LETTER

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. 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). 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. 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 prosurvival 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 re-suspended in RPMI 1640, 50 U/ml streptomycin and penicillin G, and 10% autologous serum before incubation (5% CO2, 3% O2, 92% N2). Eosinophils were incubated with the eosinophils for 1 h before the addition of the GC. Eosinophil adhesion to the Costar 96-well plates was assessed in the presence or absence of GCs. Eosinophil apoptosis was assessed in the presence or absence of GCs. Eosinophil adhesion was measured in the presence of GCs and propidium iodide (PI) was used to assess apoptosis. Eosinophil apoptosis was assessed using PI as a marker of apoptosis.

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 plates was assessed in the presence or absence of IL-5 over 24 h according to Oliver et al. 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. (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. 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. In conclusion, this study suggests a mechanism underlying poor responsiveness of eosinophils to GCs and may help define therapeutic approaches to overcome GC resistance.

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Sven Brode, Neda Farahi, Andrew S Cowburn, Jatinder K Juss, Alison M Condliffe, Edwin R Chilvers
Respiratory Medicine Division, Department of Medicine, University of Cambridge, Cambridge, UK
Correspondence to Professor Edwin R Chilvers, Department of Medicine, University of Cambridge, Box 157, Addenbrooke’s Hospital, CUH NHSFT, Hills Road, Cambridge CB2 0QQ, UK; erc24@cam.ac.uk
SB and NF contributed equally to this work.
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REFERENCES
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