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Ceftaroline modulates the innate immune and host defense responses of immunocompetent cells exposed to cigarette smoke

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ABSTRACT

Background: Cigarette smoke, the principal risk factor for chronic obstructive pulmonary disease (COPD), negatively influences the effectiveness of the immune system's response to a pathogen. The antibiotic ceftaroline exerts immune-modulatory effects in bronchial epithelial cells exposed to cigarette smoke.
Aims and methods: The present study aims to assess the effects of ceftaroline on TLR2 and TLR4 expression, LPS binding and TNF-α and human beta defensin (HBD2) release in an undifferentiated and PMA-differentiated human monocyte cell line (THP-1) exposed or not to cigarette smoke extracts (CSE). TLR2, TLR4, and LPS binding were assessed by flow cytometry, TNF-α and HBD2 release were evaluated by ELISA.
Results: The constitutive expression of TLR2 and TLR4 and LPS binding were higher in differentiated compared to undifferentiated THP-1 cells. In undifferentiated THP-1 cells, CSE increased TLR2 and TLR4 protein levels, LPS binding and TNF-α release and reduced HBD2 release and ceftaroline counteracted all these effects. In differentiated THP-1, CSE did not significantly affect TLR2 and TLR4 expression and LPS binding but reduced HBD2 release and increased TNF-α release. Ceftaroline counteracted the effects of CSE on HBD2 release in differentiated THP-1.
Conclusion: Ceftaroline counteracts the effect of CSE in immune cells by increasing the effectiveness of the innate immune system. This effect may also assist in reducing pathogen activity and recurrent exacerbations in COPD patients.

1. Introduction

The host's capacity to defend itself against a foreign invader is a complex struggle, in which eradication of infection is dictated by a robust immunologic response (Kawai and Akira, 2011). External sources, such as cigarettes (Brusselle et al., 2011), can negatively influence the effectiveness of the immune system's response to a pathogen leading to benefit pathogen establishment. Cigarette smoke is the principal risk factor for chronic obstructive pulmonary disease (COPD) (Curtis et al., 2007). COPD is a disease with high prevalence and substantial associated economic burden. The severity and the progression of COPD is strongly affected by recurrent exacerbations. Bacteria and viruses, the major causes of COPD exacerbations, may contribute to COPD progression by amplifying and perpetuating the inflammatory responses within the airways (Ko et al., 2016). The alteration of dynamic balance between the 'pathogens' (viral and bacterial) and the normal bacterial communities that constitute the lung microbiome, likely contributes to the risk of exacerbations (D'Anna et al., 2016). Exacerbations of COPD are associated with heterogeneous changes in the bronchial microbiome, with increasing species related to typical COPD pathogens and decreasing species that contribute to maintain functional homeostasis (Huang and Boushey, 2015). Acute exacerbations due to recurrence of infections in smokers and in COPD patients raise the question of the possible role of antibiotics and anti-inflammatory agents in modulating the course of the disease (Ko et al., 2016). The immune response to an infection is activated by the sensing of microbial structures through families of receptors collectively called pattern recognition receptors (PRRs) (Kawai and Akira, 2011). Toll-like receptors (TLRs) belong to the most studied family of PRRs. The activation of TLRs leads to the release of a class of cysteine-rich peptides,

Abbreviations: TLRs, toll like receptors; LPS, lipopolysaccharide; HBD2, human beta defensin 2; COPD, chronic obstructive pulmonary disease; CSE, cigarette smoke extracts; TNF-α, tumor necrosis factor alpha

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with antibacterial and antiviral activities, named defensins, including beta defensin 2 (HBD2) (Cheng-Yuan et al., 2008). These host-derived peptides kill the invading pathogens by perturbing their cell membranes and can neutralize biological activities of endotoxins. Defensins in addition to their antimicrobial properties, have the potential to influence and modulate, both directly and indirectly, the activity of various cell populations involved in inflammatory processes (Niyonsaba et al., 2004).

The new antibiotic cephalosporin, ceftaroline fosamil, is a prodrug used to treat Community Acquired Pneumonia (CAP) (Lodise et al., 2015; Stryjewski et al., 2015). We have recently shown that ceftaroline (the active metabolite of ceftaroline fosamil) is able to counteract the effects of cigarette smoke extracts (CSE) exerting immunomodulatory, anti-inflammatory, and anti-microbial host defense activities in bronchial epithelial cells (Pace et al., 2016). It is unknown whether ceftaroline exerts immune-modulatory activities in immunocompetent cells. In this regard, we previously demonstrated that HBD2 prolongs the survival of neutrophils within the airways of COPD with severe exacerbations via activation of TLR4 (Pace et al., 2011). TLR-2 is upregulated in peripheral blood monocytes collected from clinically stable or exacerbated COPD patients (Pons et al., 2006). Differently, alveolar macrophages from stable COPD patients and smokers express less TLR-2 than never smokers (Droemann et al., 2005).

In the present study we tested the hypothesis that ceftaroline may be able to counteract the effect of CSE in immuncompetent cells, such as the monocyte cell line THP-1 either undifferentiated or differentiated into macrophages by exerting immunoregulatory functions and modulating anti-inflammatory and host anti-microbial activities.

