523.11

Dose Response to a *Penicillium chrysogenum* Conidium-Associated Allergen in a Murine Model.

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Recent evidence has implicated the fungus *Penicillium chrysogenum* (Pc) in sick building syndrome (SBS). We recently showed that inhalation of viable Pc conidia by mice resulted in an allergic inflammatory response. We also have isolated a serine protease from viable Pc conidia. The present study was conducted to determine the specific dose response of C57BL/6 mice to this protease. Mice were primed with an initial dose of either 10 µg or 100 µg of protease via intraperitoneal (IP) injections with protease adsorbed to aluminum hydroxide or intranasal (IN) inoculations without adjuvant. Mice were inoculated weekly with 0.1 µg, 1.0 µg, or 10.0 µg of protease IN. Sera were collected and analyzed by enzyme-linked immunosorbent assay. Significant increases of total IgE compared to controls were detected in all animals primed IP with protease and in mice primed with 100 µg of protease IN with weekly inoculations of 0.1 µg and 1.0 µg after two weeks of treatment. After four weeks of treatment, significant increases in total IgE were detected in 10 µg and 100 µg IP-primed and 1.0 µg and 10.0 µg IN-boosted animals as well as 10 µg IN-primed and 1.0 µg and 10.0 µg IN-boosted animals. After six weeks of treatment, mice primed and boosted with 10 µg IN, and mice primed with 100 µg IN and treated weekly with 0.1 µg IN produced significantly higher IgE levels than controls. These results indicate an allergic response in C57BL/6 mice to a serine protease produced by *P. chrysogenum* conidia. This allergen could be useful for developing treatments for people exposed to high levels of *P. chrysogenum*.

523.12

Chronic Exposure of DO11.10 T Cell Receptor Transgenic Mice to Aersolized Ovalbumin Induces Airway Hyperreactivity in the Absence of Systemic Priming

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We have previously reported that DO11.10 hemizygous mice (+ /-), which bear a T cell receptor for a chicken ovalbumin (OVA) peptide on 40% of their T cells (OVA-TCR) respond to chronic aerosol exposure of heat-aggregated OVA (haOVA) by mounting a local and systemic Type 2 inunune response. We now describe that these mice also respond to chronic haOVA aerosol exposure (6 hours/day, 5 days/week for 4 weeks) by developing non-specific airway hyperreactivity (AHR) to methacholine as measured by volume-displacement plethysmography. AHR does not develop in DO11.10 -/- littermates who are exposed similarly to haOVA for 4 weeks. The development of AHR in haOVA-exposed DO11.10 + /- mice is coincident with the appearance of peak levels of serum IgE, lung eosinophilia, lung cell production of IL-4 and mucous hyperplasia. After 6 weeks of haOVA exposure, no AHR was evident in either + /- or -/- mice as compared to their AIR exposed control littermates. This was not due to a diminution of methacholine responsiveness of the haOVA-exposed DO11.10 + /- mice, but rather an increase in methacholine sensitivity in the DO11.10 + /- mice exposed to AIR when comparing mice exposed for 4 weeks to those exposed for 6 weeks. We observed a similar increase in methacholine responsiveness in DO11.10 -/- mice exposed to haOVA for 6 weeks compared to their littermates exposed for only 4 weeks. These data suggest that chronic aerosol exposure to OVA can induce a specific immune-mediated AHR in predisposed DO11.10 + /- mice. Further, the data show that prolonged aerosol exposure can induce an irritant effect in either DO11.10-/- or + /- mice that manifests itself as AHR in the absence of a measurable immune or inflammatory response.

523.13

The role of Tumor Necrosis Factorα (TNF) in Toluene Diisocyanate (TDI) Asthma

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Nearly 9 million workers are exposed to chemical agents associated with occupational asthma with isocyanates representing the chemical class most responsible. Isocyanate-induced asthma has been difficult to diagnose and control, in part because the biological mechanisms responsible for the disease and the determinants of exposure have not been well defined. Isocyanate-induced asthma is characterized by airway inflammation and we hypothesized that inflanunation is a pre-requisite of isocyanate-induced asthma with tumor necrosis factor (TNF) α being critical to this process. To explore this hypothesis, TNFα receptor knockout (TNFR) and anti-TNFα antibody treated C57BL/6J mice were sensitized by subcutaneous injection (20 µl on day 1; 5 µl, days 4 and 11), and challenged 7 days later by inhalation (100ppb; days 20, 22 and 24) with toluene diisocyanate (TDI). Airway inflammation, goblet cell metaplasia, epithelial cell damage and non-specific airway reactivity to methacholine challenge, measured 24 hrs following the last challenge, were reduced to baseline levels in TNFα null mice. TNFα deficiency also markedly abrogated TDI-induced Th2 cytokines in airway tissues indicating a role in the development of Th2 responses. Intratracheal instillation studies (50 µl, single dose, 0-58 mg/kg) with fluorescein-conjugated isothiocyanate (FITC) and intranasal studies (20 µl, single dose, 0-10%) with TDI suggested that TNFα deficiency also resulted in a significant reduction in the migration of airway dendritic cells to the draining lymph nodes. Taken together, these results suggest that TNFα plays has multiple and central roles in TDI-induced asthma influencing both non-specific inflammatory processes as well as specific immune events.

