate of lymphocytes. The infiltrate was predominantly T-cells (CD3+CD4+) with scattered CD8+ cells. A few B-cells (CD20+), macrophages (CD68+) and mast cells (mast cell tryptase+) were present. We conclude that after intrapulmonary exposure to antigen, asthmatics exhibit exaggerated delayed type skin test reactivity. Together with our previous data, this suggests that asthmatics respond more briskly than control subjects to an intrapulmonary immunization with KLH. We conclude that after intrapulmonary exposure to antigen, asthmatics exhibit exaggerated delayed type skin test reactivity. Together with our previous data, this suggests that asthmatics respond more briskly than control subjects to an intrapulmonary immunization with KLH.

197.9

ANALYSIS OF THE HUMORAL IMMUNE RESPONSE OF OVALBUMIN IMMUNIZED FLAKY SKIN MICE

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Mouse models have been extremely useful in studying the mechanisms of atopic diseases such as asthma and immediate hypersensitivity. Mice with the single gene mutation known as flaky skin (fsn/fsn) exhibit many immunolgical abnormalities which suggest an immune response skewed toward the TH2 phenotype, including high levels of interleukin 4 (IL-4), mast cells, histamine, and serum immunoglobulin. Of particular interest is a spontaneous progressive elevation in IgE, which can reach levels of 100 ug/ml or greater by the age of 10 weeks. In light of these characteristics it is believed the flaky skin mouse would be useful in the development of a mouse model of IgE-mediated lung disease. However, because the immune system of this animal appears to be in a state of hyperactivation, it was not known if an antigen-specific humoral immune response could be elicited. To address this question flaky skin mice and their normal littermates were immunized i.p. with ovalbumin. Serum samples were analyzed by ELISA to determine total IgE and IgG levels, as well as relative levels of ovalbumin-specific total Ig, IgM, IgG, IgG1, and IgE. It was found that the ovalbumin-specific humoral immune response of flaky skin mice was very similar to that of normal littermates, with two major differences: 1. A slight impairment in the IgM response of flaky skin mice, and 2. A greatly increased IgE response in flaky skin mice.

197.10

NATURAL RUBBER LATEX (NRL) ALLERGENS ARE MORE OFTEN DETECTED IN DUST THAN AIR.

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Exposure to NRL allergens plays a key role in inducing NRL allergy. We therefore compared the relative abilities of air and dust sampling to detect NRL allergen contamination in a medical facility. High volume area air samples and vacuumed bulk surface dust samples were collected onto Teflon filters in several hospital and clinic settings. Control samples were collected in a vacant office where NRL was not used. NRL allergen levels in saline filter extracts were determined by inhibition of an immunoassay for NRL-specific human IgE (Pharmacia-Upjohn Diagnostics, Uppsala, Sweden). Threshold limit of detection (LOD) for the air and dust samples was determined as the mean level in the vacant office plus 3 standard deviations. Proportions of samples exceeding the LOD are as follows:

	Dental Clinic	Operating Room	Medical Clinic	Clinical Laboratory	Office
Air	5/9*	1/9	3/9	0/10	1/11
Dust	10/10*†	7/10*†	7/9*	5/10*†	0/11

*†different at $p \leq 0.05$, Fisher Exact Test; * vs. vacant office; † air vs. dust. Thus, NRL allergen contamination in a medical facility was more often detected by sampling of bulk surface dust than by area air sampling.