LETTERS

Interleukin-5 inhibits glucocorticoid-mediated apoptosis in human eosinophils

Glucocorticoids (GCs) represent one of the most effective treatments for eosinophil-mediated inflammatory diseases such as asthma. GCs act through the GC receptor, leading to proinflammatory cytokine suppression and a reduction in the number of inflammatory cells including eosinophils and T cells.¹ However, the benefits of GCs have been limited by their side effects and the presence of GC resistance. This led to the development of more selective GCs such as fluticasone propionate (FP) and fluticasone furoate (FF).²

Increased eosinophil survival has been proposed as a mechanism underlying tissue

eosinophilia, and part of the anti-inflammatory effects of GCs has been attributed to their ability to promote eosinophil apoptosis. Interleukin 5 (IL-5) enhances eosinophil survival by inhibiting apoptosis, and increased IL-5 expression is reported in eosinophilic inflammation.³ We sought to address the ability of the 'enhanced-affinity' FF, alongside dexamethasone (DEX) and FP, to modulate eosinophil apoptosis and their potential to over-ride IL-5 pro-survival signals.

Human granulocytes were isolated from the peripheral blood of non-medicated normal and atopic donors. Approval was obtained from the Cambridge Research Ethics Committee. Neutrophils were purified using plasma–Percoll gradients and eosinophils by RoboSep (StemCell Technologies, Vancouver, Canada). Granulocytes were resuspended in RPMI 1640, 50 U/ml streptomycin and penicillin G, and 10% autologous serum before incubation (5% CO₂, 37°C). A

total of 25±1.5% of the eosinophils were apoptotic (assessed by fluorescein isothiocyanate (FITC)–Annexin V) by 24 h (data not shown). This compares closely to 31.6±3.3% when assessed by morphology.⁴

Co-incubation of eosinophils with DEX, FP or FF resulted in a concentration-dependent increase in apoptosis, with similar efficacy but different potency (mean half-maximal effective concentration (EC₅₀) and 95% CI): DEX=2.3×10⁻⁸ M (8.2×10⁻⁹ to 6.7×10⁻⁸ M), FP=4.3×10⁻¹⁰ M (9.6×10⁻¹¹ to 1.9×10⁻⁹ M), FF=1.4×10⁻¹⁰ M (8.2×10⁻¹¹ to 2.3×10⁻¹⁰ M) (figure 1A). The GC receptor antagonist RU38486 blocked the effect of these compounds (data not shown). The GCs produced concentration-dependent inhibition of neutrophil apoptosis at 20 h (mean EC₅₀): DEX=1.3×10⁻⁹ M, FP=4.5×10⁻⁹ M, FF=1.4×10⁻¹⁰ M).

As expected IL-5 inhibited eosinophil apoptosis, maximally at 0.1 ng/ml (figure 1B);

Figure 1 Induction of eosinophil apoptosis in the absence and presence of interleukin 5 (IL-5) by dexamethasone (DEX), fluticasone propionate (FP) and fluticasone furoate (FF). Apoptosis values (assessed by fluorescein isothiocyanate (FITC)-labelled Annexin V and propidium iodide) are expressed as a percentage of control apoptosis at 24 h. Dimethylsulfoxide (DMSO) was used as the vehicle for the glucocorticoids (GCs), and IL-5 was pre-incubated with the eosinophils for 1 h before the addition of the GC. Eosinophil adhesion to the Costar 96-well plates⁴ was assessed in the presence or absence of IL-5 over 24 h according to Oliver *et al.*⁶ Data represent the mean±SEM. Statistical significance was analysed by Kruskal–Wallis and Dunn post-test, and values of **p* < 0.05 were considered statistically significant. (A) Effect of GCs on constitutive eosinophil apoptosis, **p* < 0.05 vs control (*n* ≥ 4). (B) Effect of IL-5 on constitutive eosinophil apoptosis (*n* = 16), **p* < 0.05, ***p* < 0.01 and ****p* < 0.001 vs control. (C) Effect of IL-5 on eosinophil adhesion (*n* = 4), **p* < 0.05 vs control. (D–F) Effect of GCs on IL-5 inhibition of eosinophil apoptosis, *n* ≥ 4 (**p* < 0.05 vs IL-5 alone).

the reduction in the efficacy of IL-5 at 1 ng/ml reflects the capacity of this higher concentration to induce eosinophil adhesion (figure 1C). IL-5 preincubation before the addition of each GC caused a concentration-dependent inhibition of the proapoptotic effects of these compounds (figure 1D–F). This occurred without any significant shift in the GC concentration–response curves, suggesting non-competitive antagonism.

