SUNDAY

481 Augmentation of Inflammatory and Immune Responses to Inhaled Antigen by Combined Exposure to Beta-naphthoflavone (BNF)

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Combined exposures of the respiratory tract to diesel exhaust particulate (DEP) and allergen are associated with an enhanced allergen-specific IgE response. Polycyclic aromatic hydrocarbons (PAH) are thought to at least partially mediate this effect of DEP. To further evaluate the ability of PAH to modulate responses to inhaled antigen, combined exposures to a representative PAH, BNF, and an inhaled protein antigen, ovalbumin (OVA), were studied in C57BL/6 mice. BNF (100 mg/kg) dissolved in olive oil (OO) or OO alone were administered IP on days 0, 4, and 8. Both groups were also exposed to OVA by aerosol (35 mg/m^3, 30 min/day × 9 days). Serum anti-OVA IgG concentrations were not different in the BNF and OO groups at days 0 and 7, but were significantly greater in the BNF group at days 14, 17, and 21. To assess whether the BNF-treated mice were at greater risk of pulmonary inflammatory responses on re-exposure to OVA, animals from both groups underwent aerosol challenge between days 28 and 36 (35 mg/m^3 × 30 min). Mice were sacrificed and BAL examined 48 hr later. BNF-treated mice had significantly increased BAL total cell, lymphocyte, and eosinophil counts relative to OO-treated mice, with increases in BAL lymphocytes being the most marked. Trends were also noted for increased BAL macrophages and PMN. To assess the potential role of the aryl hydroxyl receptor (Ahr) in mediating the effects of BNF, exposures as noted above were performed using B6 mice congenic for Ahr unresponsiveness due to substitution of the *Ahr^d* locus from DBA mice (B6.D, *Ahr^d*). Although the differences were not as marked as those noted for C57BL/6 mice, *Ahr^d* mice treated with BNF also developed significantly greater serum anti-OVA IgG responses than those treated with OO after aerosol exposure to OVA. After aerosol challenge with OVA, *Ahr^d* mice treated with BNF had significantly greater BAL total cell, macrophage, and lymphocyte counts than those treated with OO, with the BAL lymphocyte counts being the most markedly increased. A trend was also noted for increased BAL eosinophils. These data demonstrate that exposure to at least one representative PAH can increase the level of *in vivo* inflammatory and immune responses induced by inhaled antigen. Furthermore, augmentation of response can occur even in the presence of a relatively poorly functional Ahr, suggesting an important role for other pathways in mediating PAH effect.

482 Analyses of Lyn -/- Phenotype Suggest a Central Role of Lyn Kinase in Eosinophil Differentiation and Allergen-stimulated Airway Eosinophilic Inflammation

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Lyn kinase is one of the most abundantly expressed Src-type tyrosine kinases in eosinophils. We have previously shown that Lyn is physically associated with IL-5Rα, GM-CSFRα and the common receptor subunit βc. Both IL-5 and GM-CSF can induce eosinophil differentiation. However, GM-CSF is not essential in this process. The importance of Lyn for eosinophil differentiation is unknown. In the present study we have examined IL-5-stimulated eosinophil differentiation in Lyn knockout mice. Bone marrow stem cells from sensitized Lyn -/- and control mice were cultured in the presence of IL-3 and IL-5 and eosinophil differentiation was assessed by Giemsa staining. Eosinophil differentiation was 26.2 ± 2.2% in bone marrow cultures from control mice and this differentiation was reduced to 10.5 ± 2% ($P<0.04$) in Lyn -/- mice. The total cell count in Lyn/- mice was similar to that in control cultures. Thus, the null mutation of Lyn did not impair differentiation of other myeloid lineages. The function of mature eosinophils is regulated not only by IL-5 and GM-CSF but also by CC chemokines. Recently we have shown that Lyn kinase is associated with the CC chemokine receptor-3 (CCR3) and is activated by eotaxin. CCR3 ligands (eotaxins, MCP-2, -3,-4, RANTES) are considered to play an important role in airway allergic inflammation. For this reason we studied the role of Lyn in allergen-induced eosinophilic inflammation using ovalbumin-sensitized Lyn -/- and control mice. Inhalation of ovalbumin caused intense airway eosinophilic inflammation in control mice. The BAL eosinophil count was 53 ± 4%. In contrast, BAL eosinophil number was dramatically reduced to 9.7 ± 3% ($P<0.01$) in Lyn -/- mice. Next, we examined the mechanism of inhibition of eosinophilic inflammation in Lyn -/- mice. We have previously shown that IL-5 and eotaxin activate ERK1/2 and p38 MAP kinases in a Lyn-dependent manner. In order to investigate the signaling molecules downstream of Lyn kinase that might be involved in eosinophilic inflammation, we have performed Western blotting of the allergen-challenged lung tissue with antibodies against the activated form of the kinases. Western blotting showed robust activation of ERK1/2 in control mice but not in Lyn -/- mice. In contrast, there was no difference in the Western blotting pattern of p38 MAP kinase. The phosphorylation of STAT-5, an IL-5-activable signaling molecule, was also unchnaged in Lyn/- mice. Our results suggest that the null mutation of Lyn predominantly affects the ERK1/2 pathway during airway inflammation. Because of its interaction with multiple eosinophil-active cytokine receptors and its essential role in eosinophil differentiation, we conclude that Lyn kinase plays a central role in the pathogenesis of airway eosinophilic inflammation.

483 The Role of Slp-76 Domains in Signaling Via the High-affinity IgE Receptor (FcεRI) in Mast Cells

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Products released from mast cells and basophils mediate allergic reactions after IgE mediated aggregation of the high-affinity receptor for IgE (FcεRI) by allergen. SLP-76 (SH2 domain containing leukocyte protein of 76 kilodaltons) is an adapter protein, which integrates signaling events from immune receptors such as the T cell receptor and FcεRI. We have previously shown that mast cells mature normally in SLP-76$^{-/-}$ mice. However, bone marrow derived mast cells (BMMC) from SLP-76$^{-/-}$ mice failed to release β-hexosaminidase and to secrete IL-6 after FcεRI crosslinking. SLP-76 plays therefore a pivotal role for mast cell function by integrating signals from FcεRI. SLP-76 has three major domains which link to various signaling pathways following FcεRI ligation: a N-terminal domain that contains three tyrosines (Y113, Y128 and Y145) which upon phosphorylation recruit Vav and Nck via SH2 interactions, a central proline rich region that binds to Gads and allows the translocation of SLP-76 to lipid rafts through LAT and a C-terminal SH2 domain that recruits tyrosine phosphorylated proteins such as Fyb/SLAP-130. To examine the role of SLP-76 domains in FcεRI signaling, BMMCs from SLP-76$^{-/-}$ mice were reconstituted by retroviral transfer of cDNA coding for SLP-76 and SLP-76 domain mutants. As expected, reintroduction of SLP-76 fully restored mast cell functions to normal levels as assessed by degranulation, calcium flux and IL-6 production. Mutation of all three tyrosines to phenylalanine in the N-terminal domain failed to correct the defect in degranulation and calcium flux, but partially restored IL-6 production. Tyrosine 145 (Y145) was critical, because a Y113F, Y128F double mutant restored mast cell functions. Deletion of the central proline rich domain (aa 224-244) restored degranulation and to a lesser extent calcium flux, but not IL-6 production. Deletion of the SH2 domain restored calcium flux and partially IL-6 production, but not degranulation. These results suggest that all three domains of SLP-76 are important for FcεRI mediated mast cell activation. We are currently conducting studies to precisely define the role of various molecules that interact with SLP-76 in mast cell activation.