Advances in Medical Sciences 61 (2016) 249–254

Contents lists available at ScienceDirect

Advances in Medical Sciences

journal homepage: www.elsevier.com/locate/advms

Original Research Article

Soluble CD163 modulates cytokine production by peripheral blood mononuclear cells stimulated by *Dermatophagoides pteronyssinus* allergens in vitro

Pawel Bernatowicz [a], Krzysztof Kowal [a,b,*]

[a] Department of Experimental Allergology and Immunology, Medical University of Bialystok, Bialystok, Poland
[b] Department of Allergology and Internal Medicine, Medical University of Bialystok, Bialystok, Poland

ARTICLE INFO

Article history:
Received 7 February 2016
Accepted 18 February 2016
Available online 8 March 2016

Keywords:
CD163
Dermatophagoides pteronyssinus
Asthma
Interleukin

ABSTRACT

Purpose: The CD163 is a scavenger receptor expressed exclusively on monocytes/macrophages which has been shown to exert anti-inflammatory effects. The aim of this study was to evaluate the effect of exogenous sCD163 on production of selected cytokines by peripheral blood mononuclear cells (PBMC) of house dust mite allergic patients (AAPs) stimulated in vitro with *Dermatophagoides pteronyssinus* (Dp) allergens.

Patients and methods: The study was performed in 24 AAPs and 12 healthy control subjects (HCs). Peripheral blood mononuclear cells were cultured for up to 144 h (T_{144}) in the presence of Dp extract with or without sCD163. Concentration of interleukin (IL) – 10, IL-13 and transforming growth factor beta (TGF-β) was evaluated in the cell culture supernatants using ELISA. Expression of the selected cytokines was evaluated in cell culture lysates using Taqman-based real time polymerase chain reaction (RT-PCR).

Results: Dp-stimulated PBMC from AAPs released more IL-10 and IL-13 than those from HCs. The greatest up-regulation of *IL-10* expression was seen at T_6, while that of *IL-13* was delayed. Soluble CD163 augmented production of IL-10 in response to Dp stimulation. No significant effect of sCD163 on production of IL-13 and IL-10 by PBMC of HCs could be demonstrated.

Conclusions: In AAPs sCD163 modulates the immune response to Dp allergens potentiating anti-inflammatory, homeostatic mechanisms.

1. Introduction

CD163 is a protein of 130 kD molecular weight belonging to the group B of the scavenger receptor cysteine-rich (SRCR) superfamily [1,2]. It is exclusively expressed by mononuclear phagocytes as a type I transmembrane protein [1]. The protein is shed from the cell membrane and as a soluble form (sCD163) is present in body fluids including plasma and sputum [1,3,4]. Expression of *CD163* is strongly up-regulated by anti-inflammatory mediators including corticosteroids (CS) and interleukin-10 (IL-10) [5–12]. Corticosteroids are very potent inducers of *CD163* expression and the protein, at least under certain circumstances, participates in anti-inflammatory action of CS [5–7]. Interleukin-10 is also involved in the regulation of *CD163* expression in many physiological and pathological conditions [8–12]. In fact, in experiments utilizing gene-chip technology *CD163* displays the strongest response to IL-10 among all 19 of the up-regulated genes [10]. Interleukin 10 is also responsible for up-regulation of monocyte/macrophage CD163 expression induced by CD4+CD25+Foxp3+ T regulatory cells and for rebound up-regulation of monocyte/macrophage CD163 expression after shedding of the receptor in response to Toll like receptor (TLR) stimulation [11,12]. Monocytes/macrophages expressing CD163 release broad range of anti-inflammatory mediators including IL-10 and carbon monoxide (CO) [13]. Membrane bound CD163 is responsible for fueling this anti-inflammatory response under certain clinical conditions [13]. Not only membrane CD163 but also sCD163 exerts anti-inflammatory action. Soluble CD163 inhibits in a dose-dependent

* Corresponding author at: Department of Experimental Allergology and Immunology, Medical University of Bialystok, Sklodowskiej-Curie 24a, 15-276 Bialystok, Poland. Tel.: +48 85 7468373; fax: +48 85 7468601.
E-mail address: kowalkmd@umb.edu.pl (K. Kowal).

http://dx.doi.org/10.1016/j.advms.2016.02.004
1896-1126/

manner phorbol ester-induced T cell activation and proliferation in vitro [14,15]. This anti-inflammatory function of CD163 seems to be restricted to its soluble form, as membrane-bound CD163 does not exert such an effect [16].

The anti-inflammatory effects of CD163 can be also demonstrated in vivo [17]. In particular, in patients with rheumatoid arthritis histological analysis of the inflamed joints revealed that wherever intense T cell proliferation was observed little or no CD163 was seen on tissue macrophages [17]. Moreover, lymphoid follicle macrophages, which are located in a place where intensive lymphocyte proliferation occurs, express little or no CD163 at all [18,19].

