85 Neutrophil elastase up-regulates interleukin-8 gene expression via a pathway involving interleukin-1 receptor-associated kinase in human bronchial epithelium.

Respiratory Research Division, Royal College of Surgeons in Ireland, Education and Research Centre, Beaumont Hospital, Dublin 9.

Previously it has been shown that neutrophil elastase (NE) can induce interleukin-8 (IL-8) expression in bronchial epithelial cells (BECs). We have elucidated the intracellular mechanism by which this occurs. Time course and dose response studies showed that stimulation of 16HBE14o- cells with NE (10 nM, 4h) induced maximal IL-8 promoter activity, IL-8 gene expression and IL-8 protein production as assessed by reporter gene assays, quantitative RT-PCR and ELISA, respectively. These responses were inhibited by actinomycin D, indicating a transcriptional regulatory mechanism. Using electrophoretic mobility shift assays we demonstrated that NF-kappaB, specifically the p50/65 heterodimer, was activated in response to NE and that this activation correlated with a concomitant degradation of IkappaB-alpha, a negative regulator of NF-kappaB. In addition interleukin-1 receptor-associated kinase (IRAK) was degraded following NE stimulation of 16HBE14o- cells, implicating it as a signal transducer for NE in this pathway in human BECs.

86 The IL1 receptor homologue T1/ST2 activates MAP kinases and PI3-kinase in human bronchial epithelium.

Biochemistry Dept., Trinity College, Dublin 2, Ireland.

T1/ST2, a stable marker for type 2 T helper (Th2) cells, is a member of the Toll/IL1 receptor superfamily which participates in Th2 type responses including induction of IL-4 and IL-5. A neutralising antibody to T1/ST2 attenuates Th2 driven responses in an in vivo model of asthma. The ligand for T1/ST2 is unknown. We have investigated the ability of T1/ST2 to initiate intracellular signalling pathways using in vitro overexpression studies in lieu of a physiological ligand. Overexpression of a plasmid encoding T1/ST2 in HEK293 cells or in EL4 murine thymoma cells activates p42/44 MAPK and Jun N-terminal kinase. T1/ST2 also activates AP-1 and induces a reporter gene under the control of the IL-3 promoter. A chimera comprising the extracellular domain of the type I IL-1 receptor and the intracellular domain of T1/ST2 activates NF-kappaB in response to IL-1 but only via recruitment of the IL-1 receptor accessory protein. Trypt T1/ST2 fails to activate NF-kappaB. Taken together these results identify T1/ST2 as the only member of the TIR domain containing superfamily so far tested which fails to activate NF-kappaB, consistent with a lack of a role of NF-kappaB in Th2 cell function.

87 The role of PI3-kinase in IL-3-mediated proliferation and cell cycle progression.

B. Fox, C. Edmead and M.J. Whelham.
Department of Pharmacology, The University of Bath, Bath, BA2 7AY.

Interleukin-3 acts as a growth and survival factor for haemoepoietic cells, activating many intracellular signalling cascades, including the phosphoinositide 3-kinase (PI3K) pathway. Previously, we demonstrated that regulated expression of a dominant-negative mutant of class IA PI3Ks (p85) caused a dramatic reduction in IL-3-induced proliferation, with little change in apoptosis, implicating PI3K as a key regulator of IL-3-mediated proliferation. We are now investigating the mechanism PI3K utilises to regulate IL-3-mediated proliferation.

Expression of p85, or treatment with LY29402, decreased IL-3-induced activation of MAPKs, slowed cell cycle progression and led to accumulation of cells in G1. Consistent with these findings, inhibition of PI3Ks caused a reduction in cyclin D3 levels and a decrease in IL-3-induced phosphorylation of the pocket proteins p130 and p107, all characteristic of a G1 arrest. These studies indicate that PI3K plays an important role in mediating G1/S-phase cell cycle progression. To investigate the role played by the PI3K binding protein Gab2 in these events, we expressed a Gab2 mutant deficient in PI3K binding. Expression of this mutant resulted in decreased phosphorylation of endogenous Gab2 and reduced its association with PI3K. We are currently investigating the effects of this mutant on IL-3-driven proliferation and survival.

88 Mucin-degrading glycosidase activity in Peyer’s patches and lamina propria.

A.W. Baird
Department of Veterinary Physiology and Biochemistry and *Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University College Dublin, Belfield, Dublin 4, Ireland

Peyer’s patches (PP) are portals for the surveillance of gut contents by the immune system. PP sample particulate antigens and microorganisms from the gut lumen and transfer them to the underlying mucus-associated lymphoid tissue. PP lack a substantial mucus layer. We hypothesise that mucin-degrading enzymes dynamically spread over PP from adjacent mucosa to form a continuous layer. We investigated the role of PI3-kinase in IL-3-mediated proliferation and cell cycle progression.

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