2. Materials and methods

2.1. Cell cultures

The human monocytic leukemia cell line (THP-1) (ATCC TIB-202, kindly provided by Dott. Angelo Sala) was used in this study. THP-1 cells were grown in complete RPMI 1640 medium supplemented with 10% FBS and maintained in a humidified atmosphere of 5% CO_2 in air at 37 °C. THP-1 cells were differentiated into macrophages by treating with 81 nM phorbol 12-myristate 13-acetate (PMA) from Sigma-Aldrich (St Louis, MO-USA) for 48 h (Cipollina et al., 2014). PMA-induced differentiation was monitored by measuring the percentage of adherent cells, the modulation of the side scatter (SSC, measured by flow cytometry), and the surface expression of CD68, a marker of differentiation that is known to increase after PMA treatment (See Supplementary Fig. S1 in the online version at doi:10.1016/j.toxlet.2017.07.878). Where indicated, undifferentiated and PMA-differentiated cells were incubated with 10% of cigarette smoke extracts (CSE) and different concentrations of ceftaroline alone or in combination. After 24 h, cells and supernatants were collected and used for further experiments. As shown in Supplementary Fig. S1 (See Fig. S1 in the online version at doi:10.1016/j.toxlet.2017.07.878), CSE do not induce differentiation and do not interfere with the differentiation process when looking at % adherence and SSC values. CSE appear to have a positive effect on the expression of CD68, which is consistent with the activation of TLR4 by CSE (Pace et al., 2008).

2.2. Preparation of cigarette smoke extracts (CSE)

Cigarette smoke extracts (CSE) were prepared as described previously (Pace et al., 2013) using commercial available cigarettes (Marlboro). Each cigarette was smoked for 5 min and one cigarette was used per 10 ml of PBS to generate a CSE-PBS solution. The CSE solution was filtered through a 0.22 micrometer-pore filter to remove bacteria and large particles. The smoke solution was then adjusted to pH 7.4 and used within 30 min of preparation. This solution was considered to be 100% CSE and diluted to obtain the desired concentration in each experiment. The concentration of CSE was calculated spectrophotometrically measuring the OD as previously described at the wavelength of 320 nm. As previously reported (Pace et al., 2008), the presence of contaminating LPS on undiluted CSE was evaluated by a commercially available kit (Cambrex Corporation, East Rutherfort, New Jersey, USA) and was below the detection limit of 0.1 EU/ml.

2.3. Cell viability

Cell viability was evaluated by CellTiter 96 Aqueous One Solution Cell Proliferation Assay (PROMEGA Madison WI USA) according to the manufacturer's instructions as previously described (Pace et al., 2016). One Solution reagent contains MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfopheyl)2H-tetrazolium]. Cells were plated in 96-well plate and treated with ceftaroline at increasing concentration (6 ng/ml; 60 ng/ml; 600 ng/ml; 6 μg/ml). After 24 h, 20 μl of One Solution reagent was added to each well, and incubated for 20 min at 37 °C, 5% CO_2. The absorbance was measured at 490 nm on the Microplate reader Wallac Victor2 1420 Multilabel Counter (Perkin Elmer).

2.4. Flow cytometry analyses

Flow cytometry (Becton Dickinson FACSCalibur System) was used for assessing the expression of total TLR2 and TLR4, LPS binding and surface CD68.

Monoclonal antibodies PE-conjugated anti-human TLR4 or FITC-conjugated anti-human TLR2 (both from eBioscience, San Diego, CA) were used to stain permeabilized cells. The surface expression of CD68 was measured by staining non permeabilized cells with monoclonal mouse anti-human CD68, followed by FITC-conjugated rabbit anti-mouse immunoglobulin (both from Dako).

The binding of LPS was assessed in non permeabilized cells using ALEXA Fluor LPS as previously described (Pace et al., 2013). Cells were stimulated as described above, incubated with ALEXA Fluor LPS for 30 min and the binding of LPS was evaluated by flow-cytometry. The analysis was done on 10.000 acquired events for each sample using cellQuest acquisition and data analysis software. All data obtained by flow cytometry analysis were expressed as percentage of positive cells, after gating on the cells, debris excluded.

2.5. HBD2 and TNF-α release

To assess the release of HBD2 and TNF-α, commercially available ELISA kits were used (Peprotech, London, UK for HBD2 and Cayman Chemical for TNFα) following the manufacturer's instructions.

2.6. Statistics

The analysis of results was performed at least on 3 independent biological replicates (described in figure legends). Analysis of variance (ANOVA) was used for testing differences between means. The possible association between categorical variables was evaluated by the accurate Fisher's exact test. A P value < 0.05 was considered statistically significant. The p-values were performed on log transformed data.

3. Results

3.1. Effects of ceftaroline on cell viability

The first step of our experimental design was to evaluate the effects of ceftaroline on cell viability. We found that ceftaroline at different concentrations (60, 600 ng/ml and 6 μg/ml) did not alter the cell viability in undifferentiated and in PMA-differentiated THP-1 cells (Fig. 1**A** and **B**).