It seems that CD163 may also be involved in the regulation of the inflammatory response to allergen challenge. Our previous studies demonstrated that circulating monocytes of asthmatic patients were characterized by greater expression of CD163 than those of healthy subjects [20]. Moreover, those that express high level of CD163 seem to be preferentially attracted to the airways of allergic asthma patients after allergen exposure [21]. The role of CD163 as an inflammatory molecule has been demonstrated in experimental studies using genetically modified CD163 knockout mice [22]. House dust mite-induced airway inflammation was augmented in CD163 knockout mice in comparison to their wild type counterparts. This provides evidence for important in vivo anti-inflammatory effects of CD163 in response to allergen exposure.

It was therefore of interest to investigate in vitro the effect of exogenous sCD163 on production of selected cytokines by peripheral blood mononuclear cells from house dust mite allergic asthma patients stimulated with allergen extract of a house dust mite *Dermatophagoides pteronyssinus* (Dp).

2. Patients and methods

The study was performed in 24 house dust mite allergic asthma patients (AAPs). In addition 12 nonatopic healthy subjects (HCs) were included as a control group. Asthma was diagnosed according to the GINA guidelines [23]. In all patients either forced expiratory volume within the first second (FEV_1) of less than 80% of the predicted value with at least 12% improvement 15 min after inhalation of 400 mcg salbutamol and/or significant bronchoconstrictive response to inhaled histamine was demonstrated. Patients with any other systemic diseases, suffering from respiratory tract infection within 1 month before the study or smoking were not included in the study. The study was approved by the local Bioethics Committee (R-I-002/286/2008). All participants provided written informed consent.

2.1. Skin prick tests

All persons were skin tested using prick methodology with a screening panel of aeroallergens (Allergopharma, Reinbek, Germany) as described earlier [24].

2.2. Bronchial challenge

Histamine bronchial challenge was performed according to the method previously described elsewhere [20]. Briefly, all patients inhaled doubling concentrations of histamine starting from a concentration of 0.62 mg/ml. Aerosol was generated using a DeVilbis #646 nebulizer attached to a Rosenthal – French dosimeter. All subjects performed five inspiratory – capacity breaths of given histamine concentration. Forced expiratory maneuvers were performed 90 s after each fifth inhalation. The procedure was continued until either at least 20% fall of FEV_1 or histamine concentration 32 mg/ml was reached. Bronchial reactivity to histamine is expressed as histamine concentration causing 20% fall of FEV_1 (PC_{20}).

2.3. Cell isolation and culture

Peripheral blood mononuclear cells were isolated from 20 ml of venous blood by density gradient centrifugation using Histopaque 1077 (Sigma–Aldrich, St. Louis, MO, USA). Total number and viability of isolated cells were assessed using Fuchs Rosenthal chamber and trypan blue exclusion method. The cells were cultured in 24-well culture plates at a density 1×10^{-6} cells/ml in RPMI-1640 medium supplemented with 5% heat inactivated fetal calf serum, L-glutamine, penicillin and streptomycin. The cells were cultured in the presence of Dp (Allergopharma, Reinbek, Germany) at a concentration 5000 SBE/ml with or without sCD163 (R&D Systems, Minneapolis, MN, USA) at a final concentration 0.5 μg/ml. In addition, cell cultures without any stimulation was used as a negative control. The final concentrations were established in a preliminary dose-response studies (not shown).

After 6, 12, 24, and 144 h the supernatants were separated from cells, aliquoted and stored frozen at −70 °C until tested. The cells were treated with lysing solution and stored frozen at −70 °C for further isolation of RNA.

2.4. Biochemical and immunologic assays

RNA isolation was performed using RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer instruction.

The evaluation of RNA concentration was performed using Nanodrop 2000 (Thermo Fisher Scientific, Waltham, MA, USA). Subsequently the evaluation of RNA integrity was performed using RNA 6000 Pico Kit (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer instructions and the results were read using Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA, USA). Only RNA of high quality (RNA integrity number – RIN > 8) was used for further analysis.

Taqman-based real time PCR was used to quantitatively evaluate expression of the selected genes *IL-10* Hs.193717, NM_000572.2, *IL-13* Hs.845, NM_002188.2, *TGF-β* Hs.645227, NM_000660.4, and a house keeping gene *B2M* Hs.534255, NM_004048.2. The reaction was performed in 384-well plate in a final volume of 10 μl per reaction using ABI Prism 7900HT (Applied Biosystems, Carlsbad, CA, USA) with the following thermal cycle profile: initial denaturation 10 min at 95 °C, then 40 cycles each 15 s at 95 °C and 60 s at 60 °C.

Quantitative assessment of gene expression was performed using the comparative threshold cycle (CT) method. The threshold cycle (CT) indicates the number of cycles at which the amount of amplified target reaches an exponential phase of amplification. For comparative purpose a CT of an individual gene is normalized to a CT of a house keeping gene and the result is called ΔCT. Than the difference between unstimulated and stimulated samples (ΔΔCT) is calculated by subtracting ΔCT of the former from the ΔCT of the latter. The fold change was determined using formula $2^{-\Delta\Delta CT}$.

Concentration of IL-10 and IL-13 in cell culture supernatants was evaluated using high sensitivity enzyme-linked immunosorbent assays (R&D Systems, Minneapolis, MN, USA and Abcam, Cambridge, UK, respectively) according to the manufacturer's instruction. All samples were run in duplicates.

2.5. Statistical analysis

Normal distribution was evaluated using Wilk-Shapiro test. Comparison of normally distributed continuous variables was