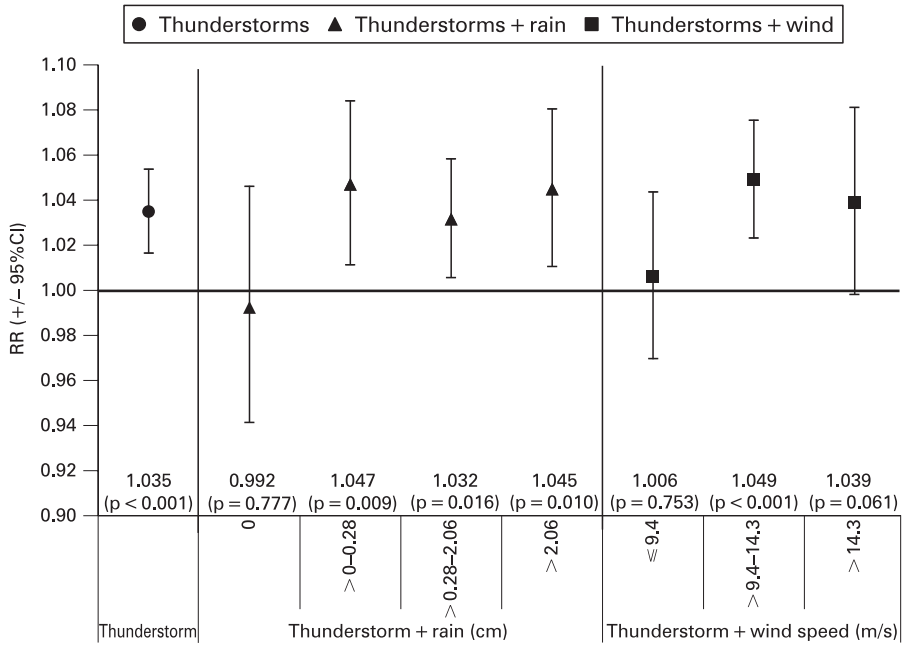

Figure 1 Relative risk of asthma emergency department visits following days with thunderstorms, thunderstorms in combination with rainfall (in four categories) and thunderstorms in combination with wind speed from maximum 5 s wind gusts (in three categories), compared with days with no thunderstorms. Relative risks (RR) and p values are presented for each model result.

When thunderstorms were stratified by wind gust levels, associations with asthma were strongest when wind gusts were intermediate and high.

Our findings corroborate previous reports of an association of thunderstorm activity with asthma exacerbation. Furthermore, our results provide preliminary evidence in support of rainfall and wind gusts playing important roles in this association. While a 3% increase in risk may seem modest, asthma is quite prevalent in Atlanta and a modest relative increase could have a significant public health impact in the population. This analysis used meteorological data from one weather station. However, thunderstorms are small scale phenomena, and these data may only represent events in close proximity to the station. Planned analyses will take advantage of data from other local stations, radar data on thunderstorm characteristics and spatial resolution of the outcome data to conduct a more refined assessment of the mechanistic basis of the observed association.

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Prolonged survival of neutrophils from patients with ΔF508 CFTR mutations

Cystic fibrosis (CF) is an autosomal recessive "channelopathy" characterised by aberrant CFTR and ENaC function resulting in widespread epithelial cell dysfunction and persistent airway infection. Studies indicating that airway inflammation precedes infection[1] and that patients with CF display exaggerated neutrophilic responses to pathogens have suggested a primary defect in innate immune responses in CF. Given the importance of apoptosis to the resolution of neutrophilic inflammation,[2] we sought to determine whether circulating CF neutrophils display normal apoptotic capacity.

Peripheral blood neutrophils were isolated from 12 clinically stable *Pseudomonas* colonised ΔF508 homozygote adult patients with CF and 12 age and sex matched healthy controls using discontinuous plasma/Percoll gradients.[3] Blood neutrophil counts were in the normal range for all subjects (CF mean 4.1 (range 5.4–8.3), controls 2.5 (3.7–6.6) $\times 10^9$/l); none of the patients with CF were taking azithromycin. CF and control neutrophils were isolated in parallel and handled identically. Cells were cultured (5×10^6/ml, 5% CO_2, 37°C) in Iscove's Dulbecco's medium with 10% autologous serum for 6 and 20 h, as detailed previously.[3] These incubations were conducted with or without tumour necrosis factor α (TNFα 10 ng/ml) or granulocyte macrophage-colony stimulating factor (GM-CSF 10 ng/ml), which have defined proapoptotic and antiapoptotic effects, respectively.[3] Apoptosis was assessed by blinded morphological assessment of May–Gründwald–Giemsa stained cytospins, confirmed by quantification of annexin-V–fluorescein isothiocyanate binding and propidium iodide staining.[3]

Neutrophils from patients with CF had delayed constitutive apoptosis and were resistant to the normal early proapoptotic effects of TNFα,[3] yet remained fully sensitive to the antiapoptotic effects of GM-CSF (fig 1A–C). Incubation of control neutrophils with 10% serum from patients with CF mimicked exactly the enhanced survival effect seen in CF cells, suggesting an acquired rather than intrinsic defect (fig 1D). These data indicate that blood neutrophils from clinically well patients with CF display a relative resistance to spontaneous and death ligand induced apoptosis when cultured with autologous serum. Using the Luminex FlowMetrix system to compare protein and cytokine profiles in CF and control sera, only C reactive protein (CRP) differed, with a 3.6-fold increase in CF sera (controls 1.6 (0.3) μg/ml, CF 5.8 (1.3) μg/ml; $p<0.05$, $n=4$). Although no direct measures of neutrophil activation were performed, there were no differences in proinflammatory cytokines or growth factors present in control and CF sera, including interleukin (IL)8, TNFα, IL6 and GM-CSF. Previous data reporting that migration inhibitory factor can inhibit CF neutrophil apoptosis may reflect lipopolysaccharide contamination of

