**Introduction and objectives** The alveolar macrophage (AM) is a key player in orchestrating the inflammatory response to inhaled pathogenic and environmental toxins. DEP causes the release of monocytes from the bone marrow, and during chronic inflammation, macrophages mature continuously from infiltrating monocytes. There are no studies investigating the chronic effects of DEP on monocytes and actions of DEP on neutrophils are unclear. We investigated how DEP modulate neutrophil and monocyte function and macrophage differentiation.

**Methods** Monocytes and neutrophils were obtained from healthy volunteers by density gradient centrifugation and negative magnetic selection. Monocyte-derived macrophages (MDMs) were generated in the presence or absence of DEP. Apoptosis and cell loss of MDMs were assessed by microscopy, propidium iodide positivity and alamar blue reduction assays. Killing assays of Streptococcus pneumoniae were performed. Cytokine generation in response to varied TLR agonists was assessed by ELISA and surface marker expression of receptors including TLRs, and CD14 was measured by FACS.

**Results** Monocytes and MDMs avidly phagocytosed DEP but neutrophils neither phagocytosed DEP, or markedly responded to DEP. However, chronic exposure to DEP in vitro had a detrimental effect on monocytes, with marked enhancement of apoptosis and cell loss. Despite this, the ability to kill an important respiratory pathogen S pneumoniae was preserved. Cytokine generation to TLR agonists was reduced, and this phenotype was associated with a reduction in CD14 surface marker expression.

**Conclusions** DEP directly activate monocytes but not neutrophils, and chronic exposure may have detrimental effects on the host by enhancing loss of monocytes recruited to the airways and modulating aspects of the inflammatory response to pathogens.

### REGULATION OF NEUTROPHIL APOPTOSIS BY PHOSPHOINOSITIDE 3-KINASES

**Introduction** Neutrophils are an essential component of the innate immune response. However, their microbicidal mechanisms (generation of reactive oxygen species and release of proteolytic enzymes) may contribute to tissue injury. Neutrophil-mediated tissue damage is a cardinal feature in the pathogenesis/progression of COPD, cystic fibrosis and certain types of asthma. Apoptosis is the key determinant of tissue neutrophil longevity and is critical to the resolution of granulocyte inflammation; pharmacological acceleration of neutrophil apoptosis can promote resolution of inflammation in animal models. The cytokine GM-CSF drives the aberrant neutrophil survival phenotype observed in patients with ARDS, and recent studies suggest the phosphoinositide 3-kinase (PI3K)/AKT pathway is pivotal in signalling GM-CSF-mediated neutrophil survival.

**Hypothesis** Given the emerging evidence that individual Class I PI3K isoforms (α, β, δ and γ) exert non-redundant signalling roles and represent promising therapeutic targets in inflammation, we hypothesised a distinct contribution of individual Class I PI3K isoforms in mediating constitutive neutrophil apoptosis and the GM-CSF cytoprotective effect.

**Methods** We established techniques to isolate peripheral blood neutrophils from humans and from knockout/transgenic mice lacking functional PI3K isoforms to 95% purity, and used pan-PI3K inhibitor (LY294002), pan-Class I PI3K inhibitor (PI-103) and novel PI3K isoform-selective small molecule inhibitors (YM-024, TGX-221, IC87114 and AS605240) to determine precise involvement of Class I PI3K isoforms (α, β, δ and γ) in constitutive and GM-CSF-delayed neutrophil apoptosis. Apoptosis was quantified using morphology and annexin V-FITC/propidium iodide staining.

**Results** GM-CSF-mediated neutrophil survival was reversed by pan-PI3K inhibition but not by individual PI3K isoform inhibition. Combinatorial experiments suggest there is near-complete functional redundancy amongst Class I PI3Ks with regard to GM-CSF-mediated inhibition of neutrophil apoptosis; additionally, neutrophils derived from double knockout PI3K-α/δ and PI3K-β/δ mice had normal constitutive apoptosis and GM-CSF mediated survival, but were sensitised to inhibition of the remaining isoforms.

**Conclusions** Thus Class I PI3Ks mediate GM-CSF survival of human and murine neutrophils but there is complete functional redundancy of the PI3K isoforms, necessitating multiple isoform inhibition to reverse GM-CSF-induced neutrophil survival. This finding informs our understanding of the mechanisms regulating neutrophil apoptosis and suggests neutrophil survival would be resilient to individual isoform-selective PI3K inhibitors.