523.14

INHIBITORS OF TYROSINE KINASE SIGNALING CASCADE ATTENUATED AIRWAY INFLAMMATION

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Effects of inhibitors of the tyrosine kinase signaling cascade on (i) antigen challenge of guinea pig airways in vitro, (ii) thrombin-induced guinea pig airway smooth muscle cell proliferation and (iii) cytokine-stimulated expression of CC chemokine receptor 1 (CCR1) in human monoblastic U937 cells were examined. Protein tyrosine kinase inhibitors genistein (30 µM), tyrphostin 47 (50 µM) and piceatannol (100 µM); phosphatidylinositol 3 kinase inhibitor LY294002 (10 µM); as well as mitogen-activated protein kinase kinase inhibitors PD 098059 (30 µM) and U0126 (3-30 µM) significantly reduced peak anaphylactic bronchial contraction and facilitated relaxation of the contracted bronchi. These inhibitors markedly prevented antigen-induced release of both histamine and peptidoleukotrienes from chopped lung preparations. On the other hand, genistein (10 µM), tyrphostin 47 (10 µM), piceatannol (1-10 µM), PP2 (1-10 µM), a selective Src kinase inhibitor, PD098059 and U0126 (1-10 µM), as well as LY294002 (3-10 µM) significantly reduced DNA synthesis (i.e. 3H-thymidine incorporation) in airway smooth muscle cells. Immunoblot analysis showed that these inhibitors concentration-dependently suppressed the expression of cyclin D1 in the airway smooth muscle cells. In addition, PD 098059 and U0126 (1-10 µM), and LY294002 (1-10 µM) abolished the up-regulation of CCR1 in U937 cells induced by cytokines at the gene and protein levels, and the cell chemotaxis to macrophage inflammatory protein-1α, a ligand for CCR1. Taken together, our data show that inhibitors of the tyrosine kinase signalling cascade may possess therapeutic potential for the treatnent of allergic airway inflammation.

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523.15

Extracts of Diverse Algae Inhibit Rat Mast Cells

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Finding appropiate inhibitors of mast cells has been in part limited by the difficulty of the assay, which I alleviated in recent assay modifications. To evaluate micoassays for histamine release from mast cells in the search for anti-inflammatory drugs and mechanisms, extracts of algae, a farmable resource, were examined to evaluate potential sources of biologicals that could serve as lead compounds that inhibit the release of histamine from mast cells. Samples of algae were either laboratory grown or collected from the North Atlantic seacoast. Methanolic extracts were made, filtered, and stored at -20C. Freshly harvested rat peritoneal mast cells were used in microplate histamine release assays as described previously. Of eleven algae surveyed, most had some activity, with extracts of the brown algae the most active. Significant activity in Spirulina had been reported by others and was confirmed in these studies. Varying activity appeared in the filtrate of 30KD, 10KD and 3 KD filters, indicating substantial diversity of agents. Similarly, liquid/liquid partitioning of the Spirulina activity showed significant dispersion in the water, butanol and ethyl acetate fractions, mostly distributed out of water. Activity was enhanced by longer exposure (10 min versus 1 hour) to the mast cells before challenge stimulation using compound 48/80 as the control stimulant. Mass distribution of microalgal extract eluant from an LH-20 column in methanol as measured by absorbance at 214 nm was dispersed. Activity was stable in acid methanol suggesting RP-HPLC as a safe future method of isolation. It was concluded that the microassay is an effective screening tool; in the Spirulina methanolic extract as an example, that methods of analysis all indicate that there are multiple activities present of varying chemistries; and unrelated algae have mast cell inhibiting activity of some kinds in amounts allowing further study. While these findings indicate that algae could be high priority sources for bioprospecting, the complexity indicated by probable multiple activities suggests they also will be a challenge.

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ABSTRACTS 2.1-537.42
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