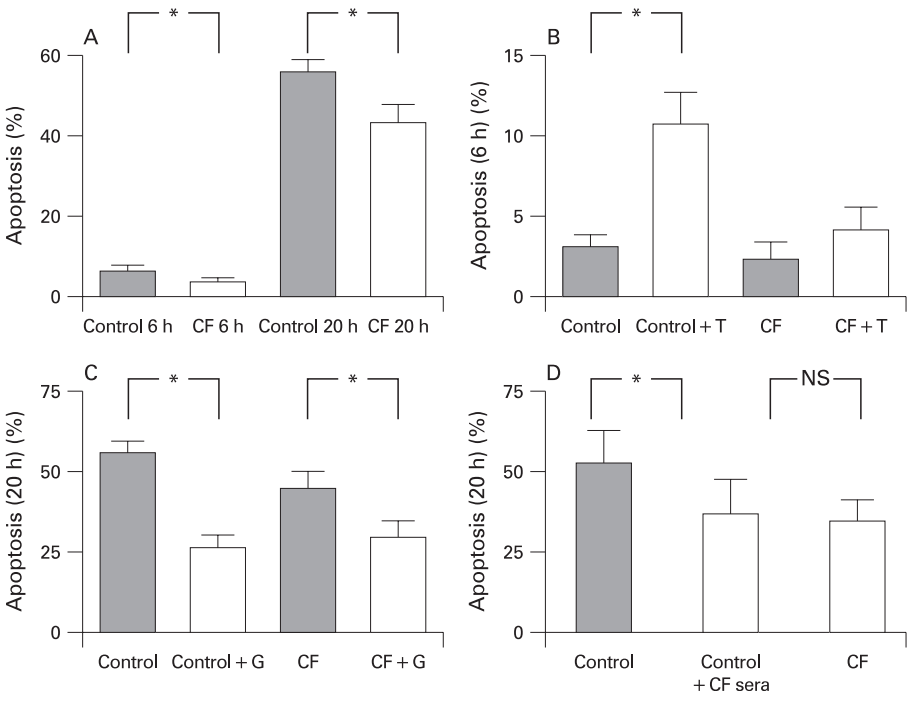

Figure 1 Human peripheral blood neutrophils from patients with cystic fibrosis (CF) and matched control subjects were incubated for 6 or 20 h in DMEM containing 10% autologous serum (unless otherwise stated) and then assessed for apoptosis using morphological criteria. (A) Delay in constitutive apoptosis in CF neutrophils at 6 and 20 h (*$p<0.05$, $n = 12$). (B) Loss of the early proapoptotic effect of tumour necrosis factor α (T) at 6 h (*$p<0.05$, $n = 5$). (C) Preserved prosurvival effect of granulocyte macrophage-colony stimulating factor (G) in CF neutrophils (*$p<0.05$, $n = 11$). (D) Ability of sera from patients with CF to delay apoptosis in normal neutrophils (*$p<0.05$, $n = 4$). Parallel assessment of apoptosis using annexin-V– fluorescein isothiocyanate binding and propidium iodide staining[3] resulted in essentially identical data (note shown). Data are expressed as mean (SEM) of (n) separate experiments, each conducted in triplicate and analysed using non-parametric (Mann–Whitney) calculations of significance.

the cytokine preparation.[4] Moreover, the delay in constitutive apoptosis in CF neutrophils was inhibited by LY294002 (10 μM), a phosphoinositide 3-kinase (PI3 kinase) inhibitor (% apoptosis 20 h: control 54 (2), control+LY294002 61 (5), CF 33 (7), CF+LY294002 50 (9), $n = 3$).

These findings add to the body of data suggesting broader defects in innate immune responses in CF. Factors present in CF serum appear to inhibit both constitutive and TNFα induced apoptosis, which would be predicted to impair the physiological removal of these cells at inflamed sites. A potential role for CRP is supported by reports that monomeric CRP, which is generated in inflamed tissues, can inhibit neutrophil apoptosis via a mechanism involving activation of FcγRIII (CD16) and PI3 kinase.[5] Together, these results suggest that CF neutrophils have an impaired capacity to undergo apoptosis, even prior to migration to the lung.

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NOTICE

New drugs and targets for asthma and COPD

19–21 November 2008, Imperial College London at the National Heart & Lung Institute, London, UK. In collaboration with the Royal Brompton Hospital, this course is suitable for physicians or scientists with an interest in the therapeutics of asthma and COPD and those working in the pharmaceutical industry (course organiser, Professor Peter Barnes). Enquiries to: Karina Dixon, Academic Events Office, National Hearth & Lung Institute, Imperial College London, Guy Scadding Building, Royal Brompton Campus, Dovehouse Street, London SW3 6LY, UK; tel: +44 (0)20 7351 8172; fax: +44 (0)20 7351 8246; email: shortcourses.nhli@imperial.ac.uk