Exchange protein directly activated by cyclic AMP (EPAC) activation reverses neutrophil dysfunction induced by β_2-agonists, corticosteroids, and critical illness

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Background: Neutrophils play a role in the pathogenesis of asthma, chronic obstructive pulmonary disease, and pulmonary infection. Impaired neutrophil phagocytosis predicts hospital-acquired infection. Despite this, remarkably few neutrophil-specific treatments exist.

Objectives: We sought to identify novel pathways for the restoration of effective neutrophil phagocytosis and to activate such pathways effectively in neutrophils from patients with impaired neutrophil phagocytosis.

Methods: Blood neutrophils were isolated from healthy volunteers and patients with impaired neutrophil function. In healthy neutrophils phagocytic impairment was induced experimentally by using β_2-agonists. Inhibitors and activators of cyclic AMP (cAMP)–dependent pathways were used to assess the influence on neutrophil phagocytosis *in vitro*.

Results: β_2-Agonists and corticosteroids inhibited neutrophil phagocytosis. Impairment of neutrophil phagocytosis by β_2-agonists was associated with significantly reduced RhoA activity. Inhibition of protein kinase A (PKA) restored phagocytosis and RhoA activity, suggesting that cAMP signals through PKA to drive phagocytic impairment. However, cAMP can signal through effectors other than PKA, such as exchange protein directly activated by cyclic AMP (EPAC). An EPAC-activating analog of cAMP (8CPT-2Me-cAMP) reversed neutrophil dysfunction induced by β_2-agonists or corticosteroids but did not increase RhoA activity. 8CPT-2Me-cAMP reversed phagocytic impairment induced by Rho kinase inhibition but was ineffective in the presence of Rap-1 GTPase inhibitors. 8CPT-2Me-cAMP restored function to neutrophils from patients with known acquired impairment of neutrophil phagocytosis.

Conclusions: EPAC activation consistently reverses clinical and experimental impairment of neutrophil phagocytosis. EPAC signals through Rap-1 and bypasses RhoA. EPAC activation represents a novel potential means by which to reverse impaired neutrophil phagocytosis. (J Allergy Clin Immunol 2016;137:535-44.)

Key words: *Neutrophil, β_2-agonist, cyclic AMP, exchange protein directly activated by cyclic AMP, hospital-acquired infection*

Neutrophils are central to the pathogenesis of a wide variety of common and important clinical conditions, including chronic obstructive pulmonary disease and asthma (particularly corticosteroid-resistant asthma).[1-3] Patients with chronic obstructive pulmonary disease and asthma are commonly prescribed long-term inhaled β_2-agonists and corticosteroids. The detailed effects of these treatments on neutrophil phagocytic function are relatively poorly understood, although it has been suggested that β_2-agonists can impair neutrophil function.[4]

It is also increasingly recognized that acquired neutrophil dysfunction is common in critically ill patients[5] and independently associated with a significantly increased risk of subsequent hospital-acquired infection (HAI).[6] Impaired neutrophil function can be restored to normal *ex vivo* through administration of GM-CSF.[6] This suggests the potential to develop pharmacologic

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Abbreviations used
AKAP: A kinase anchoring protein
BALTI-2: Beta Agonist Lung Injury Trial-2
cAMP: Cyclic AMP
EPAC: Exchange protein directly activated by cyclic AMP
HAI: Hospital-acquired infection
MTT: 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PGE_1: Prostaglandin E_1
PKA: Protein kinase A

strategies to restore specific defects in neutrophil function for clinical benefit. The potential importance of developing non–antibiotic-based pharmacologic prevention strategies is enormous. For example, at a conservative estimate, there are 1.7 million HAIs in the United States annually[7] at a time when antibiotic resistance is a global concern and few new antibiotics are in development.

Therefore the aims of this study were to characterize the effects of β_2-agonists, corticosteroids, and critical illness on neutrophil function and to identify novel ways of modulating the pathways involved in the impairment of neutrophil phagocytosis in the hope of identifying candidates with the potential to restore efficient phagocytosis.