This is the first demonstration of the proapoptotic capacity of FF in eosinophils and confirms the high potency of this compound compared with DEX. Despite this higher potency, IL-5 could still over-ride the proapoptotic effects of FF, suggesting that the IL-5 transduction pathway has GC-resistant elements.⁵ Whether this can be modulated by strategies to overcome resistance such as phosphatidylinositol 3-kinase and mitogen-activated protein kinase inhibitors is as yet unclear.¹ In conclusion, this study suggests a mechanism underlying poor responsiveness of eosinophils to GCs and may help define therapeutic approaches to overcome GC resistance.

Acknowledgements We thank Drs M Johnson and S N Farrow for helpful discussions.

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Funding This work was funded by a non-commercial grant from GlaxoSmithKline, Asthma-UK, Papworth Hospital R&D and NIHR Cambridge Biomedical Research Centre. JKK holds a NIHR Cambridge BRC Research Training Fellowship.

Competing interests None.

Ethics approval This study was conducted with the approval of the Cambridge Research Ethics Committee, UK 06/Q0108/281.

Provenance and peer review Not commissioned; externally peer reviewed.

Accepted 28 December 2009

Published Online First 30 August 2010

Thorax 2010;**65**:1116–1117.

doi:10.1136/thx.2009.124909

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Vigilance is also required for pulmonary tuberculosis in UK-born subjects

Over and above the recommendation that 'Clinicians should have a higher index of clinical suspicion of extrapulmonary tuberculosis in non-UK born cases',¹ due to an increasing proportion of non-UK-born cases during 1999–2006,¹ we also need to be vigilant for the possibility that UK-born individuals who have escaped early detection of pulmonary tuberculosis might subsequently present with advanced pulmonary tuberculosis (APT), as was the case in the USA during the period 1993–2006. During that period 160 661 notified cases of pulmonary tuberculosis were subdivided into those with APT (35 584 cases), characterised by cavitation on chest radiograph and acid-fast bacilli smear-positive sputum, and those without advanced disease, the latter amounting to 125 077 cases. Further analysis revealed that, during that period, the proportion of patients with APT 'increased greatest among whites (65.4%), the employed (63.3%), and US born (59.2%)'.² Accordingly, a recommendation, which also has relevance to the UK, was made that 'Additional efforts should concentrate on reducing time to treatment initiation in low-incidence areas and among groups traditionally seen as being at low risk for tuberculosis disease'.² Cautionary tales already abound in the tabloid press^{3–4} of white UK-born young (age range 25–41) professionals (and, in one instance, a law student) in whom the diagnosis of pulmonary tuberculosis was delayed because of under-recognition of the significance of presenting symptoms in primary care and, in one instance, also in secondary care. What also needs to be recognised is that extrapulmonary tuberculosis itself poses a threat of transmission of the disease to other people, given the fact that, in the event of positive sputum culture results despite normal chest x-ray findings,⁵ 'transmission of tuberculosis from smear-negative, culture-positive patients has been well documented in other settings'.⁵

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Competing interests None.

Provenance and peer review Not commissioned; externally peer reviewed.

Accepted 28 December 2009

Published Online First 1 October 2010

Thorax 2010;**65**:1117. doi:10.1136/thx.2009.132605

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Authors' reply

Our study examined trends in extrapulmonary tuberculosis in the UK and investigated the factors associated with recent changes highlighting this to clinicians.¹ Jolobe summarises reports from the USA and anecdotal media coverage of patients in whom the diagnosis of pulmonary tuberculosis was delayed due to the perception of low risk in native-born patients who subsequently presented with advanced pulmonary disease. We have previously reported delayed diagnosis in these groups in the UK.² As pulmonary tuberculosis is the infectious form of the disease with significant consequences of delayed diagnosis on the control effort and possible adverse clinical outcomes, we agree that the index of suspicion in patients with signs and symptoms suggestive of pulmonary tuberculosis should remain high.

This, however, raises an important clinical conundrum about uncommon illnesses. Among UK-born white adults aged 15–45 years, only 299 of 375 cases were reported with pulmonary tuberculosis. The number of cases in this subgroup of the population has remained relatively stable. A significant proportion of these will be individuals with other risk factors for tuberculosis such as homelessness and drug use or identified as contacts of a case of infectious tuberculosis. If every person with, for example, a cough lasting 3 weeks is investigated for tuberculosis, this will result in significant unnecessary investigation, especially in primary care. With <9000 cases in a population of 60 million, the majority of whom are resident in urban areas,³ the average general practitioner in a rural area in