METHODS

Reagents

Salbutamol, salmeterol, isoprenaline, fluticasone, beclomethasone, budesonide, SQ 22536, ICI 118,551, atenolol, prostaglandin E_1 (PGE_1), Y27632, zymosan derived from *Saccharomyces cerevisiae*, and Giemsa staining solution were from Sigma-Aldrich (Gillingham, United Kingdom). KT5720, mouse anti-human GM-CSF receptor antibody (IgG_{2a}), and murine IgG_{2a} negative control antibody were from Merck (Darmstadt, Germany). St-Ht31 and St-Ht31 control peptide were from Promega (Madison, Wis). Antibodies against RhoA and protein kinase A (PKA) were from Cell Signaling (Hitchin, United Kingdom). Dextran was from PHARMACOSMOS (Holbaek, Denmark). Percoll Plus was from GE Healthcare (Little Chalfont, United Kingdom). Iscove modified Dulbecco medium was from Life Technologies (Paisley, United Kingdom). 8CPT-2Me-cAMP, N^6-benzoyladenosine-cAMP, and GGTi 298 were from Tocris Bioscience (Bristol, United Kingdom). PKA inhibitor IV was from Santa Cruz Biotechnology (Dallas, Tex). ESI-09 was from BioLog (Bremen, Germany). Rho G-LISA was from Cytoskeleton (Denver, Colo). The PKA activity assay was from Abcam (Cambridge, United Kingdom).

Ethical approvals

Ethical approval to obtain neutrophils from healthy volunteers was granted by the County Durham and Tees Valley Research Ethics Committee. Approval to obtain neutrophils from patients was granted by the Yorkshire and Humber-Leeds West Research Ethics Committee. Ethical approval relating to the Beta Agonist Lung Injury Trial-2 (BALTI-2) was granted by the Oxford A Research Ethics Committee.

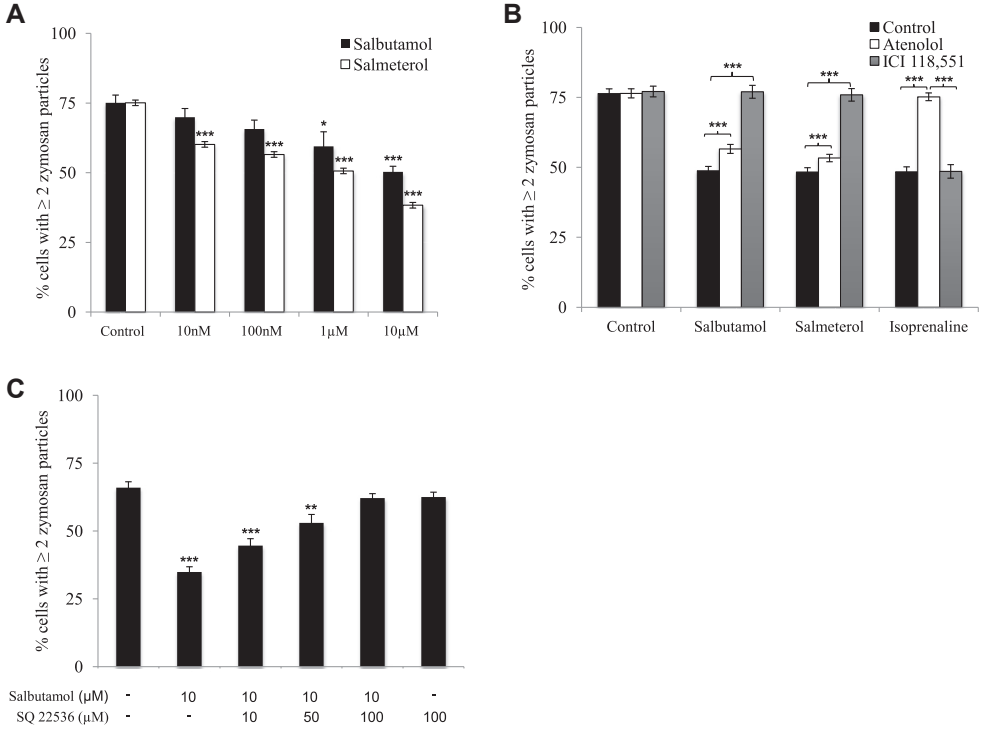

FIG 1. β-Adrenergic receptor agonists inhibit human neutrophil phagocytosis in an adenylate cyclase–dependent manner. **A,** Neutrophils were preincubated with the short-acting β_2-agonist salbutamol or the long-acting β_2-agonist salmeterol, and phagocytosis of zymosan was quantified. Statistical comparisons are with bars of the same color in the *left-hand column*. **B,** In a variation neutrophils were first treated with atenolol (100 μmol/L) or the selective β_2-agonist ICI 118,551 (10 μmol/L) for 30 minutes before exposure to either salbutamol (10 μmol/L), salmeterol (10 μmol/L), or the β_1-agonist isoprenaline (10 μmol/L). Statistical comparisons are between the bars indicated. **C,** In a separate variation neutrophils were preincubated with the adenylate cyclase inhibitor SQ 22536 before incubation with salbutamol (10 μmol/L). Statistical comparisons are with the *left-hand column*. **P* < .05, ***P* < .01, and ****P* < .001.